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THE VALUE OF TUBERCULIN TESTS IN MAN

WITH SPECIAL REFERENCE TO THE INTRACUTANEOUS TEST

By P. D'ARCY HART

LONDON
PUBLISHED BY HIS MAJESTY'S STATIONERY OFFICE
1932

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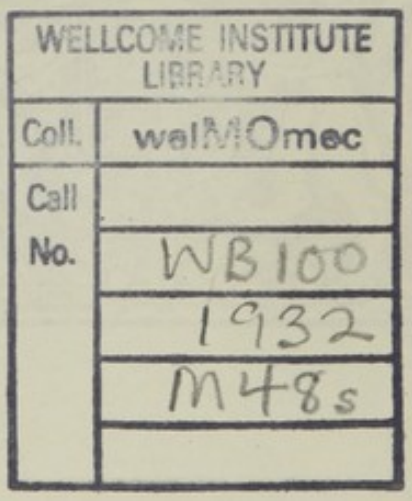
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PREFACE

Koch's use in 1890 of the 'tuberculin' then newly isolated from tubercle bacilli was greeted with the keenest interest on account of its promise in two directions. It gave hope of an effective vaccine for the treatment and cure of established tuberculous disease, and it provided a new diagnostic method by the localized response which it was found to excite when injected through the skin of an infected patient. But the hopes so raised have never been fully satisfied in either direction. As the years have moved on, tuberculin seems to have been used less rather than more, either for treatment or for diagnosis, by clinicians in Britain.

Since the War the Medical Research Council have taken an active interest in both aspects of the use of tuberculin, and in 1920 they appointed a Tuberculin Committee to whom they have been indebted for the initiation or supervision of much work of which the results have been published in this series of Reports and elsewhere.

The present Report by Dr. D'Arcy Hart reviews the present position only with regard to tuberculin as a diagnostic agent. It took its origin in a London investigation, made in 1929 by Dr. Bernard Schlesinger and himself, of the tuberculin reactions of 118 children coming from tuberculous households in contrast with those of 513 children from non-tuberculous homes. The investigation was at the same time extended to a wider field by Dr. Hart, and this Report finally gives his observations of the reactions of 1,030 clinically tuberculous patients of all ages and all types of infection, in contrast with those of 751 clinically non-tuberculous patients of all ages. Data given by many of the children examined in the former inquiry are included in these total figures.

Dr. Hart gives good evidence here that the tuberculin test is one of scientific accuracy, provided that the intracutaneous or Mantoux test be used and not the cutaneous test of von Pirquet. The positive reactions are not yet capable of such quantitative grading as might serve to distinguish active tuberculosis, as such, from the benign results of infection that has at some time or another been implanted in most members of an urban population. On the other hand, however, a negative reaction, given adequate dosage of tuberculin, is at all ages of diagnostic value for excluding the presence of tuberculous disease, the error being only about 2 per cent. The negative reaction is most

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likely to be obtained in children who have not yet become tuberculin-sensitized through chance exposure, and it may therefore be of great value for guiding hygienic measures of control in households where a tuberculous patient has been living. The accuracy of the test should make it possible now to obtain trustworthy measurements of the tuberculin sensitization of a population at different ages, and to find whether this is actually falling as a result of modern conditions or modern methods that improve our control of the infection—a point upon which Dr. Hart reviews the epidemiological evidence.

This independent work by Dr. Hart is in harmony with conclusions already published by the Tuberculin Committee upon the use and value of the intradermal test with tuberculin in cattle, and the Council are glad to include this account of his investigation in this Series.

MEDICAL RESEARCH COUNCIL,
38 Old Queen Street,
Westminster, S.W.1. 21*st December*, 1931.

THE VALUE OF TUBERCULIN TESTS IN MAN, WITH SPECIAL REFERENCE TO THE INTRACUTANEOUS TEST.[1]

BY

P. D'ARCY HART, M.A., M.D., M.R.C.P.

(Assistant to the Medical Unit,
University College Hospital, London)

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Definition of Terms used in this Report.

1. THE word *tuberculin* is used throughout this report to indicate 'preparations of fluid media on which *B. tuberculosis* has been grown in artificial culture and which have been freed by filtration from the bacilli' (*Therapeutic Substances Regulations*, London, 1927, p. 21). In the majority of instances the actual preparation is Old Tuberculin;

[1] Begun while the writer was Medical Registrar to the Royal Northern and Royal National Orthopaedic Hospitals and was a Research Student at the Hospital for Sick Children, Great Ormond Street. The major part of this report formed the substance of a thesis for the degree of M.D. (Cantab.).

less frequently it is a tuberculin prepared from a synthetic medium. The preparations BE and TR (classed as 'Tubercle Vaccines' in these regulations), when injected intracutaneously, subcutaneously, or intravenously into sensitized guinea-pigs, produce effects qualitatively indistinguishable from those caused by Old Tuberculin (Parish & Okell, 1929). The constituent of tuberculin and of tubercle bacilli responsible for eliciting the typical skin reaction is referred to as the active principle or the active constituent.

2. By the term *positive tuberculin skin reaction* is meant the typical delayed, persistent, inflammatory response. The term does not include other classes of response that may follow the injection of tuberculin, such as immediate, evanescent, urticarial reactions, possibly due to anaphylactic sensitization and atypical non-specific reactions (see p. 53). Unless otherwise stated, *tuberculin sensitization, sensitivity, hypersensitiveness*, and *reactivity* also refer to the typical response.

3. '*Infection* [is] any association of parasite and host in which the reaction between them involves any damage, however slight, to the host's tissues.' (Topley & Wilson, 1929 a.)

The term *tuberculous infection*, which appears repeatedly in this report, signifies that the individual is infected with the tubercle bacillus. This does not necessarily imply that an active focus is present, nor does it imply that the individual has had, or ever will have, clinical or manifest tuberculosis. Tuberculous infection includes primary infection and reinfection.

Clinical or *manifest tuberculosis* or *tuberculous disease* may be defined as tuberculous infection active enough to give rise to significant symptoms. This definition is admittedly imperfect. Manifest tuberculosis may be active, quiescent, arrested, or clinically cured.

Subliminal tuberculous infection is defined as infection unaccompanied by significant symptoms, i.e. infection as it exists in the clinically non-tuberculous. This definition corresponds closely to Opie's (1927 a) use of the term 'latent tuberculosis'. Subliminal infection may be active or inactive.

Although tuberculous infection includes clinical or manifest tuberculosis, these terms must be differentiated. Thus, the majority of the urban population become infected before reaching adult life, but comparatively few of those infected develop clinical tuberculosis; in the others infection remains subliminal.

4. The *incidence of tuberculous infection* in a community is defined as the percentage of the population that is infected with tubercle. The term *infected population* is used in the same sense.

The *attack-incidence of tuberculous infection* in a community is defined as the percentage of the population that has at some time been infected with tubercle. The degree of *tuberculization* of a community is the extent to which its members have been so infected.

The *attack-rate of tuberculous infection* in a community is the ratio between the annual number of new persons infected and the mid-year population expressed in hundreds.

5. The *tuberculosis death-rate* or *mortality-rate* in a community is

the ratio between the annual number of deaths from tuberculosis and the mid-year population expressed in thousands, ten thousands, or hundred thousands.

6. The *fatality of tuberculous infection* is the proportion of individuals infected that die of the disease.

The *fatality-rate of tuberculous infection* in a community is defined as the ratio between the annual number of deaths from tuberculosis and the mid-year infected population expressed in hundreds.

7. The *incidence of tuberculin sensitization* or *sensitivity* in a community is the percentage of the population that is capable of reacting positively to tuberculin. The terms *tuberculin-sensitive* and *sensitized population* are used in the same sense.

8. The term *home-contacts* is used to denote children in whose homes persons suffering from clinical tuberculosis have lived. It does not include children who have been exposed, in their homes or elsewhere, to tuberculous relatives, &c., if the latter have not resided with them.

The term *tuberculous households* is used in the same sense, and the expressions *non-contacts* and *non-tuberculous households* in exactly the opposite sense.

9. The term *hospital class* conveniently designates the average type of patient attending the public wards and clinics of state or voluntary hospitals. The cultural and economic conditions of this class are naturally liable to change, and vary from place to place. This definition recognizes in urban populations a class whose social and economic status is lower than that of the average hospital patient (e.g. inmates of workhouses or orphanages).

10. *Public school* is used in the sense accepted abroad, i.e. to indicate a state school, and not in the special sense it has in this country.

11. The terms (a) *cutaneous* and *von Pirquet test*, (b) *percutaneous* and *Moro test*, (c) *intracutaneous* and *Mantoux test*, and (d) *local subcutaneous test* and *stichreaktion* are used synonymously.

12. The word sibling is used to indicate a brother or sister having the same father and mother (Gould's *Medical Dictionary*).

Introduction.

During the last forty years a number of tests have been employed in medical practice for the detection of tuberculosis, having as their common basis the allergic response of tubercle-infected individuals to products of the growth of the bacillus. The subcutaneous test of Koch (1890, 1891) was the first of these tuberculin reactions. Shortly afterwards Epstein (1891) and Escherich (1892) described the *stichreaktion*, or needle-track reaction, which is a local phenomenon of the subcutaneous tissue produced by a dose of tuberculin too small to give a general response. This test was revived and modified by Hamburger (1908, 1912 b), and is often referred to as the local subcutaneous test. Escherich's reaction was followed by the von Pirquet (1907 a) cutaneous test, the conjunctival method of Calmette (1907) and Wolff-Eisner (1907), and the percutaneous or inunction reaction of Moro (Moro & Doganoff, 1907; Moro, 1908). A year later, Mendel (1908),

Mantoux (1908), and Moussu & Mantoux (1908 a, 1908 b) described the intracutaneous method. There are various other tests, mentioned by Baldwin, Petroff & Gardner (1927 c). The small amount of attention that they have attracted is doubtless a measure of their value, and they will not be considered in this report.

With the passage of time and the enormous accumulation of published work on the subject it has become possible to form a clearer estimate of the usefulness of tuberculin tests, and of the relative value of the various methods.

Tuberculin tests have been used in man principally for the following purposes:

(1) For detecting tuberculous infection in early childhood (Section IV), and for investigating its spread among the children of tuberculous households (Section VI).

(2) To estimate the distribution of tuberculous infection in communities in different parts of the world, a point of fundamental importance in considering the epidemiology of tuberculosis. For these tuberculin surveys it is necessary to test adequate numbers of subjects of all ages, selected at random so as to form a fair sample of the population under consideration, and to record the results in age-groups (Section VII).

(3) As an aid to the physician in the diagnosis of clinical or manifest tuberculosis in individual cases (Sections IV and V).

(4) To estimate the prognosis in cases of clinical tuberculosis (Section IV).

Before considering in detail these applications of the tuberculin test it will be well to discuss the present views of the significance of a positive and a negative tuberculin skin reaction.

PART I

DIAGNOSTIC APPLICATIONS

Section I. The Significance of a Positive Tuberculin Skin Reaction.

CONTENTS.

1. The tuberculin reaction: a hypersensitiveness phenomenon . . . 9
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1. THE TUBERCULIN REACTION: A HYPERSENSITIVENESS PHENOMENON.

WHILE knowledge of the constitution of tuberculin and of the nature of the tuberculin reaction is far from complete, the available data allow certain statements to be made. (For a comprehensive review of the subject the reader is referred to Tytler (1930).)

Both the active principle responsible for eliciting the tuberculin reaction and the factor responsible for the sensitization have been sought for in various preparations derived from tubercle bacilli.

(*a*) *Non-protein Fractions of Tuberculin.*

A non-protein fraction was obtained from tuberculin by Mueller (1926), working with material prepared from a synthetic protein-free medium. This substance has also been obtained by extraction from the bodies of the bacilli themselves (Laidlaw & Dudley, 1925). It is stated to be a polysaccharide, probably a gum (Laidlaw & Dudley, 1925; Mueller, 1926), and appears to belong to the group of bacterial products to which Zinsser & Parker (1923) have given the name 'residue antigen', extending the term antigen from the generally accepted meaning (Wells, 1929 a), namely, a substance that causes the formation of antibodies, to include any substance that will react specifically with antibodies. These complex carbohydrate products are not antigens in the usual limited sense, since they do not appear to be capable of producing antibodies when injected parenterally (Zinsser & Parker, 1923; Laidlaw & Dudley, 1925; Zinsser & Mueller, 1928). On the other hand, they react readily by precipitation or by complement-fixation with the antibodies present in the serum of animals and human beings immunized with the specific organism. This property was found in material obtained from tubercle bacilli by Zinsser & Parker (1923), Laidlaw & Dudley (1925), and Mueller (1926), from pneumococci by Dochez & Avery (1917), and Avery & Heidelberger (1923, 1924, 1925), and from streptococci by Hitchcock

(1925), Lancefield (1925), and Zinsser & Tamiya (1925). Intracutaneous injection of the residue antigen present in tuberculin fails to elicit the characteristic positive reaction in tubercle-infected animals (Mueller, 1926; Zinsser & Mueller, 1928).

(*b*) *Protein Fractions of Tuberculin.*

Protein-containing fractions, retaining most of the substance responsible for eliciting the typical skin reaction, have been obtained from tuberculin by various investigators since the time of Koch (Koch, 1891; Dienes & Schoenheit, 1925; Mueller, 1926; Long & Seibert, 1926; Seibert, 1928 a). A protein product separated by Mueller gave strong specific skin reactions in tuberculous guinea-pigs, but almost no precipitin reaction with the sera of rabbits that had been immunized with heat-killed bacilli. On the other hand, Seibert (1930), using very active protein fractions of a tuberculin prepared from a protein-free medium, obtained strong precipitin reactions with sera of tuberculous guinea-pigs, and (1927, 1930, 1931) also produced precipitins and anaphylactic sensitization in normal animals by intraperitoneal or intravenous injection. In their antigenic properties Seibert's products resemble other foreign proteins. It should be stated here that crude Old Tuberculin has been found by most observers to have little or no power of inducing antibody formation (Fleischner, Meyer & Shaw, 1919; Dienes & Schoenheit, 1925; Zinsser, 1927), possibly on account of its low protein content.

While it has been generally held that the active principle concerned in the tuberculin reaction is protein in nature (although Eberson (1925 a, 1925 b, 1926) obtained a non-protein substance from tuberculin, which he claimed to contain this principle), until recently attempts towards isolating an active protein have been unsuccessful. Seibert (1927, 1928 b), however, following on Long & Seibert's (1926) series of studies of the protein fractions of tuberculin, reported the isolation in crystalline form of a water-soluble coagulable protein. The protein was considered to be formed directly by the tubercle bacillus, since the tuberculin used in its preparation was made from a protein-free synthetic medium. This product gave strong and characteristic cutaneous reactions in tuberculous guinea-pigs, and Seibert suggested that it might be the active principle responsible for eliciting the tuberculin reaction. Further work is clearly necessary before this point can be decided. In her most recent paper (Seibert & Munday, 1931) the view is advanced that the skin reaction is associated with a specific portion of the protein molecule, present in the early cleavage products obtained during hydrolysis.

(*c*) *Bacillary Protein Fractions.*

Crude protein fractions, capable of eliciting the typical skin reaction in tuberculous animals, have been obtained from the bodies of dead tubercle bacilli by various methods of extraction. Recently, attempts towards further separation of the active constituent have met with some measure of success, and water-soluble protein material possessing

high tuberculin potency has been obtained from bacillary extracts in greater quantity and state of purity than hitherto achieved (Dienes, Schoenheit & Scheff, 1925; Coghill, 1926; Johnson & Renfrew, 1928; Tytler, 1930). The active protein fractions separated from bacillary extracts and those obtained from culture filtrates such as Old Tuberculin (described under the previous heading) seem to be very similar (Seibert & Coghill, 1928; Tytler, 1930).

The proteins obtained from the bodies of tubercle bacilli possess antigenic properties qualitatively similar to those of other foreign proteins. Thus, their injection into healthy animals can induce the formation of antibodies (Petroff, 1923; Zinsser & Mueller, 1928; Pinner, 1928), though the latter may have a low titre; and as a result of the same procedure anaphylactic sensitization may develop (Baldwin, 1910; Krause, 1916; Zinsser, 1921; Pinner, 1928), and while in this state an immediate evanescent urticarial response can sometimes be elicited by intracutaneous injection of tuberculin (Zinsser, 1921). In addition, it has been noted by Baldwin (1910), Zinsser (1921), Zinsser & Tamiya (1926), and others that a slight cutaneous response to tuberculin, resembling somewhat in appearance the typical reaction seen after tuberculous infection, may be obtainable after the previous injection of large quantities of suitably extracted bacillary protein fractions into healthy animals. This skin response to tuberculin, which Krause & Willis (1919) were unable to obtain even after previous repeated injections of bacillary protein fractions, is, however, inconstant, transient, and of less intensity than the prolonged and necrotizing reactions obtainable in tuberculous animals, and, according to Baldwin, Petroff & Gardner (1927 a), is distinguishable from the latter (but see discussion by Lange, 1924).

(*d*) *Autolytic Products.*

The biological properties of bacterial autolysates throw some light on the nature of the tuberculin reaction. Zinsser & Grinnell (1927) found considerably increased antigenic properties in the 'bacterial nucleoprotein' of pneumococci, if obtained by the relatively non-destructive method of bile extraction. Intense systemic sensitization, together with marked cutaneous sensitivity, was induced by injection of this substance into guinea-pigs. Zinsser & Mueller (1928) point out that no satisfactory method of autolysing tubercle bacilli *in vitro* has yet been elaborated. The nearest approach is provided by an experiment of McJunkin (1921), who obtained a tuberculous antigen with highly sensitizing properties from the Berkefeld-filtered exudate that resulted from intraperitoneal injection of tubercle bacilli into guinea-pigs suffering from tuberculous peritonitis. With this autolytic product he was able to evoke a certain degree of cutaneous sensitivity to tuberculin by injection into normal guinea-pigs, which sensitivity he considered similar to that present in tuberculous animals. In view of this work, confirmed by Zinsser & Tamiya (1926), using tuberculin instead of bacilli for the intraperitoneal injection, and by Lange (1924), using filtered extracts of tuberculous tissue, the distinction between

reactions to tuberculin following injections of bacillary protein extracts into healthy animals and those obtainable in tuberculous animals may be only quantitative. The observations of Tytler (1930) are interesting in this respect, for, after injecting normal guinea-pigs intraperitoneally with dry bacillary protein material under conditions that produced severe peritonitis, he obtained strong and almost characteristic tuberculin reactions in some cases, though in others only atypical responses resulted.

From the preceding discussion it may be concluded that the power of eliciting the typical skin reaction in tuberculous animals is retained in various preparations ultimately derived from tubercle bacilli, and it is very probable that the active constituent is identical in each case, and is protein in nature.

Tuberculin sensitization is an example of the type of allergy that Coca & Cooke (1923) have called 'hypersensitiveness of infection'. Some authorities (e.g. Rich, 1929; Tytler, 1930) would limit the term allergy to this type of heightened reactivity, which should in any case be distinguished from true anaphylactic hypersensitiveness, induced by the previous injection of any foreign protein antigen, and dependent on a true antigen-antibody reaction.[1] The latter state, however, can be produced with proteins from tuberculin and with proteins from the bodies of tubercle bacilli (see above), and may, but does not necessarily, exist in an infected animal at the same time as tuberculin hypersensitiveness (Baldwin, 1910; Zinsser, 1921; Smith, 1923). But while the various proteins of the tubercle bacillus can elicit anaphylactic reactions in animals sensitized either by injection of these proteins or by infection, the active constituent capable of eliciting the typical skin reaction is borne by only a small fraction of the total protein.

Tuberculin must be differentiated from the toxins, since it neither has great toxicity for normal animals, nor is able to induce the formation of anything comparable to the antitoxins that prevents its local or systemic toxic effects in tuberculous animals (see Wells, 1929 b).

Evidence is accumulating to show that tuberculin hypersensitiveness may be accompanied by cutaneous reactivity to various non-specific protein substances, e.g. meat extracts, 'peptones', &c., though the skin reactions to non-specific products are usually less intense than to tuberculin, and larger amounts are required to elicit a response. These observations make it necessary to regard the specificity of the tuberculin reaction as quantitative, rather than absolute (see Long, 1928; Tytler, 1930; Hart, 1932).

2. Tuberculin Hypersensitiveness in Man the Result of Tuberculous Infection.

The evidence on which the conclusion that tuberculin hypersensitiveness in human beings is the result of tuberculous infection has been based is as follows:

(1) Necropsies on human subjects and cattle whose tuberculin

[1] See Zinsser (1921), Krause (1925 b), Calmette (1928), Wells (1929 c), Tytler (1930), Scott (1931).

reactions had been ascertained during life have been performed by a number of observers. While in some of the work the presence of tuberculous infection was considered to be proved by the detection of bacilli in stained smears, or by cultural or biological tests, in other cases the finding of anatomic tubercle (macroscopic or microscopic) alone was taken to indicate that infection (i.e. the invasion of the tissues by living bacilli) had occurred at some time. Further, in some instances living bacilli were found in the apparently healthy tissues of individuals with no recognizable tuberculous lesions at all, but it is not clear that the tissues had been examined microscopically for tubercles in all these cases.

Von Pirquet (1907 b) examined 100 children who had been tested by his method (1 : 4 dilution). He found at post-mortem undoubted macroscopic evidence of the occurrence of tuberculous infection in 30 out of the 31 positive cases (in 21 cases tuberculosis was the cause of death), while, conversely, of the 52 cases in which no post-mortem evidence of infection was found, all but one had given a negative reaction. Complete confirmation of positive von Pirquet reactions at necropsy was reported by Grüner (1908), among others, in a series of 45 children, by Müller (1909) in 22, and by Knox (1917) in 45, similar cases; while Feer (1910) found macroscopic post-mortem evidence that infection had taken place in 22 out of 24, and Radziejewski (1911) in 149 out of 153, Pirquet-positive children. Using the intracutaneous method, Aptekmann (1909) reported that necropsy confirmed all the 17 positive reactions in his series.

Similar results were reported in cattle by Moussu & Mantoux (1908 b), using the intracutaneous test. More recently, the Medical Research Council Tuberculin Committee (1925, 1928) carried out post-mortem controls (including staining of smears, and biological tests) on 114 cattle to which the intracutaneous test had been applied immediately before slaughter. Thirty-seven of the 39 positive reactors were proved tuberculous by inoculation of guinea-pigs. The other 2 cases showed macroscopic lesions that were considered to be in all probability tuberculous, but inoculation failed to kill guinea-pigs; in one of these cases a stained smear revealed bacilli. In several instances animals that had reacted to the tuberculin test showed no naked-eye post-mortem evidence of the disease, but were stated to have been proved to be tuberculous by stained smears or guinea-pig inoculation. Conversely, the 74 cattle that were free of tuberculosis macroscopically, and whose smears and guinea-pig inoculation tests were negative, were non-reactors to tuberculin. (The agreement between the post-mortem findings and the results of intracutaneous tests in cattle resembles that found in veterinary practice by earlier workers, using the subcutaneous test. The extensive literature is quoted by Kolmer (1923 a), who states that in 15,000 instances only 2–3 per cent. of contradictions were noted.)

It will be seen, therefore, that post-mortem findings have, in the main, confirmed the positive tuberculin reactions obtained during life. From these and similar observations it has been inferred that under natural conditions tuberculin hypersensitiveness is probably constantly associated with tuberculous infection.[1]

(2) Post-mortem statistics of the attack-incidence of tuberculous

[1] In a recent paper Hastings, Beach & Thompson (1930) report that they were able to culture acid-fast bacilli from the tissues of a number of cattle that were tuberculin-positive, but showed no lesions of tuberculosis post-mortem. A number of these cultures gave negative guinea-pig tests but produced evanescent cutaneous sensitization to tuberculin, unaccompanied by lesions, when injected subcutaneously into tuberculosis-free cattle. The authors conclude that a positive tuberculin test in cattle is not absolute proof of tuberculous infection, but may be due to invasion of the tissues by other mycobacteria.

infection (as defined on p. 6) show a degree of tuberculization consistent with that indicated by statistics of positive tuberculin reactions in the living, provided that the two sets of figures are obtained about the same period and from comparable samples of the population, and that the accuracy of the technique employed is taken into consideration.

The results of various tuberculin surveys are given in Section VII of this report, where it is shown that the percentage incidence of positive reactions in the majority of civilized communities increases with age, and that by the time adult life is reached most of the urban working-class population respond to the test positively.

A similar distribution of tuberculous infection is indicated by post-mortem statistics.[1] For such figures to be reliable the post-mortem technique is obviously of the greatest importance, as small lesions may easily be overlooked. Omission of microscopic sections and of biological tests, and reliance on naked-eye examination alone, are liable to give underestimates of the real incidence of infection, and faulty technique may thus account partly for the unusually small percentages of persons showing pathological changes due to tuberculous infection that have been reported by some workers (consult Fowler, 1921 a; Fishberg, 1922 a).

It should be pointed out that the majority of the post-mortem figures for children were obtained in the first decade of the century, and from the populous quarters of various European cities where tuberculosis was very prevalent. There is some evidence from a few cities that the incidence of positive tuberculin reactions in the living has fallen during the last twenty years (p. 84). If this be so, post-mortem investigations in the future are likely to reveal a correspondingly lowered incidence of tuberculous lesions in children.

The fair agreement between post-mortem statistics of the attack-incidence of tuberculous infection and statistics of positive tuberculin reactions lends support to the inference made in the preceding subsection, namely, that under natural conditions tuberculin hypersensitiveness in man is probably constantly associated with tuberculous infection.

In seeking to establish the specificity of the tuberculin reaction in human beings by means of necropsies the attention of workers has been mainly concentrated on countries where tuberculosis is the only common endemic disease caused by an acid-fast bacillus. The possibility that other acid-fast infections, unaccompanied by tuberculous infection, may produce cutaneous hypersensitiveness to tuberculin in man cannot, therefore, be completely excluded. Paneth (1928) has recently suggested that leprous infection without clinical disease may be widespread in communities in which leprosy is endemic, just as tuberculous infection, as opposed to clinical tuberculosis, is widespread

[1] Consult Kuss (1898), Naegeli (1900), Macfadyen & MacConkey (1903), Ganghofner (1905), Burkhardt (1906), Gaffky (1907), Hamburger (1907), Harbitz (1913), Eastwood, Griffith & Griffith (1914), Opie (1917), Reinhart (1917), Hahlo (1924), Ophüls (1926), Still (1927), Todd (1927), Korns (1928).

in European populations. This suggestion led Cummins & du Pré le Roux (1930) to determine whether leprous infection might be a significant factor in causing the high percentage of positive reactors to tuberculin found among apparently healthy native mine recruits arriving at Johannesburg, for the extensive sensitization appears to be 'unaccompanied by any corresponding power to resist the development of tuberculosis while in industry'. The result of a quantitative comparison of skin reactivity to tuberculin and to extracts of leprous skin did not support this hypothesis, although, as these authors state, the evidence was not conclusive.

(3) Debré, Paraf & Dautrebande (1921) claimed that they could detect the development of focal lesions in children by clinical or radiographical examination simultaneously with the development of cutaneous hypersensitiveness to tuberculin. Opie & McPhedran (1926) have compared the frequency of latent pulmonary foci of tuberculosis radiographically with the frequency of tuberculin reactions. They find that the incidence of both increases with age, and more rapidly in contact children than in non-contacts. This observation supports the view that tuberculin hypersensitiveness and tuberculous infection are closely related.

(4) Experimental work on animals, correlating infection with the development of cutaneous hypersensitiveness to tuberculin, has been carried out by a large number of observers, whose results demonstrate that tuberculous infection induces tuberculin hypersensitiveness, which fluctuates according to the varying phases of the infection.

On the other hand, the active principle of tuberculin has no action in healthy animals in any way comparable to its effect in tuberculous animals (Tytler, 1930). Skin reactions to tuberculin, resembling in appearance to a varying extent those obtained in tuberculous animals, but usually less in intensity, have been reported in animals infected or treated with other acid-fast bacilli (Hagan & Zeissig, 1929; and see Tytler, 1930, and footnote, p. 13). Reactions have also been reported after experimental infection with certain organisms outside the acid-fast group, e.g. *Trichophyton* (Urbain & de Potter, 1926) and *B. abortus bovis* (Zinsser & Tamiya, 1926; and see Long, 1928), but their relation to the typical response seen after tuberculous infection is at present uncertain. According to Zinsser & Tamiya they represent overlapping of specificity. It is doubtful whether cross-reactions to tuberculin after infection by unrelated bacteria occur in man with sufficient frequency, intensity, and similarity to the specific response to contribute to any significant extent to the number of positives recorded in routine tests.

It has been shown experimentally by many workers[1] that true and lasting skin hypersensitiveness to tuberculin can be obtained in animals not only by infection with living tubercle bacilli but also by the injection of organisms killed by heat, and that this hypersensitive-

[1] See Petroff (1924), who summarizes the contradictory previously published work, Zinsser & Petroff (1924), Zinsser & Tamiya (1925), Petroff & Stewart (1925), Tytler (1930).

ness is identical in both cases. Even so, as Baldwin, Petroff & Gardner (1927 b) and Tytler (1930) point out, such sensitivity, induced by dead bacilli, may be associated with the formation of anatomic tubercle histologically similar to that following infection with living organisms. On the other hand, Baldwin (1911) had previously shown that even the introduction of living tubercle bacilli would not result in cutaneous hypersensitiveness if they were separated from the tissues of the host by capsules. Krause (1916) summarized contemporary opinion concisely by the statement 'There is no cutaneous hypersensitiveness [to tuberculin] without a focus (tubercle)', although this might require careful search, and if the words 'under natural conditions' are added, and possible cross-reactions within the species are excepted, this view is held by most workers to-day.

The production both of true tuberculin hypersensitiveness and of anatomic tubercle by the injection of dead tubercle bacilli, i.e. without infection, raises the question whether or not a similar process occurs in nature by absorption of dead bacilli from the respiratory or the alimentary tract. The question is discussed by Krause (1925 b) with reference to human beings. He concludes that, while hypothetically possible, practically the contingency is open to grave doubt. If the possibility that tuberculin sensitization arises in nature from the absorption of dead tubercle bacilli be dismissed, it is justifiable to take the presence of anatomic tubercle in human subjects at necropsy to indicate that infection (i.e. the invasion of the tissues by living bacilli) has taken place, and to conclude that under natural conditions tuberculin hypersensitiveness in man is the result of tuberculous infection.

A cutaneous response to tuberculin is, however, obtainable under favourable experimental conditions without tubercle formation or actual infection, for the protein material obtained from the bodies of the tubercle bacilli may, as described above (p. 11), if suitably extracted and injected in large quantities into a healthy animal, evoke slight though inconstant and transient cutaneous sensitivity to tuberculin; and similar results have been reported by Zinsser & Tamiya (1926) and Bohart (1930) after repeated intraperitoneal injection of relatively large amounts of Old Tuberculin or of tuberculin protein fractions. Sensitivity is more marked if an autolysate of tubercle bacilli is used, as in McJunkin's experiment (p. 11). Nevertheless, Baldwin, Petroff & Gardner (1927 a) hold the view that the transient cutaneous response to tuberculin obtained in uninfected animals is distinguishable from true tuberculin hypersensitiveness, which they consider is only evoked by living or dead bacilli.

Although in the natural state characteristic sensitivity to tuberculin appears, with a few possible exceptions, to be constantly associated with 'tubercle tissue', and its experimental production without this formation is open to question, it must not be concluded that tubercle tissue, as such, is an essential causative factor in the production of hypersensitiveness. Tytler (1930) points this out and cites some of the objections to such a view. It is at present wiser merely to consider that

the conditions necessary for the successful action of the sensitizing factor in the natural state are also those that produce tubercle tissue.

3. Non-production of Tuberculin Hypersensitiveness by Tuberculin.

Although a few workers have succeeded in obtaining a cutaneous response to Old Tuberculin after previous repeated injection of this substance into animals (p. 16), the relationship of the response to the typical reaction seen after tuberculous infection is uncertain. The present position with regard to the sensitization of animals by tuberculin will be made clear by quoting two recent statements. Rich & McCordock (1929) conclude that 'Up to the present no one has been able to produce anything satisfactorily comparable to tuberculin allergy by parenteral sensitization with tuberculin', and Tytler (1930) writes, 'The evidence for the production of allergic sensitivity by tuberculin is quite inconclusive, and most modern writers consider it as being entirely in the negative'. In other words, tuberculin seems unable to sensitize to itself in a tubercle-free animal so that a typical response is obtainable.

The same appears to be true in man. Hamburger (1912 a) injected increasing doses of tuberculin, up to as much as 1,000 mg., into children not infected by tubercle, but did not elicit general or specific local reactions. Debré, Paraf & Dautrebande (1921) conclude that 'chez les enfants sains, la répétition indefinie, pourrait-on dire, de cuti-réactions successives, ne provoque jamais l'apparition d'une réaction positive, si faible soit-elle'.

It not infrequently happens that a patient who fails to react to the von Pirquet test, even when undiluted tuberculin has been used, gives a definite response when the test is repeated in the same way some days later.

Lapage (1912), using 1 : 4 tuberculin, repeated the test on 67 patients who gave negative responses at first; 19 of them (28 per cent.) reacted to the second test. Peters (1921), using 1 : 1 tuberculin, obtained reactions in 27 per cent. of patients who were negative to the first test. Thinius (1924), who also used undiluted tuberculin, found an additional 12 per cent. of positive reactors on re-testing 500 children in this manner. Since less than half of these children were primary negative reactors and, therefore, received a second dose, this would mean that over 20 per cent. of those who gave negative responses to the first test reacted positively on re-test. Heimbeck (1929 c) found that only 2 per cent. of 500 children, but 18 per cent. of a group of adults, who were negative in the first instance reacted positively to a second test. (See also von Pirquet, 1909; Fishberg, 1914; Bass, 1918; Nehring, 1922; Ström, 1928.)

These 'secondary reactions' of the von Pirquet test might be interpreted as evidence that the application of tuberculin can induce tuberculin sensitivity in an individual who has not been infected. Ellermann & Erlandsen (1909 a), however, demonstrated that individuals who gave secondary reactions on re-test were already slightly sensitive to tuberculin at the time of the primary negative test, i.e. had been infected by tubercle, but that although potential reactors they did not

respond at that time because the quantity entering the skin was too small to promote a reaction. These authors held the view that although the primary test was negative, the contact with tuberculin increased the slight sensitivity already present sufficiently for the skin to give a visible reaction to the second test, even though the same quantity of tuberculin entered it as before. Thus, according to these workers, a secondary von Pirquet reaction, obtained on re-testing a primarily negative reactor some days later, is due to an increase of sensitization already present (but too small for a primary reaction to be given), and does not represent the origin, *de novo*, of tuberculin hypersensitiveness. It should, however, be mentioned here that Smith (1929) considers that these secondary reactions of the von Pirquet test are due less to sensitization than to technical error inherent in the method and causing failure at the first application.

The sensitization hypothesis of the origin of secondary von Pirquet reactions can be readily applied to the intracutaneous test of Mantoux. The latter test is not much more sensitive in cattle than von Pirquet's method is in man, and, consequently, secondary intracutaneous reactions are common in cattle, so much so that the double intracutaneous test, with a 'sensitizing injection' and a 'diagnostic injection' at two to three days' interval was recommended by the Medical Research Council Tuberculin Committee (1925, 1928) as a routine procedure for herd testing. In man, on the other hand, the intracutaneous test, as will be shown in a later section (III), is more sensitive than von Pirquet's method. We should, therefore, expect sensitized subjects to be more likely to react to a first intracutaneous test, and secondary reactions to a re-test should in consequence be less frequent.

Such is found to be the case. If the intracutaneous tuberculin test is employed, secondary reactions occur comparatively seldom when a patient who fails to respond to the usual dose (0·1 c.c. of 1 : 1,000 tuberculin = 0·1 mg.) is re-tested with the same quantity (Bass, 1918; Downing & Higgins, 1926; Smith, 1929). The writer has made similar observations. Thus, of 37 tuberculin-negative patients subjected to this procedure only 2 (5·5 per cent.) reacted positively on re-test. If, on the other hand, the dose used for the second injection is larger than for the first (negative) test a reaction commonly occurs, especially if the first dose is small, just as it frequently happens that when two concentrations of tuberculin are applied simultaneously the patient reacts to the stronger and not to the weaker.

These considerations suggest that a positive skin response to tuberculin in a subject who has failed to react to a previous application of the same dose, if it be not the result of a chance or deliberate variation in technique, is due to one of the following causes. Either the patient has become infected since the first test, or else he was then already infected but was insufficiently sensitized at the time to give a reaction to the dose and method used. In the latter case the primary ineffective application of tuberculin has increased the degree of sensitization sufficiently to give a positive reaction on re-test with the same dose (or, rarely, the increase of sensitization is endogenous,

being the naturally developing allergy from an infection that had occurred shortly before the first (negative) test).

The failure of tuberculin to induce cutaneous sensitivity to itself carries us a stage farther in the conception of the specificity of the reaction. Not only does the reaction fail to occur under natural conditions in an individual who has not been infected, but apparently it cannot be produced artificially by the injection of tuberculin.

4. Supersensitization by Tuberculin.

In a primary positive reactor an increase of response, due to sensitization, frequently occurs if the test is repeated. This 'positive sensitization' or 'supersensitization' has been noted in the subcutaneous and ophthalmic tests by Cobbett & Griffith (1913), and in the cutaneous test by von Pirquet (1907 b), Ellermann & Erlandsen (1909 a), Ström (1928), and many others; and it occurs also when the Mantoux intracutaneous test is used (Mantoux, 1910; Bass, 1918; Bandelier & Roepke, 1922).

Ellermann & Erlandsen (1909 a) observed that the increase of sensitivity began a few days after the primary positive reaction had been elicited, and lasted many months. On the other hand, using the Mantoux test the writer found some evidence of a period of decreased reactivity or 'negative sensitization', lasting for a few days after the first skin test. Furthermore, skin sensitivity to tuberculin can be greatly diminished or abolished by giving repeated intracutaneous injections at suitable intervals (Borok & Pick, 1926), a change that has long been known to occur in cattle shortly after subcutaneous administration.

Positive sensitization may occur in clinically non-tuberculous subjects. Thus, after about a fortnight's interval, the writer re-tested 18 clinically non-tuberculous adult patients who had reacted positively to a first test made with 1 : 1,000 or 1 : 10,000 tuberculin. The dose for the second test was the same as for the first. An increased size of local reaction was noted in 13 out of the 18 patients. In the remaining 5 cases the reaction to the re-test was about the same size as that produced by the first injection. Lobban (1930), on the other hand, found exactly similar reactions on repeating tests in clinically non-tuberculous patients, but apparently the intervals were greater.

When tuberculin tests are repeated at intervals during the course of clinical tuberculosis, an increase or decrease in intensity of the response may be obtained. These changes may be due to positive or negative sensitization by the previous dose, as occurs in clinically non-tuberculous individuals; or they may be dependent upon variations in the patient's resistance, in the activity of the disease process, or in extrinsic factors such as intercurrent infections; or, finally, if an inaccurate method is used, they may be merely the result of a chance or deliberate variation in technique. The changes of allergy which repeated tuberculin tests are supposed to reveal have been used by some workers to estimate the prognosis in tuberculosis;

an increase of reaction is considered to be a favourable sign, while a decrease of intensity is taken to indicate a depression of the immunity process. This point will be considered again when the value of tuberculin tests in prognosis is discussed (p. 34).

5. The Latent Period preceding Sensitization.

The incubation or latent period preceding tuberculin sensitization varies from a few days to several weeks in guinea-pigs, and from a few weeks to several months in children, depending on the size of the infecting dose and on other factors. Since tuberculin reactivity increases gradually (Hamburger, 1910), the more sensitive the tuberculin test used, the earlier is sensitization apparent.[1]

6. Summary.

1. A positive tuberculin skin reaction indicates hypersensitiveness of the tissues to a product of the tubercle bacillus. This product is probably protein in nature.

2. Under natural conditions, at any rate in Europe, the typical response to tuberculin in man is probably only seen after tuberculous infection accompanied by the formation of histological tubercles.

3. In an individual not previously infected, the injection or cutaneous application of tuberculin has not been conclusively shown to be capable of inducing the typical reactivity of the skin to tuberculin.

4. In a subject who has been infected by tubercle, the degree of skin reactivity to tuberculin may be increased by repeated cutaneous application or intracutaneous injection of Old Tuberculin.

5. There is a significant interval between primary infection and the appearance of tuberculin skin reactivity.

If these statements are accepted, it follows that a positive tuberculin skin reaction indicates that the individual has at some time been infected by the tubercle bacillus. The question now arises whether it is justifiable to assume that he is still infected, i.e. that living bacilli and unhealed tuberculous foci are still present in the tissues.

The data are as yet insufficient to answer this question definitely. There is some evidence[2] to show that in man, as in laboratory animals,[3] tuberculous infection may become extinct, with death of the bacilli and complete healing and even obliteration of the anatomical lesion: on the other hand, living bacilli may be found in apparently healed foci at necropsy (Opie, 1927 b). Animal experiments[4] indicate that as the tuberculous process regresses tuberculin sensitivity wanes,

[1] See reports of experimental work by Roemer & Joseph (1909, 1910), Nobécourt, Mantoux & Perroy (1909), Hamburger & Toyofuku (1910), Esch (1914), Krause (1916), Debré, Paraf & Dautrebande (1920), Parish & Okell (1929); observations on children by Siegert (1911), Hamburger (1912 d), Dietl (1912), Kleinschmidt (1916), Debré & Jacquet (1920), Breckoff (1924), Selter (1925), Epstein (1926); and discussions by Bernard (1927), Myers (1930 f), and Tytler (1930).

[2] See Bugge, Opie (1924, 1927 a, 1930), Opie & Aronson (1927).

[3] See Gardner (1922), Krause (1922, 1925 a, 1927), Willis (1928).

[4] See Krause (1916, 1927), Willis (1928).

and these experiments, together with some clinical observations,[1] suggest that complete disappearance of reactivity may follow extinction of all infection (primary infection and reinfection) in man. It is, however, possible that reactivity was never completely lost in the cases described, for the full sensitiveness of the tuberculin test was not utilized. Thus, some of Austrian's (1927) patients, also negative to the von Pirquet test, were found to react positively when the intracutaneous method was used, and Hamburger's case was found to be positive on repetition of the intracutaneous test a week later (see p. 51).

It should be mentioned here that Willis (1928) found in guinea-pigs that with the subsidence of infection skin sensitivity to tuberculin became reduced to a point where an intracutaneous reaction was not obtained with the usual test dose, but allergy could be shown to be still retained to a slight degree by the elicitation of a response with larger doses of tuberculin, or by the demonstration of a very prompt return of the scarcely detectable allergy to former high levels after fresh reinfection.

If extinction of all tuberculous infection in man is commonly followed by the complete disappearance of detectable allergy, a positive tuberculin reaction indicates not only that infection has taken place, but that it is still present. Until, however, there is clearer evidence of this sequence it appears wiser to assume from a positive response merely that infection has occurred at some time in the individual's life, without attempting to decide whether it is present or extinct.

This question has much more than academic interest, for upon it depends the significance of the tuberculin surveys that have been made in various parts of the world. Assuming that every individual capable of reacting to tuberculin is detected by means of the most sensitive technique (an assumption that from the evidence presented in subsequent sections appears to be approximately true when the intracutaneous method is used in sufficient dosage), the percentage of positive reactors in a community represents its sensitization or sensitivity incidence, as defined on p. 7. Now, if extinction of infection is followed by complete loss of skin reactivity to tuberculin, the sensitization incidence of the community corresponds to the percentage of persons that is infected by tubercle, i.e. the infection incidence (p. 6). If, on the other hand, skin reactivity persists in slight amount after complete healing, and is not lost through some other cause (see p. 22), the sensitization incidence corresponds to the percentage of persons that has at some time been infected, i.e. the attack-incidence of infection (p. 6), *and may include some in whom infection has died out.* On account of the present uncertainty of this question a conservative view will be taken in this report, and the sensitization incidence, rather than the incidence or attack-incidence of infection, will be referred to in describing tuberculin surveys (Section VII).

[1] See Hamburger (1912 c), Austrian (1924, 1927), Krause (1925 a), Opie & McPhedran (1926), Heimbeck (1927, 1928), Opie (1930).

Section II. The Significance of a Negative Tuberculin Skin Reaction.

While for practical purposes every individual who shows a positive tuberculin skin reaction may be considered to have been infected with tubercle, the significance of a negative response cannot be so simply stated. Its significance depends both on the type of case tested, and on the method and dosage employed; if, however, the most sensitive technique available is used, a negative reaction approximately excludes the presence of tuberculous infection.

The evidence for this statement is fully discussed in subsequent sections of this report, but may be summarized here: (*a*) by improving the technique a positive response has been obtained in many of the commoner conditions where tuberculous infection is reputed to be accompanied by non-reactivity to tuberculin; (*b*) with this improved technique practically every tuberculin-sensitive individual is detected; (*c*) a negative response to the test, when this method is used, has been found to be very unusual in an average case of clinical tuberculosis. (For description of technique see Appendix 5.)

(*a*) The conditions that have been stated to give rise to non-reactivity to skin tuberculin tests in the presence of tuberculous infection are collected in Table 4. The conditions in italics have only been reported to be responsible for non-reactivity in individuals with subliminal infection; the others are said to produce non-reactivity in clinical tuberculosis as well. There is little doubt that complete tuberculin insensitiveness occurs during the latent period following tuberculous infection (p. 20), after desensitization with tuberculin, and in the so-called anergic tuberculous skin diseases. But there is reason to believe that many of the other conditions tabulated owe their apparent non-reactivity to an insufficiently sensitive technique. The majority of the reports have been based on experience with von Pirquet's method, or, if the intracutaneous route has been employed, the solution of tuberculin has not been stronger than that contained in the usual test dose. But, as will be shown in the next section, the von Pirquet test, as ordinarily performed, is less sensitive than the intracutaneous test, the latter eliciting positive reactions in patients negative to the former; furthermore, the sensitiveness of the intracutaneous method is significantly increased by testing individuals negative to the standard concentration of tuberculin (1 : 1,000) with graded stronger solutions, even if necessary up to the maximum concentration, that is, undiluted tuberculin (Section V and Appendix 5). In this way it has been demonstrated that in tuberculosis complicated by marked toxaemia, miliary dissemination, and meningitis, conditions which are generally reputed to be prone to abolish reactivity to tuberculin, reactivity is, as a rule, only depressed and not entirely lost (p. 27, and Section V).

(*b*) If the percentages of positive reactors to the intracutaneous test are plotted against the strengths of tuberculin used, a curve is obtained, which, as the solution approaches the maximum con-

centration, flattens out and becomes nearly parallel with the base-line. This suggests that when the full sensitiveness of the intracutaneous test is utilized almost every potential reactor is detected (pp. 49, 53).

(*c*) In a miscellaneous series of over a thousand patients known to have clinical tuberculosis, and tested by the writer, almost one hundred per cent. were positive to the intracutaneous test, used in maximal doses where necessary (Section V).

If it be assumed that, when a satisfactory method is employed, a negative tuberculin reaction approximately excludes the presence of tuberculous infection, the question arises whether it indicates also that infection has never occurred. This is the converse of the question raised at the end of the preceding section. In view of the possibility, there pointed out, of the complete disappearance of tuberculin hypersensitiveness after the extinction of all infection (primary infection and reinfection) in man, it would seem wiser, in the present state of knowledge, not to make this further assumption.

Section III. The Sensitiveness of the Tuberculin Tests.

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The tuberculin test of choice should, as far as possible, comply with the following requirements: it should be safe, easily performed, capable of quantitative application, sufficiently sensitive to detect every tubercle-infected individual, and reliable in the diagnosis of clinical tuberculosis. The sensitiveness of the various methods will be considered in this section.

1. The Subcutaneous Test.

This test is no longer widely used. It was considered to be unwieldy and difficult of interpretation by the Medical Research Council Tuberculin Committee (1925, 1928) appointed to inquire into the tests for tuberculosis in cattle. The method has similar disadvantages, with the added element of danger, in human, especially out-patient, practice, and is totally unsuited for mass investigation. (See Bardswell, 1921; Finkelstein, 1921; Fowler, 1921 b; Marfan, 1923; Krogsgaard, 1925; Burhans, 1927; Calmette, 1928.) Depending for its interpretation on the production of pyrexia, the test cannot be used easily in febrile subjects. Although considered by some workers (e.g. Stolkind, 1910; Swenigorodsky, 1913) to be more sensitive than the other methods, it is doubtful if it is more accurate than the graded quantitative intracutaneous test (Klopstock, 1919).

2. The Local Subcutaneous Test (Stichreaktion).

The *stichreaktion* of Escherich has never been popular outside Austria and Germany, where Hamburger has been its chief supporter. It is usually employed supplementarily to the cutaneous (von Pirquet) test. An injection of 0·1 c.c. of a dilution of tuberculin is made in the superficial subcutaneous tissue, usually of the forearm. The dose varies from 0·01 mg. to 10 mg., but the usual upper limit is 0·1 or 1·0 mg. Redness and infiltration about the area of injection constitute a positive reaction (Hamburger, 1908, 1922, 1926; Hamman & Wolman, 1912 e).

Since it is a local response this test is more closely allied to the intracutaneous than to the subcutaneous reaction. There is evidence that the *stichreaktion* is a considerably better test than von Pirquet's cutaneous method (Nothmann, 1910; Rominger, 1920; Selter, 1921; Hamburger, 1922), but it appears to offer no advantage, either in sensitiveness or in convenience, over the quantitative intracutaneous test, provided that the latter is applied in proper doses.

Hamburger (1908) apparently believes that the local subcutaneous test is about 1,000 or 10,000 times as sensitive as the cutaneous test, concentration for concentration. In 1909 this author and Monti reported the results of an investigation into the tuberculin reactions of children in Vienna. Those who were negative to the cutaneous (von Pirquet) test were re-tested with the *stichreaktion*. The figures, as Hamburger (1922) points out, were considerably higher than those obtained in Vienna about the same time and on similar clinical material by von Pirquet (1909), using the cutaneous method alone.

Nothmann (1910) reported a similar discordancy from Düsseldorf, comparing his results, obtained with the *stichreaktion*, with those of Engel & Bauer (1907), obtained with the von Pirquet test two years previously.

Rominger (1920), in Freiburg, recorded more than twice as many positives with the von Pirquet test, followed by the *stichreaktion*, as with the cutaneous test alone.

3. The Conjunctival Test.

This test has been abandoned by most workers. Not only is it unjustifiably painful (Hamill, Carpenter & Cope, 1908), but the results are unreliable. Roepke (1908 a, 1908 b) found positive reactions in only half of his series of early active pulmonary tuberculosis, and concluded that a negative result did not prove the absence of active tuberculosis. Similar unsatisfactory results were reported by Morelli (1908). Hamman & Wolman (1912 b) have collected the figures obtained by various workers. The failure of the test in the clinically tuberculous varied from 7 per cent. to as much as 47 per cent.; the error was 16 per cent. in their own series of cases, nearly all of which were adults. These authors also (1912 d) give the results of comparisons made between the cutaneous and conjunctival tests when performed simultaneously. Only about half of those who reacted to the former gave positive responses to the latter test in most of the series quoted. It should be mentioned, however, that Missbach (1909), who, according to Krogsgaard (1925), is the only observer who has compared the conjunctival with the intracutaneous test, found a dis-

cordancy of only 4 per cent. in favour of the latter, a figure that is hardly significant.

4. The Percutaneous (Moro) Test.

The Moro percutaneous test, as originally performed, also appears to be inaccurate. It is made by rubbing an ointment consisting of equal parts of Old Tuberculin and anhydrous lanolin into the skin of the chest or abdomen (Moro, 1908; Bandelier & Roepke, 1913). The area of skin should be about 5 cm. in diameter. An efflorescence of papules constitutes a positive response.

Hamman and Wolman (1912 c, 1912 d) give some of the published results of tests made on clinically tuberculous patients by this method. The percentages that failed to react in the three series of any size were 17, 24, and 48, respectively. The percutaneous test proved considerably less sensitive than von Pirquet's method when both were applied to the same individuals. On account of its frequent failure in clinically tuberculous patients Bandelier & Roepke (1922) consider that a negative response to the percutaneous test does not prove the absence of tuberculosis.

Recently, however, a more sensitive method of performing the percutaneous test has been introduced in Germany and Austria, and is described by Lovett (1929). The main improvements are that the skin is first rubbed with ether to remove as much fat as possible, as suggested by Widowitz (1922), and that a concentrated form of Old Tuberculin, prepared originally by Hamburger & Stradner (1919), is used as the ointment. Lovett claims that this modified percutaneous test is as sensitive as the von Pirquet method. She admits, however, that the error is from 15 per cent. to 20 per cent., and consequently advises that in certain cases the test, if negative, be followed by the *stichreaktion*. Its practical value in excluding tuberculous infection is, therefore, questionable.

A modified inunction test of a totally different kind, known as the 'contrast reaction', has been employed in dermatology with some success since 1913, and is described by Jadassohn (1929). It is based on the observation that the application of tuberculin to some tuberculous skin lesions produces a greater local reaction than when applied to an area of healthy skin.

5. The Cutaneous (von Pirquet) and Intracutaneous (Mantoux) Tests.

The cutaneous and intracutaneous methods are the only two tuberculin tests widely used at the present time. Examination of the literature reveals a large number of investigations where the sensitiveness of the two tests has been compared in the same series of individuals. These investigations will be briefly described in chronological order.

Aptekmann (1909), Macé de Lépinay (1909), and Mantoux (1910) were the first workers to make this investigation in man. They compared the cutaneous with the intracutaneous test by performing both on a mixed series of tuberculous and non-tuberculous cases. They used 1 : 100 tuberculin for the von Pirquet reaction

and 0·05 c.c. of 1 : 5,000 (0·01 mg.) tuberculin for the Mantoux test. They found a discordancy of 12, 4·6, and 21 per cent., respectively, in favour of the Mantoux test. Among those reacting positively to the intracutaneous test but negative to the von Pirquet test were some cases of clinical tuberculosis, which would, therefore, have been passed over had the latter method been employed alone. These observations have been quoted by Krogsgaard (1925) to demonstrate that the Mantoux test is more sensitive than the von Pirquet reaction. In fairness to the latter method, however, it must be stated that, as von Pirquet himself recognized about the same time, the solution of 1 : 100 tuberculin used by many workers for his cutaneous test was too weak. Thus, von Pirquet (1909; and see also 1907 d) recommends undiluted tuberculin for preference. Consequently, since Aptekmann, Macé de Lépinay, & Mantoux used a weak solution for the von Pirquet reaction, as well as what is now considered to be a small dose (0·01 mg.) for the intracutaneous test, too much stress must not be laid on their observations. The majority of those who have worked with the von Pirquet cutaneous test since 1909 have employed undiluted tuberculin, and, unless stated to the contrary, this may be assumed to have been used for the observations about to be described. (In some instances, unfortunately, authors have omitted to give the strength of their solutions.)

McNeil (1909, 1923), by modifying the original technique of the von Pirquet test, obtained reactions where the original method had failed, and concluded that this test was more accurate than was generally believed.

Engel (1911) called attention to the number of cases of tuberculosis in which the von Pirquet test failed to give a reaction. These included heavily infected cases, patients with cachexia, miliary tuberculosis, and meningitis, and cases complicated by measles, scarlet fever, or pneumonia. It was often just in these cases, he considered, that the diagnosis might be most needed, and in his experience the Mantoux intracutaneous test, if used in large enough doses, might succeed where the von Pirquet test had failed. Engel unfortunately gave no statistics, but only quoted examples from his clinical experience in support of this view.

Monti (1912) performed both the cutaneous and intracutaneous tests on 58 clinically tuberculous children, and on 316 patients who were clinically non-tuberculous or doubtful cases. He used 0·05 c.c. of 1 : 5,000 (0·01 mg.) tuberculin for the intracutaneous test. All of the first group who reacted to one of the tests reacted to the other. In the second group there was a discordancy of 9 per cent. in favour of the intracutaneous test. While, therefore, from his observations there seemed nothing to choose between the two methods for the diagnosis of clinical tuberculosis (his series, however, was very small and the intracutaneous dose inadequate), the intracutaneous test appeared to be preferable for detecting infection in the clinically non-tuberculous.

Bandelier & Roepke (1913), reviewing the various tuberculin tests, state that in their opinion the intracutaneous Mantoux test is more accurate than the cutaneous reaction of von Pirquet for indicating tuberculosis.

Gade (1913) performed both tests on 51 children. He found a 60 per cent. discordancy in favour of the intracutaneous test. Krogsgaard (1925) suggested that an error of technique accounted for this huge difference.

Jeanneret (1913), as a result of an extensive comparison, concluded that the Mantoux test was much superior to the von Pirquet reaction.

Schloss (1917 a) compared the two tests on 191 orphans from a very poor quarter of Berlin. For the intracutaneous method he used doses of 0·01 mg., 0·1 mg., and 1 mg. tuberculin. He found a discordancy of 13 per cent. with 0·01 mg., 22 per cent. with 0·1 mg., and 26 per cent. with 1 mg. tuberculin, all in favour of the intracutaneous test.

Bass (1918) tested 206 clinically non-tuberculous children repeatedly with the von Pirquet test. Those finally negative were injected intracutaneously with 1 : 1,000 tuberculin, and then, if still negative, with 1 : 100 tuberculin. By this procedure another 15 per cent. of positive reactors were discovered, showing the relative insensitiveness of von Pirquet's method.

Garrahan & Iraola (1918) found the Mantoux test (0·1 mg.) often positive with a negative von Pirquet test, even in cases of active tuberculosis.

Reiss (1918), who made comparative tests on a series of children, found that the von Pirquet reaction was never positive in the absence of a positive Mantoux reaction, but that the reverse did not hold.

af Klercker (1920) found that the two tests agreed in 156 cases. But he used a weak solution for the Mantoux reaction (0·1 c.c. of 1 : 10,000 = 0·01 mg.).

Davidsohn (1920) compared these tests in 68 children aged 1–5, chosen at random from a Berlin orphanage. Ten of them were negative to the von Pirquet test but reacted to the Mantoux test, the dose of the latter being 0·1 c.c. of 1 :1,000 (= 0·1 mg.). One child who failed to react to the Mantoux test gave a doubtful positive reaction with the von Pirquet test. There was thus a 15 per cent. disagreement in favour of the intracutaneous method. Further observations of this worker pointed indirectly in the same direction. He tested 100 of the orphans, aged 1–5, by the intracutaneous method, and compared the results with those obtained in 450 children from the same institution tested during the same period by von Pirquet's method. At every age the Mantoux test gave higher figures than did the von Pirquet test. Davidsohn concluded that the intracutaneous was more sensitive than the cutaneous method.

Nehring (1922) compared the two tests on 60 school children. He recorded a discordancy of 10 per cent. in favour of the Mantoux test when doses of 0·1 c.c. of 1 : 1,000 (= 0·1 mg.) tuberculin were used.

Happ & Casparis (1922) tested a series of children suffering from miliary tuberculosis with or without meningitis. Of these children thirty-six per cent. more reacted to the intracutaneous tuberculin test than to the von Pirquet test, when solutions stronger than 1 : 10,000 (= 0·01 mg.) were used for the former. In strengths of 1 : 10,000 tuberculin only 50 per cent. reacted to the intracutaneous test, a percentage that was the same as that found with the von Pirquet reaction. On the other hand, 80 per cent. responded to a dilution of 1 : 1,000, and 94 per cent. to 1 : 100, and, if sufficient concentrations were used, 100 per cent. of this type of case reacted to the Mantoux test. These authors concluded that the von Pirquet test was equivalent to an intracutaneous dose of 0·1 c.c. of 1 : 10,000 (= 0·01 mg.) tuberculin, and that with larger doses the intracutaneous method was far more sensitive than the cutaneous reaction. Their work confirms the prevailing view that the von Pirquet test fails frequently in miliary tuberculosis and meningitis, but shows that this fault is due to the relative insensitiveness of the method and can be obviated by employing the intracutaneous test.

Hamburger (1922), who as early as 1908 pointed out that von Pirquet's test gave figures that were too low, writes that this method is entirely unreliable, having an error as great as 40 per cent. in detecting tuberculous infection, and deplores its continued popularity.

Wahl & Gerstenberger (1923) found the Mantoux intracutaneous test more sensitive than von Pirquet's method in tuberculous cases.

Mensi (1923) used the von Pirquet test on 629 mixed cases and found 26 per cent. positive, and performed the Mantoux test on 885 individuals, finding 34 per cent. positive. He does not give the doses used, nor does he state whether both tests were performed on the same patients. If they were not, the absence of details of ages makes this author's conclusion that the intracutaneous is the more sensitive test quite unwarranted by his evidence, although this statement has been accepted in one review.

The Medical Research Council Tuberculin Committee (1925, 1928) concluded that the intracutaneous method was an accurate test for tuberculosis in cattle, the percentage error being extremely small, and that it was the best of all tests for field work.

According to Krogsgaard (1925), Brinchmann (1924) performed both tests on 47 apparently healthy children under 2 years, who were tuberculosis contacts. He found a 24 per cent. disagreement in favour of the intracutaneous test, which he concluded was the more sensitive method.

Krogsgaard (1925) carried out an investigation on 382 children, aged 0–14 years, selected at random from the out-patient clinic. They included 125 certain or suspect cases of clinical tuberculosis. He used undiluted tuberculin for the von Pirquet test, and 0·1 c.c. of 1 : 100 (= 1 mg.) tuberculin as a maximum dose for the Mantoux test. Each patient was first tested by the former method, and, if

negative, the test was repeated and the Mantoux test was carried out at the same time. This procedure was based on the assumption, justified by the experience of previous workers, that while some individuals negative to the von Pirquet test might prove positive when subjected to the intracutaneous method, the reverse did not occur. Krogsgaard found that 23 children (6 per cent.) were negative with von Pirquet's method and positive to the Mantoux test; 5 of these were undoubted, and 7 suspected, cases of clinical tuberculosis. From these observations it appeared that the intracutaneous test was more sensitive than the von Pirquet reaction when the latter was carried out with the maximum concentration of tuberculin (i.e. undiluted tuberculin), and would give positive reactions in some cases of clinical tuberculosis which the von Pirquet test would miss. It should be mentioned that Brinchmann (1927) criticized this paper; and Krogsgaard (1927) replied to the criticism.

Forbes & Green (1926) tested 131 orphans, aged 6 months to 15 years, with the von Pirquet test (undiluted tuberculin). Those who were negative were then tested with 0·2 c.c. of 1 : 20,000 tuberculin (= 0·01 mg.) by the intracutaneous method. No further positive reactions were obtained. The majority of the non-reactors were then tested with 0·2 c.c. of 1 : 2,000 tuberculin (= 0·1 mg.) intracutaneously, and 6 per cent. reacted positively. There were, furthermore, two cases that responded to the von Pirquet test but not to 0·01 mg. tuberculin intracutaneously; but both these cases gave marked positive responses to 0·1 mg. tuberculin administered by the latter route. These observers concluded that the von Pirquet test was as sensitive as 0·01 mg. tuberculin intracutaneously, but less sensitive than 0·1 mg. tuberculin given in this manner.

Tisdall & Brown (1926) carried out the intracutaneous and von Pirquet tests simultaneously on 177 tuberculous patients. Eighty-five per cent. of them reacted to the former (0·1 c.c. of 1 : 400 tuberculin = 0·25 mg.), and 58 per cent. to the latter test.

Stewart & Collins (1926) found that 13 per cent. of 53 children with negative von Pirquet reactions gave positive responses to the Mantoux test, while only 4 per cent. of 109 with positive cutaneous tests reacted negatively to the intracutaneous test. These children showed no evidence of active tuberculosis. Three of 14 cases of clinical tuberculosis that gave positive Mantoux reactions failed to respond to the von Pirquet test. These authors omit to state the concentration of tuberculin used.

Schnipenkötter (1926) states that the intracutaneous test is a million times more sensitive than von Pirquet's method.

Austrian (1927) repeated von Pirquet tests on some patients who had changed from positive to negative, and at the same time performed the intracutaneous test with 0·01 mg. tuberculin. All of them reacted positively to the latter but were again negative to the former test.

Rueda (1928), as a result of comparisons on infants in the Argentine, concluded that the Mantoux test was incomparably superior to the von Pirquet test, and that investigations made on infants with the latter were unreliable.

Ström (1928) noted serious discrepancies in different sets of statistics of the von Pirquet reactions of Norwegian children, and determined to investigate the cause. He pointed out that repetition of the von Pirquet test after some days was essential in negative cases, especially in adults, as this procedure might produce a positive response (secondary reaction). Ström performed the von Pirquet and Mantoux tests simultaneously on 123 hospital patients, using 0·1 c.c. of 1 : 1,000 tuberculin (= 0·1 mg.) for the latter test in 66 of them, and 0·1 c.c. of 1 : 100 tuberculin (= 1·0 mg.) in the remaining 57. There was agreement between the two tests in all cases. Nevertheless, the necessity for repeating the von Pirquet test in non-reactors makes it difficult to accept this author's conclusion that it is preferable to the intracutaneous test for clinical purposes.

According to Cummins (1929), even if the technique of the von Pirquet test is modified so as to make it more delicate, it is only equivalent to 0·1 c.c. of a 1 : 1,000 dilution (= 0·1 mg.) of tuberculin administered by the intracutaneous route, so that the latter is still a much more sensitive method.

Smith (1929) reported tests made on over 3,000 cases by the Mantoux and von Pirquet methods simultaneously. The dose of tuberculin used for the intracutaneous test apparently varied. His material consisted of nearly all the children (aged

0–13 years) admitted into the wards of a New York hospital over a period of eight years. The results showed that at all ages the intracutaneous test gave over twice as many positives as did the von Pirquet reaction. In the whole series there was a discrepancy of 9 per cent. in favour of the intracutaneous method.

Heimbeck (1929 b), a strong supporter of the von Pirquet test, injected 1 mg. of tuberculin intracutaneously into 50 Pirquet-negative individuals under 22 years, but obtained no fresh positive reactions.

Ustvedt (1929) compared the von Pirquet and Mantoux tests in 320 cases, using undiluted tuberculin for the former and 0·1 c.c. of 1 : 10,000 (0·01 mg.) tuberculin for the latter. The von Pirquet reaction was alone positive in 35 per cent., while in no instance did a patient react to the Mantoux test alone. The author also compared both methods on 414 persons, using 1 : 1,000 (0·1 mg.) tuberculin for the intracutaneous test. The von Pirquet test was positive, and the intracutaneous reaction negative, in 2·5 per cent., while the reverse occurred in 1·5 per cent. Ustvedt concludes that the von Pirquet test is more sensitive than the intracutaneous method when 0·1 c.c. of 1 : 10,000 (0·01 mg.) tuberculin is used for the latter, and approximately equivalent when a 1 : 1,000 dilution (0·1 mg.) is employed. From earlier experiments he considers that a few individuals negative to a first von Pirquet test will react to a 1 : 100 dilution (1 mg.) given intracutaneously, but that the advantage is very small if the von Pirquet test is repeated.

It should be pointed out that most of the comparative work quoted has been carried out either on clinically non-tuberculous cases, or on mixed series in which the number of clinically tuberculous patients included is not specified. Monti, Happ & Casparis, Krogsgaard, Tisdall & Brown, and Stewart & Collins, however, gave separate results for manifest tuberculosis. Monti found these cases as likely to react to one test as to the other, but he used a weak solution for the intracutaneous method. On the other hand, the observations of the other authors suggest that the intracutaneous test is more likely to elicit a positive response in clinical tuberculosis, a view that is confirmed indirectly in a subsequent section (V).

The evidence afforded by the published work may be summarized as follows. The intracutaneous or Mantoux test, used in doses of 0·1 c.c. of 1 : 1,000 (= 0·1 mg.), is at least as sensitive as, and in bigger doses more sensitive than, the cutaneous or von Pirquet test, when the maximum concentration of tuberculin (undiluted) is used for the latter method. The intracutaneous test is thus 1,000 or more times as sensitive, if concentration be compared with concentration. The objection that it is too delicate cannot be upheld, because the sensitiveness of the method may be reduced at will by dilution until equal to or less than that of the von Pirquet test.[1]

The greater sensitiveness of the intracutaneous method gives it a reserve of strength not possessed by the von Pirquet test. Since undiluted tuberculin is generally used in the first instance for the latter test at the present time, repetition with the same solution is the only means of procedure in case of doubt. In the intracutaneous method it is possible to use ten, a hundred, and finally if necessary a thousand times the usual initial dose (0·1 c.c. of 1 : 10,000 or 1 : 1,000) when re-testing patients.

The two tests differ in respects other than sensitiveness. In the von Pirquet reaction the volume entering the skin is variable and cannot be measured, so that the quantitative use of the method is limited. Even if two tests are performed simultaneously in an apparently identical manner a response may be obtained to one and

[1] The applications of the intracutaneous test are considered in subsequent sections, and its technique is described in detail in Appendix 5.

not to the other. Hamburger (1922) complains of the variety of instruments devised and the uncertainty of depth of wound. Wahl & Gerstenberger (1923), Ström (1928), and other workers have found a difference in the percentages of positive reactions when two minor modifications of the von Pirquet test are used. Wahl & Gerstenberger made simultaneous tests by the 'burr' and 'tattoo' methods, and Ström compared tests made with a 'borer' and a scalpel. These discrepancies are probably due to variations of the average quantity of tuberculin entering the skin according to the instrument used.

In contrast to the von Pirquet test, however applied, the intracutaneous method affords an accurate means of administering a known quantity of tuberculin at a known depth of the skin. It can, therefore, be used quantitatively in graded doses, and the results compared with considerable accuracy. In Opie's (1927 a) opinion it is 'the only means by which sensitiveness to tuberculin can be measured so accurately that two successive tests can be compared'.

Finally, the quantitative possibilities of the intracutaneous test make it suitable for the biological titration of tuberculin.

O'Brien (1925) considered that the quantitative intracutaneous method represented the most useful means of standardizing tuberculin on guinea-pigs. Okell & Parish (1927) came to the same conclusion, expressing the view that the von Pirquet test in guinea-pigs gave gross errors, and that the subcutaneous was less accurate than the intracutaneous method. Moreover, the intracutaneous method was chosen by the Tuberculin Committee of the Health Section of the League of Nations (see Calmette & de Potter, 1926) as the best means of standardizing tuberculin on the guinea-pig.[1]

The intracutaneous test is thus more sensitive and more free from technical error than the von Pirquet test, and the dose can be more accurately regulated.

Section IV. The Value of the Positive Reaction in Diagnosis and Prognosis. The Choice of Method for the Positive Diagnosis of Clinical Tuberculosis.

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1. Qualitative Tests.

Qualitative skin tuberculin tests are usually made with the same concentration of tuberculin in each case. The result of the test is

[1] In a recent paper Philip, McKendrick and others (1930) claim that the cutaneous (von Pirquet) method in human subjects is as accurate a means of standardization as the intracutaneous method in guinea-pigs.

either reported merely as positive or negative, or is roughly classed according to the intensity, and sometimes the character, of the reaction.

The assessment of the diagnostic value of positive tuberculin tests in clinical practice is a problem of peculiar difficulty. Much has still to be learnt about the interaction of such factors as dose, virulence, and site of lodgement of the bacilli, on the one hand, and resisting power of the host, on the other, which decides the course taken by tuberculous infection in the body. Thus, infection is apparently innocuous in most individuals, but in some it progresses either primarily ('childhood type'), or after a period of years in the form of reinfection ('adult type'), into a sufficiently gross state of activity to be recognizable by the physician as 'tuberculosis'.[1] Between this manifest or clinical tuberculosis (tuberculous disease) and minimal infection consistent with good health throughout life, every grade of latency and activity may be found. While tuberculin sensitivity is one of the earliest features of first infection, its high incidence among apparently healthy people, reported by von Pirquet (1909) and contemporary workers, makes it clear that the mere discovery of a positive skin response cannot in general decide at which end of this scale of activity an individual is to be placed, and does not show that he is suffering from tuberculous disease. (The clinical value of the positive reaction in early life is discussed later in this section.)

There have been many attempts to determine the value of the character and intensity of the positive reaction in diagnosis and prognosis.

Wolff-Eisner (1908) classified the various types of von Pirquet reaction according to intensity, time of appearance, and duration, and suggested a different meaning for each, correlating them with different phases of activity of the tuberculous process. These types included 'normal', 'strong and normal', 'rapid weak and ephemeral', and 'late and lasting' reactions. Von Pirquet (1909) found that torpid and secondary reactions occurred principally in cases that were clinically non-tuberculous (latent tuberculosis) and only exceptionally in manifest tuberculosis. More recently, Cantani (1920), as a result of several thousand tests, came to the conclusion that negative or slightly positive cutaneous tests were indications of tuberculous lesions cured or in process of cure, respectively, while manifestly or intensely positive reactions characterized the initial and active forms of the disease.

It is, however, probable that the character as well as the intensity of the response to tuberculin is dependent upon the amount of this substance entering the skin, on the one hand, and upon the degree of skin reactivity, on the other hand. The degree of reactivity can be more accurately estimated by quantitative than by qualitative methods.

From these considerations it appears that a positive reaction to a qualitative test establishes that infection has taken place, but that, except in early life, further information about the diagnosis and prognosis is likely to be better obtained by using a quantitative procedure.

[1] For the nomenclature of tuberculosis adopted in 1929 by the American Sanatorium Association consult Myers (1930 f).

Possibly, however, when the conditions under which infection protects against, rather than produces, active disease are more fully understood, a positive reaction in a healthy person may sometimes be considered to indicate partial immunity (see p. 88).

2. Quantitative Methods.

Quantitative skin tuberculin tests are made (*a*) with tuberculin of fixed concentration, the result of the test being classed according to the measured intensity of the reaction; or (*b*) with a series of graded dilutions, the test being reported as positive or negative to a particular dilution. Methods (*a*) and (*b*) are frequently combined.

Measurement of the intensity of the reaction involves a large personal factor, for observers differ in their methods, some measuring the extent of the oedema, others that of the erythema; some noting the diameter, and others the area of the reaction. Moreover, since reactions change in intensity in a few hours it is often difficult to obtain the maximum reading. On this account the quantitative method of choice is probably to employ graded dilutions of a tuberculin of known potency and merely to note the presence or absence of a reaction to each.

Quantitative tests afford a means of estimating the degree of skin reactivity to tuberculin. They have been used with the object of differentiating active tuberculous disease from the benign infection that most town-dwellers have at some time during their lives, and for gauging the activity and probable course of tuberculous lesions.

(a) *Quantitative von Pirquet Tests.*

The von Pirquet test was applied quantitatively by Erlandsen (1908) and Ellerman & Erlandsen (1909 b, 1910), and similar methods have been used by many others. The most general assumption appears to be that, except in certain conditions, patients with active tuberculosis react to weaker solutions of tuberculin, or give bigger responses, than patients in whom the disease is quiescent. Bandelier & Roepke (1922), among others, conclude that quantitative von Pirquet tests are open to too many inexactitudes and inaccuracies to be reliable for distinguishing the two phases of the disease. Gunter (1928), however, in a recent book advocates the use of these tests in pulmonary tuberculosis in order to estimate activity.

The main technical objection to the quantitative application of the cutaneous test is the uncertainty of the amount of tuberculin that enters the skin. The intracutaneous test is free from this objection and merits more detailed consideration.

(b) *Quantitative Intracutaneous Tests.*

The value of quantitative intracutaneous tests was investigated by Roemer and Joseph (1909, 1910), af Klerker (1920), and Luker (1920), who believed that they could distinguish between active and inactive tuberculosis. Jeanneret (1913) considered that the size of the reaction helped in the diagnosis of activity. Opie and McPhedran (1926), and Opie (1930), as the result of careful correlation of

radiographical and clinical manifestations in children with their tuberculin reactions, concluded that the weaker the solution of tuberculin to which a child would respond, or the more severe the reaction, the greater was the chance of his having grave tuberculous infection, with the danger of manifest tuberculosis developing. By directing attention to the gravity of the infection the test might enable latent pulmonary tuberculosis, unaccompanied by the usual symptoms, and only discoverable by X-rays, to be diagnosed (see also p. 71). Atsatt (1927) believes that it should be possible by quantitative methods to find a critical threshold that will usually help to establish the diagnosis of active tuberculous disease. Lobban (1930) considers that the earlier the stage of the disease, and the less the degree of infection, the greater is the dilution of tuberculin with which a reaction can be obtained.

On the other hand, Monti (1912), Klopstock (1919), Krogsgaard (1925), and Ustvedt (1929) were unable to obtain any useful information as to activity and prognosis from quantitative methods. Combe (1916) believes that a single reaction does not give any indication as to prognosis, no matter how mild or severe the response may be. Dickey (1929) finds that race and age are important factors in determining the size of the reaction, and points out that the relation between this size and the total skin surface area must be taken into consideration. In a recent address on the value of tuberculin tests in dermatology Jadassohn (1929), discussing the different degrees of reactivity that tend to be associated with various tuberculous skin diseases, emphasizes the extreme caution necessary before concluding that a lesion is tuberculous or estimating its probable progress.

The most hopeful line of approach would seem to be that of Opie and his associates, and their papers should be consulted; but more extended trial of their method is required before a just appreciation of its value as a routine procedure can be reached. The present writer has attempted to test the hypothesis that the weaker the solution of tuberculin to which a patient will react positively, the more likely he is to be suffering from clinical tuberculosis rather than from subliminal tuberculous infection (see definition, p. 6). The curves shown on Chart 2 give graphical support to this hypothesis. They are prepared from the writer's figures (Sections V and VII) and show the relation between the percentage of positive reactors and the concentration of tuberculin used among the clinically tuberculous and the clinically non-tuberculous. It will be noted that a smaller proportion of both groups respond to the weaker solutions of tuberculin than to the stronger, as would be expected, but that the curves of the clinically tuberculous fall more slowly with increasing dilutions of tuberculin than do the curves of the clinically non-tuberculous. This suggests that the latter curves will meet the base-line first and that, therefore, a sufficiently dilute solution of tuberculin may be found to give positive reactions in the clinically tuberculous only. Unfortunately, however, with so weak a solution it is likely that too few cases of clinical tuberculosis would react positively to allow the method to be used as a practical routine procedure, except in dermatology, where examples of clinical tuberculosis associated with extreme cutaneous sensitization are met. The curves of the percentage of positive reactors to different dilutions of tuberculin among patients with tuberculous cutaneous lesions fall more slowly as the dilution increases than is the case with most other forms of tuberculosis; whereas the curves of the percentage of positive reactors to different dilutions of tuberculin among the clinically non-tuberculous will, of course, be the same as

on Chart 2. These considerations suggest that when the latter curves meet the base-line, a significant proportion of the clinically tuberculous patients will still react positively. From Jadassohn's (1929) paper weak dilution methods do appear to be sometimes useful in the diagnosis of certain tuberculous skin affections, e.g. lupus vulgaris and the tuberculides, but considerable caution seems necessary in interpreting the results. This author uses dilutions as weak as one in a million, and quotes Scholtz who uses dilutions up to one in a thousand million. Such dilutions must, of course, be freshly prepared.[1]

Much attention has been directed to the changes in skin reactivity to tuberculin found by repeating tests at intervals in health, and during the course of tuberculosis. (Consult Bandelier & Roepke (1922), Krogsgaard (1925), and Schnippenkötter (1926).) The relationship of these changes to the prognosis does not seem sufficiently constant for the method to have much practical value. A complication is introduced by the number of outside factors that are stated to cause variations in skin reactivity, such as intercurrent infection, seasonal variations (Hamburger, 1920; Peyrer, 1921), pregnancy, menstruation, &c. (see Table 4). In a recent paper, however, Lobban (1930) has reintroduced this method, using repeated intracutaneous tests with varying dilutions. From the results obtained he believes that clinically tuberculous patients who show increasing reactivity to tuberculin tend to improve, whereas patients who fail to react as strongly as they did originally, or who require a lesser dilution to produce a positive response, have an unfavourable prognosis. Lobban bases his conclusions partly on a series of clinically non-tuberculous patients, most of whom gave exactly similar reactions on repeating the test. This result is opposed to the experience of the present writer (p. 19), who usually found an increase of response on re-testing clinically non-tuberculous individuals at the end of a fortnight. This discordance may be due to a difference in the interval between the injections, and further work is required before the value of the method of repeated tests can be decided.

The introduction, therefore, of quantitative skin tuberculin tests into clinical practice has, in the main, proved disappointing, so far as the positive reaction is concerned. The interpretation of positive reactions to various dilutions of tuberculin, when the diagnosis of clinical tuberculosis or the prognosis of a patient with this disease is in question, calls for considerable experience, and the information given is at the best only probable. Nevertheless, a method of this type, suitable for routine work, may ultimately be developed.

Quantitative tests with very high dilutions of tuberculin were stated above to be most likely to prove useful in dermatology, and the modification known as the 'contrast reaction' (see p. 25) also has a limited use in the diagnosis of skin tuberculosis. In addition, a

[1] The most recent method of this type is described by Blair & Galland (1931), who use a series of dilutions ranging from 1 : 10,000 to 1 : 10,000,000. They consider a positive reaction to a dilution of 1 : 100,000 and over to indicate active tuberculosis. Unfortunately the type of tuberculous case on which this claim is based is not made clear.

positive tuberculin reaction has a very special significance in certain skin diseases, e.g. lupus pernio, lupus miliaris, and the sarcoids. A positive response to a weak solution of tuberculin in a patient suspected of suffering from one of these conditions actually suggests an error of diagnosis, a negative response being the rule (see also p. 22).

When discussing the value of the positive reaction in diagnosis and prognosis it is usual to place infants in a special category, and the same course is followed here.

3. The Fatality of Tuberculous Infection and Clinical Value of the Positive Reaction in Infancy.[1]

Many recent writers express the view that a positive response to tuberculin in an infant (under one or two years of age) has an unquestionably serious, if not fatal, significance, and usually indicates active tuberculous disease (Much, 1921; Blau, 1923; Wahl & Gerstenberger, 1923; Marfan, 1923; Davis, 1924; Schlutz, 1926; Carr, 1929; McIntosh & Whitby, 1930; Nicholson, 1930).

That tuberculin sensitivity in infants is frequently unassociated with manifest tuberculous disease is, however, shown by the presence of a significant percentage of positive reactors in groups of young children who show no clinical evidence of tuberculosis (Hamburger & Monti, 1909; Mantoux & Lemaire, 1909; Calmette, Grysez & Letulle, 1911; Veeder & Johnston, 1915; Bass, 1918; Spolverini, 1919; Asserson, 1927; and Hart (Table 11)). The percentage of positive reactors in these series varied from 1·5 to 11 among infants under a year, from 6 to 22 in those a year old, 3 to 15 in the age-group 0–1, and 6·5 to 18 in the age-group 0–2.

A number of observers, moreover, have made careful records of the physical condition of positively reacting infants, with a view to discovering the proportion showing clinical evidence of tuberculosis. Asserson (1927) studied the records of the infants in two New York hospitals and clinics between 1920 and 1926, many of whom had been brought up because of symptoms. Of 460 positive reactors under two years 70 per cent. had no manifest disease. Myers & Kernkamp (1930) examined 236 infants under two years of age with positive reactions, who were brought up mainly because of exposure to the disease. Only 5 per cent. presented physical signs, and 18 per cent. positive X-ray evidence, of tuberculosis.

Analysis of the published reports on the fate of infants showing positive tuberculin reactions also leads to a more favourable view of tuberculous infection in early life. The results of some of these reports are collected in Table 1. It will be seen that (1) the immediate outlook does not appear hopeless; (2) nevertheless, a high mortality while under observation is recorded by many workers; on the other hand, a few observers (Walquist & Myers, Myers & Kernkamp, Asserson, and Gasul) have reported a comparatively low tuberculosis mortality during an

[1] The importance of the positive reaction in methods of prophylactic immunization is mentioned in Section VI.

TABLE 1.

Reported mortality among infants showing positive tuberculin reactions.

Reference.	*Age.*[1]	*No. of cases.*	*Tuberculosis deaths per cent.*	*Period observed.*	*Remarks.*
Pollak (1911)	0–6 mos.	30	86[2]	1–3 yrs.	
,,	6–12 ,,	62	59[2]	,,	
Nassau & Zweig (1925)	0–3 ,,	20	100		
,,	3–6 ,,	16	87		
,,	6–12 ,,	13	31		
Gasul (1929)	0–6 ,,	16	19[2]	1–8 yrs.	Positive reaction was sole evidence of tuberculosis when tested.
,,	6–12 ,,	44	2[2]	,,	,, ,,
Bartlett & Wollstein (1924)	0–6 ,,	—	75		Even if no symptoms of the disease when tested.
,,	6–12 ,, *Age last birthday.*[1]	—	50		
Calmette, Grysez & Letulle (1911)	0	10	10	7–16 mos.	
Brown (1913)	0–1	114?	70		All were cases in which tuberculosis was suspected clinically.
Salvetti (1915)	0	13	85[2]		
,,	1	17	30[2]		
Umber (1917)	2–4		20		In-patients. 50% clinically tuberculous.
Bernard & Debré (1920)	Infants	40	40	2–24 mos.	Separated from tuberculous parents.
Bernard, Debré & Lelong (1925)	,,	171	7·5	4 yrs.	Separated from tuberculous parents, but 'rigorously selected'.
,,	,,	66	82	,,	Left with tuberculous parents.
Barchetti (1921)	,,	37	71	2–4 yrs.	Infants of tuberculous mothers; many being suckled.
McLean & Jeidell (1922)	0	147	55		In hospital for symptoms.
,,	1	151	38		,, ,,
Roepke (1923)	0	23	26		Home-contacts of tuberculosis.
Davis (1924)	0	20	70	½–3 yrs. or more	Children from chest clinic, the majority being contact cases.
,,	1	25	24	,,	,, ,,
Reuben & Smith (1924)	0	64	68[2]		
Nassau & Zweig (1925)	0	49	78		
Lesné & Coffin (1926)	0–1	—	70–80[2]		Estimated rate. Drawn from poorest homes; many ill when first seen.

[1] Age when tuberculin sensitivity was first discovered.
[2] In these series the general mortality is apparently referred to.

Reference.	*Age.*[1]	*No. of cases.*	*Tuberculosis deaths per cent.*	*Period observed.*	*Remarks.*
Walquist & Myers (1926)	0–1	71	8		Infants, most of whom were brought up mainly on account of exposure and not because of symptoms. 73% had Tb. parents, 11% Tb. siblings, and 11% had several sources of contagion.
Myers & Kernkamp (1930)	0	71[2]	6 } 2·5	1–9 yrs.	History of exposure in 82%. At time of test only 5% had physical signs, and 18% positive X-ray evidence, of tuberculosis. 27% untraced.
,,	1	165[2]	1·5 }		
Asserson (1927)	0–1	268[3]	3	1–5 yrs.	A third from tuberculous homes. No clinical evidence of disease when tested. 32% died from other causes. Fifty untraced cases.
,,	0–1	137[3]	83	,,	Clinically tuberculous when tested. Seven untraced cases.
,,	Infants		8		Negroes.
,,	,,		3·5		'Americans'.
,,	,,		1		Jews.
Gasul (1929)	0	60[3]	7 } 3[4]	1–8 yrs.	Positive reaction was sole evidence of tuberculosis when tested. Many untraced cases.
,,	1	114[3]	1 }		

extended period of observation; (3) the chance of life varies directly with the age of the infant when tuberculin sensitivity is first discovered, the mortality falling rapidly as this age increases.

It is probable that these divergent results are mainly due to differences in clinical material, although variation in the length of the period of observation may play a part. Much of the work has been carried out on children brought to hospital on account of symptoms, many even being clinically tuberculous when first seen. There is fairly general agreement that even at the present time the prognosis of tuberculous infection in infants is unfavourable, once it has progressed to the stage of manifest tuberculosis (Hahlo, 1924; Asserson, 1927; Gittings, Lathrop & Anderson, 1927; see also review by Myers & Kernkamp, 1930), and it is, therefore, not surprising that a high mortality is found among infants who show symptoms as well as tuberculin sensitivity (see Table 1).

[1] Age when tuberculin sensitivity was first discovered.
[2] Including untraced cases.
[3] Excluding untraced cases.
[4] In these series the general mortality is apparently referred to.

Where, on the other hand, the group investigated has consisted mainly or entirely of children in whom the only sign of tuberculosis is a positive reaction, a much lower mortality from this disease has been recorded.

Walquist & Myers (Table 1) studied 71 children under two years, 'most of whom were brought up mainly on account of exposure and not because of symptoms'; 8 per cent. died from tuberculosis while under observation. Myers & Kernkamp followed up a series of 236 similar infants for from one to nine years; only 5 per cent. showed physical signs of the disease on first examination. Out of 160 who could be traced only 6 died from tuberculosis during this period. Asserson's first group was composed of infants under two years with no clinical evidence of tuberculosis when tuberculin sensitivity was first discovered. She recorded a tuberculosis mortality of only 3 per cent. during a period of from one to five years; 32 per cent. of these infants, however, were dead from other causes. The fate of this group may be contrasted with that of Asserson's second series, which was composed of infants with clinical tuberculosis as well as tuberculin sensitivity; 83 per cent. of these infants died from the disease. Finally, Gasul reported a mortality of only 3 per cent. in a group of infants less than two years of age, in whom the sole evidence of tuberculosis was a positive von Pirquet reaction; the period of observation was from one to eight years.

The figures recorded by these authors are apparently the lowest published for the tuberculosis mortality among infants showing positive reactions and living at home. Although the untraced cases introduce an unknown error, it is suggested that they represent the immediate prognosis of tuberculin-sensitive infants in general at the present time more closely than do the high mortalities found by previous workers, which should be considered to apply rather to sick infants.

The influence of environment in determining the fatality of tuberculous infection in infancy is receiving increasing attention. Reuben & Smith (1924) find that cases in private practice are more likely to survive infection than are infants of the hospital class. Myers (1930 c) advocates the avoidance of further exposure to contagion, even if manifest disease has developed, and in support of this view there is some evidence to show that infants who have been infected by tuberculous parents are more likely to die of the disease if allowed to remain at home, than if separated as soon as the occurrence of infection has been discovered by the tuberculin test (see Bernard, Debré & Lelong, 1925; Bernard, 1927; Asserson, 1927; and Section VI, p. 61). It is indeed possible that all infants infected by some one living in their homes are less likely to survive, whether separated subsequently or not, than infected infants in non-tuberculous households, on account of the risk of the infection being both massive and repeated (Lemaire, 1924; Asserson, 1927; Opie, 1930).

4. Estimated Tuberculosis Death-rate in the Tuberculin-sensitized Population, based on Statistical Evidence.

The ratio between the annual number of tuberculosis deaths in a community at each age-period and the mid-year population gives the age distribution of tuberculosis mortality. The figures for various countries and at different epochs have been discussed in a recent report (League of Nations, 1931). In all countries and

at all epochs the highest mortality from tuberculosis in children takes place during the first year of life. The curve of mortality then falls rapidly, reaches a minimum between 5 and 14, and increases rather abruptly from 15 to 25. After that age the mortality-rate either remains high or decreases, depending on the type of community, the country, and the period.

On the other hand, the ratio between the annual number of tuberculosis deaths in a community at each period of life and the mid-year *sensitized* population would give the tuberculosis death-rate in the sensitized population. This ratio differs from the tuberculosis mortality-rate, because the sensitized population, and not the total population, is used. It also differs from the fatality-rate of tuberculous infection, as defined on p. 7, in that the sensitized population is used instead of the infected population. The latter term is avoided here because, as pointed out in a previous section (p. 21), there is not yet clear evidence whether the incidence of tuberculin sensitization in a community, which can be determined by the tuberculin test if the most accurate technique is used (p. 75), corresponds to the infection incidence, or whether it only indicates the proportion of persons who have at some time been infected, i.e. the attack-incidence of tuberculous infection. (These terms are defined on p. 6.)

The results of recent tuberculin surveys with the intracutaneous test indicate that the incidence of tuberculin sensitization among the poorer classes or general population of most urban communities increases gradually with age, from zero at birth to ninety per cent. or more in adult life (Section VII).

If the tuberculin-sensitized proportion of a community increases with age, and the age distribution of tuberculosis mortality is as stated above, then, assuming that only the sensitized (or, practically only the sensitized) develop clinical tuberculosis, one can draw the following inferences:

(1) That rates of mortality based upon the sensitized populations in age-groups will in the earlier age-groups be much larger than when the rates are based upon the whole populations.

(2) That the death-rate from tuberculosis among the sensitized children is highest in infancy, but then falls rapidly. It remains comparatively low during middle childhood and until the age of puberty, after which it may rise or fall, depending on the mortality-rate and sensitization incidence of the population.

(3) That the difference between tuberculosis mortality in infancy and middle childhood will be greater, and between that in middle childhood and adult life less, when figures are based upon sensitized populations, than when the rates are based upon the whole populations.

Tuberculosis mortality rates have shown a steady decline in most civilized countries since the middle of the last century (except during the War), and it is to be hoped that the figures will continue to fall.[1] The reduction in mortality in the first few years of life is particularly striking. There is also some evidence to support the view that the tuberculin sensitization incidence of the community is decreasing. If this change takes place more slowly than the fall in mortality-rate, the death-rate in the sensitized population will also decline. Should this decline principally affect infants, tuberculous *infection* may cease to be most fatal in early life.

5. Summary and Conclusions.

1. A positive tuberculin reaction indicates that tuberculous infection has taken place, whatever be the age of the individual or the method used.

2. The positive reaction has its chief clinical value in infancy, when, from direct observation, tuberculous infection is more likely than at other ages to be associated with, or to progress into, active tuberculous disease, and to lead to a fatal issue. The younger the age, the worse is the prognosis. Unfavourable home conditions appear to affect the

[1] See footnote, p. 84.

outlook adversely. The detection of tuberculin sensitivity in infants must, however, be taken only as contributory evidence of the presence of clinical tuberculosis; and, moreover, the mortality from tuberculosis among these infants—under the present conditions of living—appears from recent work to be lower than it was formerly believed to be, indicating that control and arrest of infection are possible.

3. The following is suggested as a practical diagnostic procedure at the present time for patients of the hospital class:

(*a*) The intracutaneous, rather than the cutaneous, method is recommended for the detection of tuberculin sensitivity in early life, on account of its greater delicacy (for technique see Appendix 5).

(*b*) An infant under two years showing a positive reaction, but without symptoms, should be kept under observation for some years, on account of the possible development of clinical tuberculosis. Meantime the prognosis should be optimistic though guarded. If, however, obscure and persistent symptoms (e.g. unexplained pyrexia) are present, a positive response should be regarded as important evidence, but not proof, that these are tuberculous in origin, and a serious view should be taken until time shows this to be unwarranted.

(*c*) A positive reaction in a young child aged two to five with persistent symptoms should suggest that these symptoms are tuberculous in origin.

(*d*) Where a patient over five gives a positive reaction to a qualitative test, it should not be concluded that clinical tuberculosis is present, even in a suspicious case.

4. In patients over five the positive reaction has its principal diagnostic value when elicited by quantitative intracutaneous tests made with weak solutions of tuberculin. This procedure is especially indicated in dermatological conditions. The methods, however, require considerable experience, and until more information is forthcoming they are not recommended for routine work.

5. Quantitative tests are of doubtful value for estimating the prognosis in clinical tuberculosis.

6. In a community in which the tuberculin sensitization incidence rises gradually from infancy to manhood, and in which the mortality from tuberculosis has the usual age distribution, the estimated mortality from this disease in the sensitized population has a similar age distribution, except that the difference between middle childhood and infancy is enhanced, and between middle childhood and adult life is diminished.

Section V. The Value of the Negative Reaction in Diagnosis. The Choice of Method for the Negative Diagnosis of Clinical Tuberculosis.

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1. INTRODUCTION.

In the preceding section it was stated that a positive tuberculin skin reaction had a very limited value in the diagnosis of clinical tuberculosis, except in early life. Consequently, the usefulness of the tuberculin test in diagnosis must come mainly from a negative response, in so far as it excludes this disease. Mantoux defined the position as he conceived it when he wrote of the intracutaneous test (1910), 'A negative test except in measles, meningitis, miliary tuberculosis and advanced cases with marked toxaemia, is an argument of the first order in excluding [clinical] tuberculosis. . . . Contrary to most clinical methods the value of the intracutaneous test lies in negative results'.

At the present time, however, the tuberculin test is little used for this purpose—in this country perhaps less than in any other. One might suggest two reasons for this neglect: one, the belief that the test is unreliable in excluding clinical tuberculosis, seeing that a negative reaction may be obtained in patients with undoubted evidence of the disease: the other, the belief that, whatever be the implication of a negative test, it can, like the positive reaction, be of value only in early life. These views, as will be shown in this section, can no longer be justified.

2. The Error of the Cutaneous (von Pirquet) Test in the Negative Diagnosis of Clinical Tuberculosis.

A large number of observations have been made on the error of a negative von Pirquet reaction in excluding clinical tuberculosis. The procedure has been to test a series of definite cases of clinical tuberculosis and to find the percentage that fails to react. These observations indicate that as ordinarily performed the error of this method is very high.

Von Ruck (1909) collected 2,192 cases from the earlier literature; 341 of these cases failed to react, giving an error of 16 per cent. Von Ruck's figures include a series of 460 cases (Petruschky, 1908), containing suspects as well as definite cases of clinical tuberculosis. When these are excluded there remain 1,732 cases of clinical tuberculosis with an error of 15 per cent.

No review appears to have been published since von Ruck's paper. The writer has therefore abstracted the results of 42 authors who between 1907 and 1928 tested altogether 4,787 cases of undoubted clinical tuberculosis, including all types of the disease, with the von Pirquet test (Table 2 and Appendix 1). Of these patients 766 were reported to have given negative reactions, the error thus being 16·0 per cent. The largest series of mixed cases is that of von Pirquet himself (1907 d, 1909), comprising 286 cases of various types, with a 7 per cent. error.

The assessment of the value of the von Pirquet test by adding together the results of these authors is difficult, for three main reasons.

(*a*) The concentration of the tuberculin used in the earlier work varied, for von Pirquet at first recommended diluted tuberculin, but soon (1908 a, 1909) began to use the undiluted product.

(*b*) The difference of the brands and batches of tuberculin must also be considered, for an appreciable variation in potency has been recorded by a number of investigators (Watson, 1920; Cunningham & Ratcliffe, 1922; O'Brien, 1925; Okell, 1930). Okell states that while a large number of random samples are of approximately standard potency, occasionally one is found that is considerably below Frankfurt standard value, but very rarely does a sample exceed twice its potency. It is only within recent years that the intracutaneous method of titrating tuberculin in guinea-pigs (Roemer & Joseph, 1909, 1910; Lewis & Aronson, 1923; Eagleton & Baxter, 1923; Okell & Parish, 1927) has come to be recognized generally as the best method of standardization (O'Brien, 1925; Calmette & de Potter, 1926; Okell, 1930). The original Koch (1891) method of standardization is unreliable (Long, 1925), and the Frankfurt subcutaneous method requires a much greater number of guinea-pigs to give a degree of accuracy equal to that obtained by intracutaneous titration (Okell, 1930). Until recently the current Frankfurt standard tuberculin was that accepted as a basis of comparison. In 1927 an International Standard of very similar potency was established in Copenhagen and in the Medical Research Council's laboratory, Hampstead. The standards of the Pasteur Institute and of the American Hygienic Laboratory are of approximately equal potency to the Copenhagen tuberculin.

(*c*) Comparison is made difficult because many series are composed entirely of adults, among whom a high percentage of reactors would be expected in any case. The severest test of any diagnostic tuberculin method is in its application to young

children, among whom a comparatively low incidence of positive reactors is found if they are clinically non-tuberculous. Von Pirquet's series of 286 cases, for instance, consisted of 75 adults, of whom all but one reacted, whereas of 209 children 20 failed to react, giving an error of 10 per cent. In order to bring out these differences between the error in children and adults, the 4,787 cases were analysed according to age (Table 2). Of 2,168 cases of clinical tuberculosis in adults and adolescents 212 failed to react, an error of 9·8 per cent., while among 1,933 children there were 445 who did not respond to the von Pirquet test, making an error of 23 per cent., i.e. twice the adult error.

Even if those series of children that are composed entirely of cases of miliary tuberculosis or meningitis are excluded (607 cases with an average error of 37·5 per cent.), the error in children is greater than in adults, being as much as 16·5 per cent.

In spite of the limitations of a gross comparison of this kind it is clear that the von Pirquet cutaneous test, as ordinarily performed, is totally unsuitable for negative diagnosis in cases of suspected clinical tuberculosis. By using a different method of abrading the skin (Petruschky's modification) and by repeating the test whenever negative, Peters (1921) obtained a much smaller error. His series (not included in the above totals) consisted of pulmonary tuberculosis. Of 56 children with 'closed pulmonary tuberculosis' (all stages) all but 3·5 per cent. reacted, while only 1·5 per cent. failed to respond among 1,909 adults and adolescents suffering from the same condition. This worker noted an error of only 1 per cent. in 105 cases (age not stated) of open pulmonary tuberculosis, but he excluded heavily infected cases. Similar claims have been made by McNeil (1923). It was stated in a preceding section (p. 30) that other authors have noted that by varying the instrument used and the mode of applying the tuberculin to the skin, a significant difference in the number of positive reactions may be obtained. Instances such as these, showing the effect that slight differences of technique can produce, serve to demonstrate the unreliability of the von Pirquet test, for it is a fair argument that if slight modifications of a method produce significant differences in results, that method is unsuitable for routine work in the hands of those who are not experts. Stewart (1928), however, advocates a technique similar to that described by Lewis (1927), which he states gives results exactly corresponding to those produced by scarification, and is less objectionable. Its absolute error in clinical tuberculosis is not given.

Analysis of the published statistics show that the von Pirquet test is apt to fail in particular types of patient. Miliary tuberculosis, meningitis, moribund cases, and very acute or active tuberculosis are the most notorious examples, and an error of up to 50 per cent. has been reported in the first two conditions. These and other conditions that are cited in the literature, either as a result of personal experience, or in obedience to accepted tradition, are included in Table 4. It should be mentioned that the examples given do not account for all the failures, there being some cases of clinical tuberculosis in which negative reactions are obtained for no apparent reason, and which cannot be placed in any particular group.

3. The Error of the Intracutaneous (Mantoux) Test in the Negative Diagnosis of Clinical Tuberculosis.

A. *Historical Survey.*

Although the conclusion that the intracutaneous test is more sensitive than the cutaneous method is based on comparative work carried out by many authors during the last twenty years, and reviewed in Section III, surprisingly few investigations have been made with the object of finding the absolute error of this test in the negative diagnosis of clinical tuberculosis. A knowledge of this error is of great practical importance, in view of the inaccuracy of the von Pirquet test. The statistics on this point that the writer has been able to discover are given in Appendix 2, and summarized in Table 3. In some instances authors have re-tested the initially negative reactors with larger doses; the upper limit is indicated in the second column. The types of case that fail to react to tuberculin are similar to those insensitive to the von Pirquet test. Again, however, some cases are met with that cannot be classified, and for whose non-reactivity there is no apparent explanation. Unfortunately, some of these series include an unknown number of adults, who in any case would be expected to yield a high proportion of positive reactors; since the number of adults is not stated, the error for children cannot be calculated in these instances.

Although these statistics are fewer than those collected for the von Pirquet test, they suggest that the error, measured by the percentage of cases of clinical tuberculosis that fails to react, is less than that of the older test. Nevertheless, the error is by no means negligible, and is sufficient to make the intracutaneous method, as usually carried out, far from ideal for the negative diagnosis of clinical tuberculosis.

There are, however, indications in published work that the intracutaneous test of Mantoux, if suitably modified, is far more accurate in excluding clinical tuberculosis than is generally supposed. Engel (1911) suggested that the reason why the intracutaneous method yielded nearly as unsatisfactory results as the cutaneous method was that the dosage used (0·1 c.c. of 1 : 10,000, or 0·1 c.c. of 1 : 1,000 tuberculin, = 0·01 mg., 0·1 mg.) was too small, and that the full sensitiveness of the method was, therefore, not utilized. Engel advised, in case of doubt, re-test of patients who were negative to these doses with higher concentrations, e.g. 1 : 100 (= 1 mg.), 1 : 10 (= 10 mg.), and even undiluted tuberculin (100 mg.), before pronouncing them free of tuberculosis. With these very large doses he was able to obtain reactions in patients with cachexia, miliary tuberculosis, meningitis, and acute infections, who were negative to the usual intracutaneous doses and to von Pirquet's test.

Engel's graded quantitative intracutaneous method received little notice, presumably because he gave no figures but merely quoted individual cases. Although Kleinschmidt (1918) recommended its wider use he too gave no figures. It is, therefore, to Happ & Casparis (1922), a decade later, that credit is due for putting Engel's method

on a numerical footing. These workers tested a series of 72 cases of miliary tuberculosis and meningitis, proceeding with gradually increasing doses from 0·1 c.c. of 1 : 10,000 tuberculin (= 0·01 mg.) up to 0·1 c.c. of 1 : 100 (= 1 mg.) or more before classing a patient as a non-reactor. With this procedure 0·1 c.c. of undiluted tuberculin (100 mg.), which is 100–1,000 times as much as the quantity usually given, is the maximum intracutaneous dose. It was found that while only 50 per cent. of the patients reacted to 1 : 10,000 tuberculin—the same percentage as reacted to the von Pirquet test in this series, 80 per cent. gave a positive response to 1 : 1,000 tuberculin (the upper limit of concentration used by most workers), 93 per cent. to the 1 : 100 dilution, and 100 per cent. reacted positively when the highest concentrations were reached, even though some of the tests were made a few days before death. Happ & Casparis suggested that in the same way it might be found that positive reactions would be obtained in practically all the conditions (Table 4) in which the von Pirquet and Mantoux tests were supposed to fail, if bigger doses were employed than those generally in use. Cutaneous sensitiveness, in their opinion, is only depressed but not lost in these conditions.

These observations, which Tisdall & Brown (1926) have confirmed, suggest the desirability of re-examining on a large scale the whole subject of the graded intracutaneous tuberculin test in the clinically tuberculous, particular attention being paid to those conditions (Table 4) in which negative reactions are apt to be found with the usual doses of tuberculin. At the suggestion of Dr. R. A. O'Brien, Director of the Wellcome Physiological Research Laboratories, an investigation of the intracutaneous test on these lines was carried out by the writer in London and the Home Counties from January to December, 1929. The present time is peculiarly opportune for such an inquiry, for the recent adoption of an international method of biological standardization of tuberculin makes the results applicable in other countries.

B. *The London Investigation*, 1929.

(*a*) *Purpose of Investigation.*

The object of this investigation was to find the error of a negative intracutaneous reaction in excluding clinical tuberculosis, and to determine how this varies with the type and stage of the disease, the age of the patient, and the dose of tuberculin employed.

(*b*) *Tuberculin.*

Human Old Tuberculin (O.T.), batch T. 1586, made by the Wellcome Physiological Research Laboratories, was employed throughout this investigation. The human type was used exclusively, for there is abundant evidence, both from clinical and experimental work, that there is no qualitative difference in the reactivity of animals and man towards this and the bovine type.[1] In many instances bovine tuber-

[1] See Gauvain (1917), Tisdall & Brown (1926), Stewart & Collins (1927), Calmette (1928), Tytler (1930), Okell (1930).

culin prepared from a synthetic medium, the most complex nitrogenous constituent of which was asparagine, was employed in addition to the Old Tuberculin; the same sample (batch T. 803 made in 1915) was used throughout. There was complete qualitative agreement, although not infrequently a larger dose of this product was required than of the Old Tuberculin to give a reaction. Moreover, the general impression formed was that, when diluted, the tuberculin prepared from the synthetic medium deteriorated more rapidly than did the Old Tuberculin.

The Old Tuberculin used was prepared by the method described by Okell (1930), and was concentrated ten times over steam before being supplied for use. It was standardized by the intracutaneous and subcutaneous guinea-pig methods, as described by Okell & Parish (1927) and Okell (1930), and was found to be equal in potency to the current (1928) Frankfurt standard within the limits of the method. These are such that differences representing a 40 per cent. drop of potency are almost always detectable on a small series of guinea-pigs, and finer differences on a larger series. The present International Standard Tuberculin is based on the Frankfurt standard in question.

The tuberculin was put up fortnightly in dilutions of 1 : 10,000, 1 : 1,000, 1 : 100, 1 : 10, and 1 : 1 (undiluted). 0·5 per cent. phenol-saline was used as diluent. 0·1 c.c. was the quantity injected intracutaneously in every case. The dilution method of recording dosage, used on the Continent, was adopted in preference to the weight method, popular in America, as it was considered to be the better by the League of Nations Tuberculin Committee. Moreover, as Signy (1930) points out, the weight method depends on the assumption that 0·001 c.c. of undiluted Old Tuberculin weighs 1 mg. This assumption is not strictly true, for Old Tuberculin is an oily material with a specific gravity greater than that of water. For the convenience, however, of those who are accustomed to the weight method, the approximate quantity in milligrammes of tuberculin injected is inserted in brackets.

Thus 0·1 c.c. of 1 : 10,000 tuberculin = 0·01 mg.; 1 : 1,000 = 0·1 mg.; 1 : 100 = 1 mg.; 1 : 10 = 10 mg.; and 1 : 1 = 100 mg.

Control solutions of glycerinated veal broth were used in the majority of cases. The writer was carrying out, side by side with this investigation, an inquiry into the response of the skin of tuberculous and non-tuberculous individuals to meat derivatives. During the course of this inquiry (Hart, 1932) evidence was adduced to show that skin reactions having all the macroscopic characteristics of the tuberculin reaction were obtainable with glycerinated veal broth in suitable strength, the activity varying with different samples, but that such reactions occurred only in tuberculin-positive individuals. It would seem, therefore, that, once familiarity with the appearance and progress of the tuberculin reaction has been achieved, glycerinated veal broth controls are of little value in routine testing. It is, however, desirable to use them when performing tests with 1 : 10 tuberculin or stronger solutions, in order to avoid confusion between true tuberculin reactions and the delayed, atypical non-specific reactions (p. 53) which follow with

comparative frequency the injection of these concentrated solutions. Atypical non-specific reactions seldom give rise to difficulty when solutions of 1 : 100 or weaker tuberculin are injected.

(*c*) *Technique used.* (See also p. 52 and Appendix 5.)

A separate syringe was kept for each strength of tuberculin. The injections were made intracutaneously in the forearm or upper arm. All tests were made and read by the writer in order to ensure uniformity of interpretation. Reactions were read on the second or third days, and subsequently if negative. Where a response gave rise to doubt the test was repeated within a week with the same or a higher concentration. In one instance a positive reaction was delayed until the seventh day (miliary tuberculosis with meningitis); repetition of the test produced a prompt positive response. The reactions were measured, but are recorded here merely as positive or negative to the particular dilution employed; they usually reached their maximum on the second day. A reaction was considered to be positive if it consisted of an area of erythema or erythematous infiltration whose greatest diameter equalled or exceeded 5 millimetres.

(*d*) *Clinical material.*

This consisted of 1,030 clinically tuberculous patients of all ages, comprising the commoner types of the disease, and every stage, from early activity to the moribund state, and from approaching quiescence to clinical arrest. The majority lived in London or the suburbs, and a smaller number in the Home Counties (p. 93). All doubtful cases were excluded, as only in this way could a just estimate of the value of the intracutaneous test in negative diagnosis be formed, and the figures refer, therefore, only to patients in whom the diagnosis of clinical tuberculosis was considered to be certain by the clinician in charge. This was an easy matter in pulmonary disease where a positive sputum or unequivocal X-ray was obtained, in bone or joint disease or adenitis where the pus contained tubercle bacilli, or where the clinical diagnosis was confirmed by biopsy or necropsy. In the other cases, mainly adenitis and bone or joint tuberculosis, the diagnosis was made on physical examination, history, and X-ray. It is just in this type of case that tuberculin finds its most useful application, but in the present investigation, the object of which was to 'test the test', the last word naturally lay with the clinician. Patients who gave a definite history of past active clinical tuberculosis (other than dry pleurisy), but who were now apparently well so far as tuberculosis was concerned, were included, even if there was no present clinical evidence of disease. The majority of these cases come into the category of quiescent or arrested clinical tuberculosis. In eight of the series (seven bone or joint cases) the disease was believed to be arrested.

The distribution of the 1,030 cases according to the age of the patient and the type of the disease is shown in Table 5. There were 167 patients under 6, 389 under 11, and 689 under 21 years of age. The figures for pulmonary tuberculosis include cases complicated by

laryngitis. Otherwise, cases in which the disease was located in more than one system (e.g. lupus+joint tuberculosis, but not cases in which two joints, or one bone and one joint, were involved) are tabulated in the eighth column. This procedure avoids duplication. The numbers in brackets in the second column refer to the patients known to have had bacilli in their sputum at some period. The remainder showed unequivocal radiographical evidence of phthisis. Only 19 patients with pulmonary tuberculosis (13 with positive sputum) under eleven years of age could be tested, but there were 77 (72 with positive sputum) aged 11–15, and 340 older. The ages refer to the age last birthday. There were only 5 patients suffering from miliary tuberculosis with meningitis. Of the patients with pulmonary disease 93 were advanced cases with marked toxaemia, the majority with considerable pyrexia, about a dozen of them moribund; 3 of these patients were under 11 years of age, 19 were aged 11–20, and the remainder were adults. In the other pulmonary cases the disease was early, intermediate, or advanced, but was unaccompanied by marked toxaemia. To a rough estimate 30 had quiescent and the remainder active disease. Twelve of the whole series (8 pulmonary, 3 miliary tuberculosis with meningitis, 1 bone tuberculosis) were tested a week or less before death. Of the 454 bone or joint cases 257 were active, 125 were quiescent or approaching quiescence (including 7 believed to be arrested), and the remainder were not placed in either of these groups.

(*e*) *General Results.*

The reaction of each patient in the series to 0·1 c.c. of a 1 : 1,000 dilution (0·1 mg.) of tuberculin, given intracutaneously, was ascertained, this being the dilution used by most recent workers; 766 of these patients were tested with 0·1 c.c. of a 1:10,000 dilution (= 0·01 mg.) and as many as 93 gave a negative reaction (12 per cent.) with this dose.[1] Thirty-eight patients out of the 1,030 (3·7 per cent.) failed to react to the 1 : 1,000 dilution. These 38 patients (see Appendix 3) were then tested with 0·1 c.c. of a 1 : 100 solution (= 1 mg.); a positive reaction was obtained in 10 cases, leaving 28 (2·75 per cent.) negative. Five of these 28 patients could not be tested further, owing to death or refusal; the remaining 23 were tested with 0·1 c.c. of 1 : 10 tuberculin (= 10 mg.); 4 of them were positive, 19 were negative, and these figures, if adjusted for the whole series on the assumption that the 5 patients who were not tested would have behaved as those who were, give an error of 2·3 per cent. These 19 negative cases were finally tested with undiluted tuberculin (1 : 1 = 100 mg.), which is the maximum intracutaneous dose, with the result that only one more patient gave a positive response, so that the final and minimum error of the test, adjusted for the whole series, was 2·2 per cent. The numbers tested with the higher concentrations are naturally small since they

[1] The tests with 1 : 10,000 and 1 : 1,000 tuberculin were either made simultaneously, or the weaker solution was given first and the patient, if negative, was then tested with the standard dilution (1 : 1,000).

comprise only those cases that were negative to the previous dilutions.

The increase in the percentage of positive reactors, and consequent accuracy of the test, accompanied an increase in the strength of tuberculin in both young and old, but the error in the age-group 0–5 was greater than in other age-groups, being 6 per cent. with a 1 : 1,000 solution, 4 per cent. with a 1 : 10 dilution, and 3·5 per cent. with undiluted tuberculin.

The results for all types and stages together, arranged according to age, are given in Table 6; the figures for all ages together will be found at the bottom of this table. The same figures are summarized in Table 12, and shown graphically on Charts 1, 1 a, and 2. Chart 1 a is a simplified graph showing only the figures for the standard dilution (1 : 1,000).

A clinical summary of the 38 patients who failed to react to 0·1 c.c. of the 1 : 1,000 dilution of tuberculin (= 0·1 mg.) is given in Appendix 3, together with a note of the further behaviour of each of them when tested with larger doses.

Charts 1, 1 a, and 2 show graphically how the percentage of positive reactors increased, and the error of the test was reduced, by using larger doses, and that this occurred at all ages. Attention is drawn to the flattening of the curves of Chart 2 when the highest strengths of tuberculin were used, suggesting that the limit of potential reactors had been almost reached, i.e. that almost every tuberculin-sensitive individual had been detected.

The present observations thus go to show that a negative intracutaneous reaction to a 1 : 10,000 solution of tuberculin, or to the standard (1 : 1,000) dilution, does not exclude clinical tuberculosis with certainty. A negative test with a 1 : 10 dilution excludes the disease with greater accuracy, the error (2·3 per cent.) being very close to the minimum error (2·2 per cent.) and small compared with the figures given in Tables 2 and 3. For these reasons there is no advantage in using solutions of tuberculin stronger than 1 : 10 for excluding clinical tuberculosis. Moreover, as will appear later, non-specific effects of tuberculin become more marked with highly concentrated solutions.

(f) Results analysed according to type and stage.

The figures for all the patients with pulmonary tuberculosis (436), and for 93 of them who were advanced cases with marked toxaemia, are given in Table 7, and those for the 454 patients with bone or joint tuberculosis in Table 8. The figures for the remaining smaller groups are given in Table 9. In Table 10 the figures for active and quiescent bone or joint tuberculosis are compared.

The number of cases of each type of the disease correspond, of course, to the numbers given in Table 5. The numbers in brackets refer to patients with positive sputum. The arrangement of the results is exactly as in Table 6, already considered. In the last two columns the percentages are adjusted for the whole series, on the assumption that the few patients who could not be tested with concentrations higher

than 1 : 100 tuberculin would have behaved as the others did (see (*e*) and footnote to Tables 6–10).

Perusal of Tables 7–10 brings out the following points:

(1) The decrease of error which, as pointed out when considering the composite table (Table 6) and Charts 1, 1 a, and 2, resulted from an increase in the concentration of tuberculin, occurred in both pulmonary and bone or joint tuberculosis. The reactions of the two types were very similar (Tables 7 and 8), the minimum error being 1·7 and 2·0 per cent., respectively, but a slightly greater percentage of the bone or joint patients (89 and 97·4 per cent.) than of the pulmonary patients (87 and 96 per cent.) reacted to the 1 : 10,000 and 1 : 1,000 solutions. This difference is not significant, for the great majority of the pulmonary cases were active, while a considerable proportion of the bone or joint cases were quiescent, and when the active members of the latter group (Table 10) are compared with the pulmonary group (Table 7) the percentage of reactors is approximately the same. It will be noted (Table 8) that the minimum error for the bone or joint cases was about the same in each age-group (2 per cent.), but that the error with 1 : 1,000 tuberculin was greatest for children in the age-group 0–5 (4 per cent.). The pulmonary patients included 93 advanced cases of phthisis with marked toxaemia. When these 93 cases are taken separately (Table 7) there is a large percentage of failures (15 per cent.) with 1 : 1,000 tuberculin, but this is reduced to 8·5 per cent. with 1 : 100, and to 3·5 per cent. with 1 : 10 and 1 : 1 tuberculin. These toxaemic pulmonary cases account for most of the cachexias in the series. About a dozen were in a moribund condition when tested. Eight of them were tested a week or less before death; 7 of these patients failed to react to 1 : 1,000 tuberculin. The 7 were re-tested with 1 : 100 tuberculin; 2 were positive, 5 negative. Three of these non-reactors to 1 : 100 tuberculin died before they could be tested further. The other two were re-tested with 1 : 10 tuberculin: one reacted positively and the other gave a negative response to this strength and also to 1 : 1 tuberculin. The final minimum error is thus very great in pulmonary tuberculosis just before death (roughly 1 in 3, based on 8 cases).

If the 8 cases tested a week or less before death are excluded from the series of 93 patients with advanced phthisis and severe toxaemia, the error in the remaining 85 cases is 8 per cent. with 1 : 1,000 tuberculin, 4 per cent. with 1 : 100, and only 1 per cent. with 1 : 10 and 1 : 1 tuberculin. These figures show that in advanced pulmonary tuberculosis with severe toxaemia cutaneous sensitivity to tuberculin is depressed rather than lost, and that almost all patients, unless moribund, will react to this substance provided that the higher concentrations are used. These findings are in agreement with those of Happ & Casparis in miliary tuberculosis and meningitis, but while 100 per cent. of their patients reacted positively, two very toxic cases (one moribund) in the writer's series failed to respond even to the huge intracutaneous dose of 0·1 c.c. of undiluted tuberculin (100 mg.).

(2) The patients with lupus vulgaris all reacted to the 1 : 100 dilu-

tion, and there was a smaller error (8 per cent.) with the 1 : 10,000 dilution than in any of the other types tabulated.

(3) Tuberculous pleurisy and peritonitis gave the largest error with the strong concentrations (see also Appendix 3).

(4) All of the very small series of miliary tuberculosis with meningitis reacted to 1 : 100 tuberculin: 4 out of 5 to the 1 : 1,000 dilution. All were tested within a month, 3 within a week, of death. These figures agree with those of the much larger series (72 cases) of Happ & Casparis (1922), already quoted.

(5) Little information can be given regarding the reputed reduced allergy of old age. After 70 years tuberculosis is comparatively rare, and this series includes but few cases of this age. In 65 tuberculous patients over 50 years of age there was no evidence of reduced allergy.

(6) Comparison of the reactions of active and quiescent bone or joint tuberculosis (Table 10) shows a slight difference in reactivity. The percentage of reactors to each dilution of tuberculin was greater among quiescent cases in the age-group 0–10 than among active cases in the same age-group. A similar difference is seen to a less degree in the upper age-group (11–). These differences suggest that cutaneous sensitivity to tuberculin increases when the disease becomes quiescent. This conclusion is in disagreement with the view that sensitivity is greatest during the active phase (see p. 32).

Analysis of the 38 cases, out of the 1,030 tested, that were negative to a 1 : 1,000 dilution of tuberculin (Table 6 and Appendix 3) shows that these fall into certain groups:

(1) Advanced pulmonary tuberculosis with marked toxaemia. This group has already been considered. Most cases reacted to the higher concentrations. The persistent non-reactors were chiefly patients tested a week or less before death.

(2) Bone or joint tuberculosis with marked activity. These cases showed less tendency to react to higher concentrations if negative to 1 : 1,000 tuberculin.

(3) A few non-toxic cases of active pulmonary tuberculosis with positive sputum, and a few of active bone or joint tuberculosis with definite radiographical evidence of disease, for whose failure to react no reason can be advanced. These cases were negative even when undiluted tuberculin was used.

(4) Eight cases either quiescent or approaching quiescence, all but one of which (a joint case) were also negative to undiluted tuberculin. These included three patients with bone or joint tuberculosis, one of whom had also the diagnosis of scrofulodermia, and two children with pulmonary tuberculosis (sputum positive once). There was also a case of quiescent peritonitis, one of quiescent, or possibly arrested pleurisy with effusion, and a patient with quiescent calcified tracheo-bronchial glands. All the other quiescent cases in the series (including eight in which the disease was believed to be arrested) were positive to 1 : 1,000 tuberculin; indeed the quiescent bone or joint group showed a slightly larger percentage of reactors than did the active group. Krause (1925 a) published a case of clinical tuberculosis in which a positive

reaction to the von Pirquet test was lost after complete clinical recovery. Opie (1930) states that some children with calcified nodules in the lungs and adjacent lymph nodes fail to react to tuberculin (intracutaneous test with upper limit of dose = 0·1 c.c. of 1 : 100), and believes that the lesion is completely healed in such cases.

(*g*) *Control series.*

Seven hundred and fifty-one clinically non-tuberculous members of the hospital class of London served as controls to the 1,030 cases of clinical tuberculosis. They were selected at random from in- and out-patients attending hospitals in North and Central London (see p. 93). Six hundred and seventeen of these controls were under 21 years of age, 490 under 11, and 245 under 6. All suspected or definite cases of clinical tuberculosis, past or present, were excluded, together with patients who gave a history of dry pleurisy unaccompanied by pneumonia.

The material, dosage, and technique employed were those described for the series of clinical tuberculosis, and the investigation took place during the same period. The criterion of a positive reaction was also the same. All tests were made and read by the writer. Reactions were read on the second and fourth days, or on the third day. They reached their maximum usually during the second day. Slight reactions had, not infrequently, disappeared by the end of a week. In a few cases the appearance of the reaction was delayed to the seventh, tenth, or even fourteenth day. This delayed reaction was always a dull purple stain, and a prompt response of the usual type was obtained on repeating the test. It is possible, therefore, that a few positive reactors were missed through not examining all patients later than the fourth day.

The reaction of each of the 751 controls was ascertained to 0·1 c.c. of the standard (1 : 1,000) dilution of tuberculin (= 0·1 mg.), given intracutaneously; 295 of these controls, selected at random, were tested with 0·1 c.c. of a 1 : 10,000 solution (= 0·01 mg.).[1] A random sample of the individuals in each age-group who were negative to 1 : 1,000 tuberculin was tested with a 1 : 100 solution, and the reactions noted. In the same way a random sample of the non-reactors was tested with a 1 : 10 dilution, and finally the same procedure was carried out with 0·1 c.c. of undiluted (1 : 1) tuberculin (= 100 mg.), this being the maximum intracutaneous dose. The number of individuals tested with the higher concentrations was naturally small, owing to the gradual elimination of those who gave positive reactions with the weaker solutions.

The percentage of positive reactors in the whole series was calculated for each age-group and for each dilution of tuberculin by adjusting the figures on the assumption that those individuals who were negative to 1 : 1,000, 1 : 100, or 1 : 10 tuberculin and were not tested further with 1 : 100, 1 : 10, or 1 : 1 tuberculin, respectively, would have reacted in the same way as those who were tested further. The resulting figures, with these adjusted percentages, are given in Table 11, and shown graphically on Charts 1, 1 a, 2, and 4. They are summarized in Table 12, where the abbreviated results of the other tables will also be found.

The control series presents a contrast to the series of clinical tuberculosis, this contrast being naturally most marked in the youngest age-group (Table 12, Charts 1 and 1 a). 96·5 per cent. (adjusted) of

[1] See footnote, p. 48.

the clinically tuberculous children in the age-group 0–5 reacted to undiluted tuberculin, and 97 per cent. in the age-group 6–10; while the (adjusted) percentage of reactors among the controls was only 6·5 per cent. in the age-group 0–2, 18 per cent. among those aged 3–5, and 38 per cent. in the age-group 6–10. Seventy per cent. of the controls aged 11–20 gave positive responses and the figure was 95 per cent. for the adults of this series. The percentage of reactors thus rose evenly from birth to manhood.

Table 11 and Charts 1, 2, and 4 demonstrate that the accuracy of the test in the control group varied with the dose of tuberculin, as was found in the series of clinical tuberculosis, the variation being more marked the older the patient. Thus it will be seen that the percentage positive to 1 : 10 tuberculin was significantly greater than to the standard dilution (1 : 1,000). On the other hand, the percentage positive to the maximum concentration (1 : 1) was scarcely greater than to 1 : 10 tuberculin, suggesting that almost all the potential reactors had been detected. Judging from these observations, one cannot exclude tuberculous infection with certainty in a clinically non-tuberculous individual in whom a negative reaction to the standard dilution has been obtained, without making further tests with higher concentrations, up to a 1 : 10 solution, but there is no advantage in proceeding beyond this strength of tuberculin (cf. Schroeder, 1924; Hetherington *et al.*, 1929; Cummins, 1930).

(h) Atypical non-specific reactions.

The delayed, persistent, inflammatory response to which the term positive tuberculin reaction is limited is not the only type of skin response to the injection of Old Tuberculin. Immediate reactions of an urticarial type are said sometimes to occur, appearing within a short time, chiefly characterized by oedema and little inflammation, and fading promptly without apparent injury. (See Zinsser, 1921; Zinsser & Tamiya, 1926.) These atypical reactions are possibly due to anaphylactic sensitization, and are unlikely to cause any confusion. They will not be considered further.

More important are the infiltrative, erythematous reactions that may be met with in individuals who have not been infected with tubercle. Some of these non-specific reactions probably belong to the class of response described by Lewis (1927) and his co-workers as following any form of injury to the skin, and due to the liberation of a histamine-like substance; in other cases it is open to question whether injury is an important factor. The glycerine present in the tuberculin possibly plays a part in producing the reactions. In the present discussion these non-specific responses will be referred to as atypical non-specific reactions.[1] Their rapid development to a maximum and their rapid subsidence help to distinguish them from true tuberculin reactions. Reaching their height during the first twenty-four hours they have usually disappeared completely by forty-eight hours, when tuberculin

[1] These reactions are probably identical with the 'trauma reactions' of certain authors (e.g. Krogsgaard, 1925), and the 'Reizwirkung' of Hamburger (1912 a).

reactions are as a rule maximal, so that confusion is avoided in most cases if tests are not read before the second day. Occasionally, however, during the present investigations atypical non-specific reactions were found to be still present on the second, third, or even fourth days. These delayed non-specific responses were not seen after tests made with 1 : 10,000 Old Tuberculin, and followed less than 0·5 per cent. of injections made with 1 : 1,000 or 1 : 100 dilutions. But they were more frequent after injections of 1 : 10 tuberculin, and florid delayed reactions of this type were noted after about a quarter of the tests made with the undiluted product. Delayed non-specific reactions naturally gave rise to difficulty in interpretation, but they disappeared more rapidly than true tuberculin reactions of the same size. Glycerinated broth controls also helped to make the distinction (see also p. 6, Appendix 5, and Hart, 1932).

(*i*) *Ill-effects of the intracutaneous test.*

The only untoward effects of the intracutaneous test met with in this investigation were local vesiculation and slight systemic disturbance, but in only one instance (a patient who had a haemoptysis and died some days later) did the test appear to have any adverse influence on the course of the disease. Sloughing is apt to be a serious sequel of vesiculation, and may leave an unsightly scar.

Local vesiculation occurred in 9 cases of tuberculosis (less than 1 per cent.) and in 7 (less than 1 per cent.) of the controls. The 9 tuberculous patients were as follows: 'surgical' tuberculosis (i.e. bone or joint tuberculosis, adenitis, genito-urinary tuberculosis, peritonitis) 6; pulmonary tuberculosis 2; not classified 1. Five of the 7 clinically non-tuberculous controls were home-contacts of tuberculosis, a point of interest. One of the remaining two cases was a young adult in whom a second test had been performed after a fortnight on account of a doubtful positive first test. The violent local response that resulted was presumably due to sensitization, and suggests that, where possible, patients who show feeble positive reactions should be re-tested within a week, or else after an interval of several months.

A mild degree of fever, occasionally accompanied by malaise, was noted in a small number of cases. It subsided in 48–72 hours. The systemic disturbance occurred only in highly allergic individuals who gave strongly positive local reactions, and in cases where for experimental purposes tests were made with two or more dilutions at the same time. Moreover, systemic reactions were roughly proportional to the intensity of the local responses. These facts suggest that with attention to dosage, it is possible to produce a positive local response without a systemic reaction in every allergic individual.

To avert local and general ill-effects it is necessary, first, to make the initial dose of tuberculin sufficiently small, having in regard the type of individual (see above) that is most likely to give an intense response; and secondly, when re-testing negative reactors with graded higher strengths of tuberculin, to make the difference of strength between the solutions used for two successive tests sufficiently small. (For method see (*j*) and Appendix 5.)

(*j*) *Suggested routine procedure.*

A method based on the data given in this section, and suggested as safe and reliable for the negative diagnosis of suspect cases of clinical

tuberculosis by means of the intracutaneous test, is described in Appendix 5. A negative intracutaneous reaction, when 0·1 c.c. of a 1 : 1,000 dilution (the standard dilution) of a brand of tuberculin of potency approximately equal to that of the International Standard Tuberculin (p. 42) has been administered, must weigh heavily against a diagnosis of clinical tuberculosis, the average error in the present series being less than 4 per cent., which figure was increased to 6 per cent. in children aged 0–5. If no reaction is obtained with 0·1 c.c of 1 : 10 tuberculin (= 10 mg.), clinical tuberculosis can be excluded with an average error of a little over 2 per cent., judging from the present results, the error being only raised to 4 per cent. in children aged 0–5, and to 3·5 per cent. in advanced cases with marked toxaemia.

4. Discussion.

The small use that is made of the tuberculin test in many quarters appears to be due, in part, to a misconception of its value in the diagnosis of clinical tuberculosis. It is still commonly believed that the diagnostic applications of the test are confined to early life. As stated in the preceding section, this belief is, in the main, correct with regard to the positive diagnosis of clinical tuberculosis. On the other hand, a negative reaction, judged from the results of the investigation described in the present section, excludes this disease with almost equal accuracy at all ages (the error being 2 per cent. to 4 per cent.), provided that the intracutaneous method and suitable doses are employed. It is, therefore, profitable to perform the intracutaneous test for the purpose of negative diagnosis in any case where the patient has a reasonable chance of giving a negative reaction should his condition *not* be due to tuberculosis. This chance is determined entirely by the incidence of negative tuberculin reactions among the clinically *non-tuberculous* population. A knowledge of this incidence becomes essential in consequence. Where it is low, the chance of obtaining a negative reaction in a patient, suspected of having tuberculosis, but whose clinical condition is in reality non-tuberculous, is extremely poor; where, on the other hand, the incidence of non-reactors among clinically non-tuberculous individuals is high, the utility of the test in negative diagnosis is correspondingly great.

A résumé of mass investigations of tuberculin reactions among the clinically non-tuberculous members of various classes of the community from 1907 to 1929 will be found in a subsequent section (VII). Early inquiries revealed that in some of the large cities on the Continent half the children of the poorer classes were tuberculin-positive by 5 years of age, and about three-quarters by 10 years, while nearly all gave positive reactions by the time the age of puberty was reached. Under these conditions it was clearly waste of time to use the tuberculin test in the hope of being able to exclude tuberculosis from the diagnosis in suspect cases, if the latter were over the age of 10.

These figures naturally received great prominence, and are widely accepted, even at the present time, as capable of universal application among the urban poorer classes or general population. Although

certain recent investigations with the intracutaneous test have shown a large percentage of positives among children (e.g. a survey in Philadelphia), inquiries made with this method in London and some other European and American cities have shown a distinctly lower incidence than was reported twenty years ago in Vienna, for example. Thus, only 20 per cent. of the present London controls, drawn from the hospital class, reacted positively by 5 years, 45 per cent. by 10 years, and 70 per cent. by 16 years. The range of life through which the tuberculin test can be profitably employed for negative diagnosis seems, therefore, greater than is commonly believed, and it is worth applying for this purpose throughout childhood, at least in London hospitals. Almost all the adults in the London control group, however, were found to be positive (95 per cent.), and similar figures were found recently in Cardiff, so that little advantage would seem to be derived at the present time from using the tuberculin test in adults of the urban hospital class in order to exclude clinical tuberculosis.

The incidence of positive reactions reported among the wealthier classes and in country districts appears to be lower than among the urban poorer classes. This comparative infrequency has been reported even in adults. Thus, Krause (1928) states that in private practice many non-reactors are met with, and disagrees with the widely held opinion that almost all adults are positive. Although the majority of observers have used von Pirquet's method, their results, taken together, suggest that the tuberculin test can be used in country and private practice for the purpose of excluding clinical tuberculosis with a greater chance of success than in hospital clinics, and that under these conditions its application may be even extended to adults.

The usefulness of the tuberculin test for negative diagnosis depends, therefore, not only on the patient's age, but also on his social status, and on whether he has been brought up in town or country.

Not only do the recent tuberculin surveys of clinically non-tuberculous children of the urban poorer classes or general population show, in the main, lower figures than might have been expected from the results of some of the prominent earliest continental investigations, but there is also some direct evidence of an actual decline in the incidence of positive reactions among the healthy children of a few cities (Section VII). Should this decline become general, as a result of a diminished incidence of open tuberculosis (reflected by a fall in the phthisis death-rate), an improvement in the standard of living, and anti-tuberculosis measures such as the pasteurization of milk, the usefulness of the tuberculin test for negative diagnosis will increase correspondingly.

5. Summary.

1. The intracutaneous is the most accurate skin tuberculin test for diagnostic purposes, and should take the place of the method of von Pirquet.

2. The principal application of tuberculin tests in clinical practice is to the negative diagnosis, i.e. exclusion, of clinical tuberculosis.

3. While, except in the first few years of life, a positive skin tuberculin reaction is of very limited value in the positive diagnosis of clinical tuberculosis, a negative intracutaneous reaction excludes this disease with a high degree of accuracy, which, judged from the results of the present investigation, is almost the same at every age.

4. The average minimal error of the intracutaneous tuberculin test in negative diagnosis is obtained by using undiluted tuberculin. The error was very small—2 per cent.—in the present investigation.

5. A negative intracutaneous tuberculin reaction to a 1 : 10,000 dilution of tuberculin should not be considered to exclude a diagnosis of clinical tuberculosis, on account of the error—12 per cent. in the present series. This figure is about the same as the average published error of the von Pirquet test.

6. A negative intracutaneous reaction to the standard (1 : 1,000) dilution of tuberculin must weigh heavily against a diagnosis of clinical tuberculosis, the average error being 4 per cent. in the series investigated. If, however, the slightest doubt remains as to the diagnosis, further tests with a 1 : 100 dilution, and, if still negative, with 1 : 10 tuberculin should be made. A negative intracutaneous reaction to a 1 : 10 dilution of tuberculin excludes clinical tuberculosis with an error that, judged from the present series, is very close to the minimum error (2 per cent.), obtained by using undiluted tuberculin. On this account, and for technical reasons, a 1 : 10 dilution of tuberculin is the practical upper limit. Glycerinated broth control solutions should be used with this dilution in order to prevent confusion with delayed atypical non-specific reactions.

7. The belief, based on results obtained with the relatively insensitive von Pirquet test, that patients with advanced pulmonary tuberculosis and marked toxaemia are particularly liable to give negative responses to tuberculin, is supported by the results obtained in the present series with the standard dilution (1 : 1,000) of tuberculin, injected intracutaneously. When, however, stronger solutions of tuberculin were used, all but a very small portion ($3\frac{1}{2}$ per cent.) of these cases were found to give positive reactions, showing that cutaneous sensitiveness to tuberculin is depressed rather than lost in this condition. The non-reactors to the low dilutions were almost entirely patients tested within a week of death. If these are excluded, all but 1 per cent. of the series of advanced phthisis with severe toxaemia reacted to tuberculin administered by the intracutaneous route.

8. A large proportion (roughly a third) of the present small series of patients with pulmonary tuberculosis and severe toxaemia lost their cutaneous sensitiveness to tuberculin, even administered in maximal intracutaneous doses, a few days before death. This is in contrast to patients with miliary tuberculosis and meningitis, most of whom appear to maintain their sensitiveness to strong solutions of tuberculin, given intracutaneously, up to death.

9. Quiescent tuberculosis appears, from the series of bone and joint cases tested, to be associated with slightly greater skin sensitivity than active disease.

10. The usefulness of the intracutaneous test for the negative diagnosis of clinical tuberculosis in a given case depends not only upon the accuracy with which a negative reaction excludes this disease (98 per cent. in the present series), but also upon the chance of the patient giving a negative reaction should his condition, suspected of being tuberculous in origin, be in reality due to some other cause. This chance is determined by the percentage of negative reactors to tuberculin among clinically non-tuberculous individuals of the same age and social status as the patient, and living in a similar environment.

11. At the present time the test can be profitably applied for the purpose of negative diagnosis throughout childhood in patients of the hospital class living in London and other large cities, and possibly even in adults in country and private practice.

12. The usefulness of the test for negative diagnosis will increase in the future *pari passu* with any rise in the incidence of negative tuberculin reactions among the apparently healthy members of the population. This conclusion follows from (10).

PART II

EPIDEMIOLOGICAL APPLICATIONS

Section VI. The Value of Tuberculin Tests in the Contact Clinic. The Role of Human Contagion and Heredity in the Production of Tuberculous Infection in Childhood.

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1. INTRODUCTION.

The relative importance of heredity and contagion in the production of non-bovine tuberculosis, both in children and adults, has long been a subject of discussion. While many workers have investigated the importance of these factors in relation to the morbidity and mortality of clinical tuberculosis, using statistical evidence, their influence in producing tuberculous infection, as opposed to manifest disease (p. 6), has been less frequently studied.

There are, however, indications in the earlier literature that the extensive tuberculization of the apparently healthy children of some of the densely populated continental cities of twenty years ago, revealed by mass investigations with tuberculin tests, was largely attributable to contact with open tuberculosis in the home and its vicinity.

2. EARLIER INVESTIGATIONS.

As early as 1909, Mantoux & Lemaire, reporting 16 per cent. of reactors to tuberculin in a series of apparently healthy Paris children aged 1–2, 51 per cent. among those 2–4 years old, and 66 per cent. in the age-group 4–7, described their clinical material as 'sortant d'un milieu essentiellement misérable et fortement tuberculisé'. These authors contrasted their figures with the lower percentages obtained in a better quarter of Paris. Similar findings were recorded in Lille by Calmette, Grysez & Letulle (1911), who pointed out the extreme frequency of reactors among children in towns where familial contagion was most intense. Lille was an example of such a town, and the figures given by these workers for its general population were only slightly lower than those reported by Cohn (1910) about the same time, in another European city (Posen), for children who were drawn exclusively from tuberculous homes. Pollak (1911) found positive von Pirquet reactions in 96 per cent. of a series of infants under two years of age, living in tuberculous surroundings in Vienna, but his material largely consisted of sick infants brought up for treatment.

The usual method of studying the effect of contagion in the home in producing tuberculous infection in childhood has been to compare the incidence of positive tuberculin reactions among home-contacts of tuberculosis either with the incidence among children living in non-tuberculous households, or with that among children with no history of exposure (see definitions, p. 7).

Observations in rural communities have shown striking differences between the reactions of these two groups.

Jakob (1911), working in a rural district of Germany, found that practically all the children living in houses where there were cases of open tuberculosis reacted to tuberculin, while in the other homes a much smaller percentage of reactors was discovered. Overland (1913 a) found that the majority of the reactors in the Norwegian villages that he investigated came from tuberculous homes. Slater (1924) studied the tuberculin reactions of a wealthy farming community in Minnesota. The proportion of children giving positive von Pirquet reactions was extremely small, being only 10 per cent. for all cases under 16. When, however, his subjects were divided into those who had lived in a house with an open case of tuberculosis (61 children) and those who did not give a history of exposure (529), a very marked difference in the percentage of reactors was revealed, 80 per cent. of the former, as against 5 per cent. of the latter, being positive.

The most reasonable explanation for this marked disparity is that, owing to wide separation of houses and relative infrequency of open tuberculosis, the chance of contact for children whose homes are free of the disease, or who give no history of exposure, is reduced to a minimum.

The first comparison of the tuberculin reactions of children from tuberculous and non-tuberculous households in a large city appears to have been made in New York.

Fishberg (1914, 1915) examined a series of destitute Jewish children under 15 years of age, living in a congested tenement area of New York. These children were divided into two groups: (1) those whose parents were clinically tuberculous, and (2) those whose homes were free of clinical tuberculosis. The results of tuberculin tests were as follows:

	Group 1.	*Group* 2.
Aged 1–3, positive reactions in	55 per cent.	33 per cent.
,, 3–5 ,, ,,	69 ,,	41 ,,
,, 5–7 ,, ,,	65 ,,	50 ,,
,, 9–11 ,, ,,	71 ,,	69 ,,
,, 13–15 ,, ,,	76 ,,	76 ,,
All ages ,, ,,	67 ,,	53 ,,

Fishberg, apparently believing that the slight disparity was due to a small difference in technique, was led to conclude that 'the difference between those who lived in a tuberculous milieu and those who had no contact with consumptives in their homes, was apparently insignificant'.

A definite difference between the tuberculin reactions of children of tuberculous and non-tuberculous households has, however, been recorded in the majority of other urban inquiries. It is possible that Fishberg's results were due to the extremely congested surroundings in which the children whom he tested were living, for overcrowding would be expected to increase the risk of exposure even for those not actually living in contact with the disease.

Manning & Knott (1915) tested 228 children under 16 in Seattle, a comparatively modern town where housing conditions were much better than in the congested poorer quarters of New York. One hundred and sixty-six of these children were living in intimate contact with active adult cases of tuberculosis; the remaining 62 children had no history of exposure. The percentage of reactors in the first group was more than twice that found in the second group (51 per cent. as against 23 per cent.).

Delthloff (1916), in Bergen, compared the tuberculin reactions of a series of home-contacts of pulmonary tuberculosis with those of non-contacts of the disease (as defined on p. 7). Her cases were drawn from children of the working-class. A much greater percentage of positives was recorded in the first group.

3. Recent Investigations.

Bernard & Debré (1920) made some important observations in Paris with the von Pirquet reaction on a series of infants aged 0–2, who had been separated from their tuberculous mothers and placed in a crèche. Of 58 infants whose mothers had bacilli in the sputum, 40 (69 per cent.) were found to have been infected, as shown by a positive tuberculin reaction, while of 65 infants where bacilli had not been discovered in the mothers' sputum, or where the parents were not tuberculous, only 8 reacted (12 per cent.). All the infants who had been in contact with open tuberculosis for more than six months before separation were stated to have been infected, all but one of the uninfected group having been removed 1 week to 3 months after birth. On the other hand, many of the infected infants had been in contact for only a few months. The infected group was followed up in order to discover if there was any association between the duration of contact and the ultimate prognosis. Forty per cent. of the infants who had been infected died of tuberculosis, usually in the month following separation, and even though apparently healthy at that time. The other 60 per cent. were well when examined 2 to 24 months after separation. The duration of contact in both the survivors and the fatal cases of this group was from 2 to 3 months. The authors draw the conclusion that a certain time is required for contact to produce infection, but that infection almost invariably results if the duration is prolonged to about 6 months.[1] But their further conclusion, that separation of an infant from its tuberculous mother, even if it has already been infected, may save its life, is not warranted by the evidence given in this paper, for 40 per cent. of the infected group died, and there was nothing to show whether their fate would have been better or worse had they been left with their parents. This work does, however, suggest that the temporary removal of a contact infant from its home before it has become infected is the best way of preventing infection during the period of life when the latter is most likely to prove fatal.

In a later paper Bernard, Debré & Lelong (1925) report the results achieved by 'L'Œuvre du Placement Familial des Touts-Petits', founded in Paris in 1920. (A more recent account of the successful results of this system is given by Bernard (1927).) They compare the fate during the first four years of life of (*a*) 265 infants with negative tuberculin reactions, separated from their tuberculous parents either at birth or later, and placed in healthy homes. Infants who developed positive reactions so soon after separation that the previous family contact seemed the probable cause, i.e. who were really infected before separation, are excluded from this group. They are presumably included in Group (*b*). (*b*) 171 infants separated when already infected, but 'rigorously selected' according to the circumstances of their exposure; and (*c*) 66 infected infants who remained with their tuberculous parents. The tuberculosis death-rate of the first group was nil, and none of them developed clinical tuberculosis while under observation, nor did they show any unusual susceptibility to chance tuberculous infection. Seventeen of the 171 infants in the second group developed clinical tuberculosis, which was fatal in 13 (7·5 per cent.), meningitis and pulmonary disease accounting for most of the deaths. Eighty-two per cent. of the third group, the infected infants who were left with their tuber-

[1] In another paper (in English) Bernard (1927) states that 'it is exceptional that a contact which has lasted less than a month should succeed in bringing about infection'.

culous parents, are stated to have died of pulmonary or meningeal tuberculosis, the majority in the first year of life. The temporary separation of infants of the poorer classes from tuberculous parents before infection has occurred appears, therefore, to be of value. The advantage of separating infants already infected seems less certain, as the 'rigorous selection' of the 171 infected children for separation makes them hardly comparable with the 66 infected infants who remained with their tuberculous parents. Nevertheless, the contention of these writers that this is the proper course to take is supported by their statement that of 13 infected infants who had passed the 'rigorous test', but whose removal was refused by their parents, 8 perished. Asserson (1927), in New York, also reports successful results from removing infected infants from the source of infection.

It is interesting to compare the figures of Bernard and his co-workers with those of Walquist & Myers (1926). The latter authors followed up 71 tuberculin-positive infants under 2 years of age (21 under 1), who were attending the out-patient clinic of the Lymanhurst School for Tuberculous Children, Minneapolis. The majority of these infants had been brought up not because of symptoms, but mainly because of exposure to the disease. Ninety-six per cent. of the infants gave a history of exposure: 73 per cent. to tuberculous parents, 11 per cent. to tuberculous siblings, and 11 per cent. to several sources of contagion. A tuberculosis mortality-rate of only 8 per cent. was recorded, which was no higher than that found by Bernard and his associates among infants who were separated from their tuberculous parents after being infected. These results suggest that under favourable conditions the outlook for infected infants remaining in contact with tuberculosis is far from hopeless.

Schram (1922) investigated the von Pirquet reactions of 500 children aged 0–8 from tuberculous homes in a poor quarter of Oslo. He obtained positive reactions in as many as 53 per cent. of 113 infants under 1 year of age, and found that by 8 years four-fifths had been infected. The incidence was higher among those living in contact with sputum-positive patients than among those living in homes where a tuberculous patient had negative or no sputum; and the incidence was higher among contacts of tuberculous parents or siblings than when the source of contagion was a more distant relative or a lodger. Twenty-three per cent. of the positive reactors showed clinical signs of the disease. The value of Schram's work is unfortunately seriously affected by the lack of normal controls. Similar criticism can be made of the observations of Barchetti (1921), who reported 73 per cent. of tuberculin-positive reactors in a series of 51 infants of tuberculous mothers in Vienna.

Roepke (1923), working in Mannheim, found that the percentage of children that reacted to tuberculin in tuberculous households was greater if the source of contagion was an advanced case than if in an earlier stage of the disease. Similarly, Lampson (1923) concluded that the spread of tuberculous infection in families was greater if the infective agent had open tuberculosis than if the disease was latent or healed. The hygienic habits of the infective patient and the conditions of living also seem to be of importance (Benninghof, 1925; Dow & Lloyd, 1931).

Falk (1924) examined 68 children under 13 whose parents had open tuberculosis: 91 per cent. gave reactions with tuberculin (6 out of 10 infants less than a year old), the percentage being 100 in the case of 20 children of fatal cases. Girls seemed more likely than boys to receive infection from tuberculous mothers.

Austrian (1924) reported the results of an investigation carried out from 1915 to 1920 at a tuberculosis dispensary in Baltimore. He found that actually fewer children aged 0–9 years who had been exposed to a known case of clinical tuberculosis reacted than those with no known exposure. The figures were 67 per cent. (241 cases) and 74 per cent. (65 cases), respectively. Drolet (1924) published the tuberculin reactions of 1,234 children with a positive parental history of tuberculosis, and of 461 with a negative parental history. His cases were drawn from a tuberculosis dispensary in the East-side district of New York over the period 1912 to 1916. In the first group 48 per cent. of those under 5 years reacted, 69 per cent. of the children aged 5–9, and 78 per cent. in the age-group 10–14. The corresponding percentages for the children of non-tuberculous parents were very similar, being 47, 64, and 74. The findings of Austrian and Drolet might be

construed as evidence against the importance of family contagion in determining infection. But their controls are open to the serious objection that they were apparently all children attending a tuberculosis dispensary, to which they must have been sent because of symptoms suggesting tuberculosis to their parents, since none of them were home-contacts; whereas many, if not most, of the home-contact series had been brought up because of exposure alone. For this reason the non-contact controls would be expected to have been infected to a greater extent than the general non-contact population. The statistics of Sill (1918) appear to support the validity of this objection, for they were obtained, only a few years after Drolet's investigation, among children from non-tuberculous families of the hospital class living in the same district of New York. A much lower percentage of positives was recorded, the figures being 10 per cent. for the age-group 4–5, 16 per cent. for children aged 8–9, and 48 per cent. for those aged 10–13 years.

Myers & Magiera (1925), working in Minneapolis, applied the von Pirquet test to 761 contacts aged 0–19 years, the majority of whom had been brought up on account of exposure to infection, or for symptoms that had suggested tuberculosis to their parents. All but 12 were under 16 years of age. The results were compared with those of tests made on 784 cases (all but 25 less than 16 years old) with no known exposure to tuberculosis. Reactions were obtained in 57 per cent. of the contact group, and in 29 per cent. of the control group.

Parisot & Saleur (1925) compared children living in contact with progressive open tuberculosis with children from non-tuberculous households. Ninety to ninety-five per cent. of the home-contacts were positive reactors by 5 years, whereas only 9 per cent. of the non-contacts aged 2–5 were positive.

Klostermann (1926) found the incidence of infection among infants of tuberculous mothers to be higher than when the fathers were diseased. The reverse was noted by Dow & Lloyd (1931), who studied older children.

Opie & McPhedran (1926), in Philadelphia, made a careful study of the spread of infection among children exposed to a tuberculous parent or sibling, using the intracutaneous (Mantoux) test as an index of infection. For controls they used children of families in which there had been no known case of tuberculosis. The percentage of positive reactors among 272 children who were home-contacts of open tuberculosis (bacilli in sputum) rose from 80 per cent. in the age-group 0–5 to 100 per cent. at 20, while in 179 non-contact controls the percentage rose from 23 per cent. among those aged 0–5 to 100 per cent. at 20. Of 124 children, one or both of whose parents suffered from open tuberculosis, 11 were found to have manifest disease themselves. These workers noted that positive reactions were more frequent among children in contact with a tuberculous parent (86 per cent.) than when in contact with a brother or sister (65 per cent.). Children whose parents, brothers, or sisters had tuberculosis without bacilli detected in the sputum showed a frequency of positive tuberculin reactions no greater than that found among the non-contact controls (44 per cent.). Opie & McPhedran ascribed these differences to varying duration and intimacy of contact. In a similar manner they observed the frequency of latent pulmonary foci in both groups of children by radiographical examination, and found that, as with tuberculin reactions, the proportion rose with age, but was higher in the home-contacts than in the controls. The same differences were noted as in the reactions of children in contact with open and closed tuberculosis, except that the incidence of latent lesions among the latter group was greater than among the non-contacts.

The results of Opie & McPhedran are shown graphically on Chart 3. It will be seen that (*a*) the curve for home-contacts runs above that for non-contacts; (*b*) tuberculization occurs earlier in childhood among the former than among the latter; (*c*) the shapes of the two curves differ. The home-contact curve rises rapidly at first, and then assumes a more gradual slope; the control curve, on the other hand, is approximately rectilinear. This difference in shape may have some epidemiological interest. It suggests that the tuberculization of home-contacts has its maximum rate in infancy and early childhood, whereas the tuberculization of non-contacts occurs at approximately the same rate from birth to manhood. Such a dissimilarity is in keeping with a difference in the mode of exposure to

contagion. Children in tuberculous households are subjected to intense contagion from birth, but children whose homes are free of open tuberculosis make their contacts with the disease mainly outside the home, and with increasing frequency as they grow up.

Chadwick & Zacks (1927) found the percentage of reactions among the home-contact school-children whom they studied to be approximately twice that among the non-contact group. Dow & Lloyd (1931) noted a similar ratio in their series.

Myers & Kernkamp (1930) found that over half of a series of 428 infants under 2 with a history of exposure reacted positively; only 11 of these were clinically tuberculous. Myers (1930 a) states that the incidence of infection in infancy varies from 1 to 2 per cent. where there is no known exposure, to 100 per cent. where exposure has been definite and prolonged.

From this résumé of published work it will be apparent that care must be taken in selecting clinical material for an investigation of the influence of contagion in the home in producing tuberculous infection. Suitable controls are essential. Not infrequently they have been omitted altogether. In some of the published series the non-contact controls were composed of children brought up on suspicion to a tuberculosis dispensary, while a large proportion of the contact cases had attended only because of exposure. This is an unfair comparison. Moreover, it would seem desirable to use as controls children from non-tuberculous households rather than children with no history of exposure, on account of the memory factor involved in the latter case.

Of the published investigations that of Opie & McPhedran has attracted most attention, and in order to confirm some of their results an inquiry was carried out in London on somewhat similar lines.

4. The London Investigation, 1929.[1]

(a) *Clinical Material and Technique.*

The present investigation comprises a study of 118 London children of the hospital class, who came from tuberculous households. They were aged 0–15 years, 94 being less than 11 years old. The majority were brought up to hospital on account of exposure to the disease, or for some non-tuberculous complaint. All suspect or definite cases of clinical tuberculosis were excluded. This series was compared with 513 clinically non-tuberculous children of the same class and district, but in whose homes there were stated to have been no persons suffering from clinical tuberculosis. These non-contact controls were also aged 0–15 years (438 being under 11 years of age), and were tested over the same period (twelve months), in 1929, and by the same observer. The sources of both groups of children are given on p. 93. The non-contact series was obtained by removing all home-contacts from the series of clinically non-tuberculous subjects described in the preceding section (p. 52).

The intracutaneous test was used, and the reaction of each child to 0·1 c.c. of a 1 : 1,000 dilution (0·1 mg.) of Old Tuberculin (Wellcome Physiological Research Laboratories) was ascertained. The tuberculin,

[1] An account of this investigation is given in a paper by Schlesinger & Hart (1930). The writer is indebted to the Editors of the *Archives of Diseases in Childhood* and to Dr. Bernard Schlesinger for permission to reproduce part of this paper.

of which the same batch (T. 1586) was used throughout, was prepared and standardized as described on p. 46.

Of the 118 children under 16 years, 87 (68 under 11) were home-contacts of relatives who had pulmonary tuberculosis: in 50 of these cases (42 under 11) tubercle bacilli were known to have been present in the relatives' sputum; in the remaining 37 cases (26 under 11) the sputum result was negative or not known. Thirty-one children under 16 (26 under 11) were home-contacts of relatives who had non-pulmonary tuberculosis. The relatives with pulmonary tuberculosis were parents or siblings in all but four cases, where a grandparent (one case), uncle (one case), or aunt (two cases) were responsible. The relatives with non-pulmonary tuberculosis were parents or siblings, the latter predominating.

(b) *General Results.*

The reactions of the pulmonary contacts in the different age-groups are compared with those of the non-contact controls of the same age in Table 13, and on Chart 3. It will be seen that the percentage of positive reactors increases from infancy to adolescence in both the home-contact and the non-contact series, but that the curve of the home-contacts runs above that of the non-contacts up to the age of 16. By 6 years of age half, and by 10 years three-quarters, of the home-contact series had been infected, whereas only one-fifth and one-third of the non-contact controls were positive reactors at these respective ages. These figures may be compared with those of Opie & McPhedran, shown on the same chart: by 5 years three-quarters of their home-contacts gave positive reactions, as compared with one-quarter of their non-contacts. Thus, most of the tuberculization of home-contacts appears to take place in infancy and earlier childhood, i.e. in the home environment; whereas at the present day most of the infection of non-contacts appears to be received in later childhood and in adolescence, i.e. during a period when the principal activities of the individual are away from the home. Both groups, however, are tuberculized to the same extent when adult life is reached.

On Chart 4 the London home-contact and non-contact curves are again shown, together with the plotted figures for the clinically non-tuberculous series used in the preceding section (p. 52); this series was believed to be a fair sample of the hospital class population of London. It will be seen that the home-contact curve is higher than the curves for the general population (hospital class), but that the latter run above the non-contact curve, doubtless because the general population naturally contains some home-contacts.

(c) *Results Analysed.*

Of the 68 children aged 0–10 years, in the London series, who were home-contacts of pulmonary tuberculosis, 59 per cent. reacted positively, as compared with 18 per cent. of 438 non-contacts of the same age (see also Table 14). These differences are more marked if the pulmonary home-contacts are divided into home-contacts of

open pulmonary tuberculosis (bacilli in sputum), and home-contacts of pulmonary tuberculosis with negative or unknown sputum result. The percentage of positive reactors among children aged 0–10 in each of these groups is compared with the percentage of positives among non-contact controls of the same age in Table 14. It will be seen that positive reactions were obtained in 18 per cent. of the non-contact controls aged 0–10, in 42 per cent. of home-contacts of pulmonary tuberculosis with negative or unknown sputum result, and in as many as 69 per cent. of home-contacts of individuals whose sputum was known to have contained bacilli. These findings, which point to the greater infectivity of open, as compared with closed, pulmonary tuberculosis, are in agreement with the observations of other workers already quoted.

Thirty-four (four-fifths) of the home-contacts of open pulmonary tuberculosis, aged 0–10, had slept in the same room as the infective relative, the remainder having slept by themselves or with a non-tuberculous member of the family. The percentage of positive reactions among these bedroom-contacts was 71 per cent. (Table 14).

Comparison of the percentage of children infected after living with a parent, with that obtained when the tuberculous member of the household was a sibling, is not given, as the great preponderance of the parental contacts makes the figures of little value.

In 26 children, aged 0–10, who had lived with relatives (parents or siblings, the latter predominating) who had non-pulmonary tuberculosis, the percentage positive (15 per cent.) was no higher than in the non-contact group (438 cases, 18 per cent.) (see Table 14). These figures are in marked contrast to those of the contacts of open pulmonary tuberculosis. Though the numbers tested were small, the results suggest that human cases of non-pulmonary tuberculosis are an unimportant source of infection.

In an attempt to throw further light on the factors determining infectivity the non-contact controls were divided into those who had no known tuberculous relatives, one tuberculous relative, and two or more tuberculous relatives. Each parent was carefully questioned on these points. The relatives include any of the following: sibling, grandparent, uncle, aunt, great-uncle, great-aunt, great-grandparent, or first cousin—whether living or dead. It was not found practicable to classify according to the proximity of relationship. The comparisons are in any case crude, on account of the difficulty of eliciting family histories from hospital patients. The results for the children aged 0–10 are given in Table 15. It will be seen that 22 per cent. of those with one tuberculous relative reacted positively, as compared with 15 per cent. of those who had no known tuberculous relatives, while 46 per cent. of the children who had two or more tuberculous relatives gave reactions. While none of these relatives had lived in the same house as the children tested (i.e. they were all non-contacts in the sense used throughout this report), many of them had pulmonary tuberculosis, and occasionally or frequently met the children concerned. These children, therefore, would seem to have had a greater

chance of becoming infected than children who had no tuberculous relatives, and who in consequence seldom met tuberculous adults. It will be noted that a smaller proportion of children with tuberculous relatives not living in the same house gave positive reactions than of children who had relatives with open pulmonary tuberculosis actually living with them (46 and 22 per cent., as against 69 per cent.). On the other hand, positive reactions were found no more frequently among home-contacts of non-pulmonary tuberculosis than among children who had no tuberculous relatives at all, the percentage being 15 in both cases.

The incidence of positive tuberculin reactions among pulmonary home-contacts is lower than has been found by many workers quoted (e.g. Pollak, Schram, Falk). This may be partly accounted for by the exclusion, in the present investigation, of all suspect cases of clinical tuberculosis. It is possible also that the hygienic conditions in the homes of the children tested were better than they were in some investigations; this difference would tend to reduce the number infected.

The number of home-contact cases in each age-group of the present series is small. This is partly due to the exclusion of clinically tuberculous and suspect cases. The statistical error, however, is reduced by the adequate number of non-contact controls.

5. Discussion.

When discussing the relative significance of heredity and contagion in determining tuberculous infection it is essential to distinguish between the latter and tuberculous disease, i.e. clinical or manifest tuberculosis, as defined on p. 6. It has been shown above that children with a strong family history of tuberculosis are more likely than other children to acquire tuberculous infection. It might be suggested that this is largely due to an inherited predisposition, although contagion must finally determine the occurrence of the infection. According to such a view, if two sets of children, one with a strong tuberculous ancestry, the other free of this taint, are exposed to the same infective agent, the former will be more likely than the latter to become infected. There are several reasons for rejecting this hypothesis.

(1) Children from tuberculous households, as shown in this section, give a much larger percentage of positive reactions to tuberculin than those whose homes are free of clinical tuberculosis, the percentage being greater when the tuberculosis is active or open, than when it is closed or healed. The more severe the condition, the more likely is infection to take place. Children with tuberculous parents or siblings living in their homes are more frequently found to react positively than those whose tuberculous relatives visit them occasionally; the latter more frequently than those who have no tuberculous relatives at all. All these findings are satisfactorily explained by variations in frequency, intimacy, and duration of contact, without having recourse to a belief in hereditary predisposition to infection.

(2) The percentage of tuberculin reactions among children exposed

in tuberculous households to parents, brothers, or sisters with non-pulmonary tuberculosis appears to be no higher than among children of non-tuberculous households; nor does it even exceed the frequency of tuberculin reactions found among those who have no tuberculous relatives at all. An inherited familial tendency to tuberculous infection should be evident to some degree in children of tuberculous parents, whatever the type of tuberculosis. But the London figures, though the number of cases is small, suggest that a positive family history of tuberculosis is of importance in determining infection only in so far as it yields a potential intimate source of contagion in the form of an open pulmonary case. Further work on a larger scale should decide this point.

(3) Infants intimately exposed to a tuberculous individual who is not a member of the same family rapidly become infected. Schloss (1917 b) found that all but two of the infants in a ward where there was a nurse who had advanced tuberculosis gave positive tuberculin reactions. In another ward where this nurse had been only temporarily on duty one-third of the infants reacted positively. Opie (1927 b) quotes Holt, who cites the case of ten infants infected within fourteen months by a tuberculous midwife who used the mouth-to-mouth method of artificial respiration in the new-born.

(4) Infants separated from their tuberculous parents before infection, as shown by a positive tuberculin reaction, has occurred, and placed in healthy families, appear to be no more liable to acquire infection in early life than are infants of non-tuberculous parents.

The simplest view of the spread of infection in children of tuberculous families would appear to be as follows. Children with a strong family history of tuberculosis are more likely than other children to become infected, only because of the increased chance of exposure to an open case of pulmonary disease. This risk is naturally greatest if the patient lives in the children's home, and least when he only visits them occasionally. If the tuberculous relative has never seen the children, or is suffering from healed or non-pulmonary tuberculosis, they are probably no more likely than other children to become infected. It is also likely that if two sets of children, one with a strong tuberculous ancestry, the other free of this taint, are exposed to the same infective agent, both are equally liable to acquire infection. *Such a view is not inconsistent with a conception of heredity as a determinant of the subsequent course of this infection, once it has taken place.* The consideration of this matter, however, is beyond the scope of this section.

6. Practical Considerations.

The data presented in this section, and the subsequent discussion, have a practical bearing on the prevention of tuberculosis of human origin, for they justify or suggest definite lines of action, some of which are already in force in this and other countries.[1] It is clear that in

[1] A report, prepared for the League of Nations by Labesse (1926), contains a survey of the campaign against tuberculosis in early life in various European states.

allowing infants of the poorer classes to remain with tuberculous parents a great risk is incurred. The recent important study of the Lancashire Group of Tuberculosis Officers (Cox *et al.*, 1929, 1930) emphasizes this point, and supports the view that in early life infection from a tuberculous relative is more likely to result in active tuberculous disease than to give protection against it. These workers found that the tuberculosis death-rate in a group of young children living in tuberculous households in that county greatly exceeded that of the general population of Lancashire, which formed a control.

The death-rate[1] from non-pulmonary tuberculosis (meningitis in two-thirds of the cases) among 1,023 young children living with an adult who suffered from pulmonary tuberculosis with positive sputum was stated to be nine times greater than that of the control population in the age-group 0–1, fourteen times greater in the age-group 1–2, and nineteen times greater in the age-group 2–5. The actual tuberculosis death-rate (all forms) among these children was 1·7 per cent. in the age-group 0–1, 2·6 per cent. in the age-group 1–2, and 1·8 per cent. in the age-group 2–5[2]; the figures were 3·2, 1·6, and 3·0 per cent., respectively, where the infective (sputum-positive) adult was the mother. The substantial majority of these infants remained in contact with the tuberculous adults in their homes, except during the several months' institutional treatment undergone by some of the latter.

It should be mentioned here that the Lancashire figures, and others quoted in the same report, are much lower than those of Calmette (1928), who states that in France more than 24 per cent. of children born of tuberculous mothers die from the disease in the first year of life if left with their parents. Calmette's statistics have been criticized by Greenwood (1928). Even so, a difference in the home conditions and in medical supervision may account in part for this discrepancy.

The fate of children in tuberculous families noted by the Lancashire Tuberculosis Officers should be contrasted with that reported for the Papworth Settlement by Varrier-Jones (1927). He states that there has been 'a complete absence, during 11 years, of clinical tuberculosis in the 133 children of men and women who are definite clinical cases of the disease, and in most instances show tubercle bacilli in the sputum from time to time'. Whether the superior environmental and hygienic conditions of Papworth produce this result by diminishing the risk of massive and repeated infection, by influencing the course of the infection, or by preventing it altogether, it is impossible to decide without knowledge of the tuberculin reactions of the children in the Settlement. The researches of Bernard and his co-workers, already mentioned (p. 61), suggest that each of these factors may play a part.[3]

[1] The death-rate in a particular age-group in the Lancashire report is apparently the percentage of children who died at any time during the period in which they belonged to that age-group.

[2] In a similar investigation carried out more recently in Worcestershire by Turner (1931) the corresponding rates are given as 5·3, 3·9, and 3·6 per cent., respectively. The author compares these figures with those for a control population, and concludes that home-contacts of sputum-positive cases of phthisis have a tuberculosis death-rate significantly higher than the corresponding rate for the general population. See also Dow and Lloyd (1930).

[3] In the Annual Report of Papworth Settlement for 1926, p. 16, Varrier-Jones states that only 2 per cent. of the children have at one time or another given a positive Moro reaction. Confirmation of this observation with the intracutaneous test is desirable.

Judging from Varrier-Jones's statement, the Papworth system would appear to prevent the spread of tuberculosis in families, medical supervision and ideal living conditions compensating for the presence of a tuberculous parent. As an alternative, while trial of Calmette's method of prophylactic immunization (Calmette, 1928, 1931; Chaussinand, 1931) is being made, probably the most effective preventive measure would be to separate infant from parent before infection has taken place. The present short period of separation during the institutional treatment of an adult is shown from the Lancashire study to be inadequate. On the other hand, the modified Grancher system of separation, whereby uninfected infants are removed from tuberculous homes and placed in healthy surroundings for a period of several years, appears to be successful in France (p. 61), in Norway (Harbitz, 1927), in America (Hess, 1919), and in other countries. Formerly this system was limited to children of 3 or over. If infection of an infant by its tuberculous parent has already occurred, as shown by a positive tuberculin reaction, the value of separation is less certain, and it is possible that as much can be done at home by supervision and improvement of living conditions.

In any system of voluntary separation the tuberculin test plays an important part. A negative reaction is considered to favour separation, while a positive reaction suggests that the best opportunity has been lost. It must be remembered, however, that a significant period elapses between primary infection and the appearance of tuberculin skin reactivity (p. 20). This latent period varies from a few weeks to several months in children. Since the more sensitive the tuberculin test used the earlier is sensitization apparent, it follows that the intracutaneous test should be employed in preference to von Pirquet's method when selecting cases for separation, and no infant should be pronounced free of infection until found to be still tuberculin-negative several months after removal from all possible sources of contagion.

The use of tuberculin tests in young children of tuberculous families is indicated for other reasons; and it would seem advisable to make routine tests on infants attending a contact clinic, using the intracutaneous method, and to re-test negative cases at six-monthly intervals. (The technique described in Appendix 5 is recommended as safe and reliable.) In this way the physician will be enabled to determine when infection has passed from a tuberculous mother to her children, and his attention will be focussed on the latter. Children still negative at 4 or 5 years may be given a good prognosis (Section IV). On the other hand, the prognosis must be guarded when infants under 2 years react positively, and if obscure and persistent symptoms are present a serious view should be taken. In this manner cases of miliary tuberculosis can sometimes be recognized before they become obvious clinically.

In older contacts also tuberculin tests may give valuable information. Early pulmonary and tracheo-bronchial tuberculosis and tuberculous cervical adenitis are notoriously difficult to diagnose with certainty in

school-children, and a negative intracutaneous tuberculin reaction may be of the greatest help in excluding these diseases. On the other hand, Opie (1930), in a recent paper, advises that all school-children who give strongly positive reactions to 1 : 10,000 or weaker tuberculin, injected intracutaneously, should be submitted to an X-ray examination of the chest on the chance of discovering grave lesions, such as latent or manifest pulmonary tuberculosis of the childhood or adult type, or extensive tracheo-bronchial tuberculosis. The stronger the tuberculin reaction, and the higher the dilution, the more likely, Opie believes, are grave lesions to be demonstrable radiographically, even if no symptoms are present, but a very high degree of technical skill and experience is required for their recognition. Opie, in fact, suggests that a radiogram be made of every contact who gives a positive reaction, and that those who show an active response in weak dilution, if associated with impaired health and conspicuous underweight, be given special care in open-air schools or 'preventoria'.

Finally, an interesting indirect use of tuberculin tests arises from the data presented in this section. Occasionally an adult patient has signs suggesting pulmonary tuberculosis, but the sputum result is persistently negative, or no specimen can be obtained. If the patient has several young children, and all of these are found to react to tuberculin, it suggests that they have received the infection from their parent, since the incidence of positive reactions at this age is normally very low. Whenever, in fact, a family of young children is found to consist entirely of positive reactors, the milk supply should be investigated, and the parents and other relatives examined for evidence of pulmonary tuberculosis.

7. Summary and Conclusions.

1. Positive tuberculin reactions are more frequent among children of tuberculous households than among children who have not lived in contact with persons suffering from clinical tuberculosis.

2. Children who have lived in contact with tuberculous parents or siblings show positive tuberculin reactions more frequently than do children with tuberculous relatives who have not lived with them, the latter more frequently than children with no tuberculous relatives.

3. A larger percentage of positive reactors is found among children who have lived with relatives suffering from open tuberculosis than among children who have lived in contact with closed tuberculosis.

4. The above findings are adequately explained by variation in frequency, intimacy, and duration of contact, without assuming the existence of inherited predisposition to infection (in the sense used throughout this report).

5. In a small series of children who had lived with parents or siblings suffering from non-pulmonary tuberculosis, positive tuberculin reactions were no more frequent than among children of non-tuberculous households, and among children with no tuberculous relatives at all. If further work, embracing a larger number of cases,

confirms this finding, it will prove that children of tuberculous ancestry do not inherit a predisposition to tuberculous infection.

6. These conclusions do not affect the probability that the hereditary factor is to a large extent responsible for the unfavourable experience of the children of the tuberculous after infection has taken place.

7. Tuberculin tests are of great value in a contact clinic, both for detecting infection in infants, and for excluding tuberculosis in these and in older children. If the temporary removal of infants from tuberculous into healthy homes becomes a widely adopted measure, tuberculin tests will have an important role in determining which are the most suitable cases for this procedure.

8. Should Calmette's method of prophylactic immunization by the administration of attenuated cultures of the tubercle bacillus come into general use in this country, tuberculin tests will have a further application among contact children. A negative reaction will indicate the necessity for treatment and a positive test will be the sign of successful infection.

Section VII. The Value of Tuberculin Tests in determining the Distribution of Tuberculous Infection in the Community. Tuberculin Reactions in various sections of the Population.

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1. INTRODUCTION.

The study of the distribution of tuberculous infection in various parts of the world has helped to develop the modern views of the

epidemiology of tuberculosis. Apart from post-mortem examination the tuberculin test is the only means of determining this distribution, and of recording changes in the degree of tuberculization of a community from decade to decade. Mortality and morbidity rates cannot give the information, although they are factors that influence the further spread of infection.

(a) *Difficulty in Choice of Clinical Material.*

There are two alternative methods of attack in investigating the distribution of tuberculous infection. The first is to attempt to determine directly the incidence of positive tuberculin reactions in the general community as a whole—which includes clinically tuberculous individuals; the second to ascertain the incidence among the clinically non-tuberculous members of the population. It is difficult to obtain a truly representative sample of the general community, because most groups of individuals tend to be naturally but arbitrarily selected to some degree as to their quota of clinical tuberculosis. For instance, a random series of patients attending a hospital may contain a proportion of cases of the disease exceeding that present in the hospital class population (defined on p. 7) as a whole, while, conversely, the number may be unduly small (although probably nearer the truth) in a series composed of children of the hospital class attending a public school. The presence of this varying proportion of clinically tuberculous patients makes it very difficult to compare crude figures obtained from one random series of hospital patients (including cases of clinical tuberculosis) either with those obtained from another similar series, or with the results of tests made on school-children.

The alternative method of attack, it is suggested, is easier and more satisfactory. All suspected or definite cases of clinical tuberculosis are deliberately excluded from the series, as in the present London investigation, which, like most of the earlier, and some of the more recent inquiries, was carried out on hospital patients; or a group of individuals, such as school-children, is chosen in which clinical tuberculosis is either absent or its incidence so low that it may be practically disregarded as contributing to any significant extent to the percentage of positive reactions. By removing an uncontrolled variable in this way the comparison of figures obtained from hospital patients by one worker both with similar series of other workers, and with the results of tests made on school-children, becomes easier, although social and physical differences and differences in technique may still have to be allowed for. Accordingly, the present section deals mainly with infection as it occurs among the clinically non-tuberculous members of the community, and the tuberculin surveys that are illustrated on the charts are, as far as possible, those believed to comply with the conditions just mentioned.

The interpretation and comparison of the results of these investigations presents a number of difficulties even when the inclusion of patients with definite or suspected clinical tuberculosis is as far as possible avoided.

Some series, for example, contain home-contacts in a proportion much exceeding that found in the general community. The figures obtained from apparently healthy patients attending a tuberculosis dispensary fall into this category, and cannot be taken to represent the tuberculin reactions of any section of the population of the town concerned, because, as shown in the preceding section, home-contacts become infected to a much greater extent than do members of non-tuberculous households.

Again, since the incidence of positive tuberculin reactions, as might be expected, is found to vary among the different classes of society in the same community, attention must be given to the social status of the individuals tested.

Physical differences in the individuals tested by different workers must also be considered, since these might affect the validity of deductions made from the results obtained. Apparently healthy school-children may be suffering from under-nutrition, and children attending hospital, even if clinically non-tuberculous, presumably have various major or minor ailments. Malnutrition *per se*, however, does not appear to confer susceptibility to tuberculous infection, according to the observations of Chadwick and Zacks (1927, 1929). These workers found that the percentage of positive reactions in a large series of school-children was approximately the same whether the subjects were overweight, underweight, or of average weight. Furthermore, there is reason to believe that in many, if not all, of the non-tuberculous conditions (e.g. measles, pregnancy) in which non-reactivity to the tuberculin test has been reported (Table 4), and for which some of a series of hospital patients may be attending hospital, sensitivity is only depressed and can still be shown to be present by using sufficiently sensitive methods (p. 22).

(*b*) *Variety of Methods Employed.*

The technique employed in the various tuberculin surveys differs widely. The *stichreaktion*, von Pirquet, and Mantoux tests have all been used. The concentration of tuberculin for the von Pirquet reaction differs in the earlier work, and some observers repeat the test several times, whereas others are content with one application; while the upper limit of dose in the Mantoux tests varies. Moreover, until recently the methods of standardizing tuberculin have been only roughly quantitative, so that the potency of the material used by different workers may have varied significantly (p. 42). It is to be hoped that future inquiries will always contain a clear statement not only of the method of standardization, but also of the estimated potency of the tuberculin used in terms of the now available International Standard Tuberculin.

The object of tuberculin surveys is to detect every tuberculin-sensitive individual, and so to determine the sensitization incidence of the community (defined on p. 7). Differences in technique clearly would not matter if all the methods were sufficiently sensitive for this purpose. Such, however, is not the case; consequently, allowances must

be made for shortcomings and differences of procedure when interpreting and correlating the published figures. The greater sensitiveness of the intracutaneous, as compared with the cutaneous, test was demonstrated in Section III. The comparison of results of both methods in the same city and at the same period (Davidsohn, Smith), and similar comparisons between the *stichreaktion* and the von Pirquet test, make it clear that the latter method is likely to give a low estimate of the sensitization incidence of a community, a defect to bear in mind when considering statistics obtained by its use.

On the other hand, statistics based on the intracutaneous test or on the *stichreaktion* should carry more weight, although, as indicated on p. 53, the upper limit of dose must be taken into account. Provided, however, that the upper limit is sufficiently high, the intracutaneous test seems to be capable of detecting almost every tuberculin-sensitive individual (so that the incidence of positive reactions in a community represents approximately the sensitization incidence), and this method is therefore suitable for tuberculin surveys (pp. 22, 53).[1] The sensitiveness of the *stichreaktion*, as usually performed in tuberculin surveys, is probably at least as great as that of the intracutaneous method when the upper limit of the latter is the standard dilution (1 : 1,000), but no greater, if as great, when the upper limit is higher.

The position reached is that while high figures for the incidence of positive reactions in a group of people can be accepted, whatever the method employed, a low incidence of reactions must always give rise to uncertainty if the von Pirquet test has been used.

(c) *Meaning of Sensitization Incidence.*

Even if, by using the most sensitive technique available, practically every potential reactor is detected, and, consequently, the percentage of positive reactions in a community represents approximately the sensitization incidence, the significance of the latter is uncertain. It was pointed out on pp. 21, 23 (q.v.) that, since it was uncertain whether or not tuberculin sensitivity completely disappeared after extinction of infection, it was not possible to state whether the sensitization incidence of a population corresponded to the incidence of tuberculous infection (i.e. the percentage of persons infected at the time of the test), or to the attack-incidence of infection (i.e. the percentage that had been infected at some time in their lives). If, as some workers believe, extinction of infection is common, these two figures would differ considerably. On account of the uncertainty of this question the term 'sensitization incidence' will be used freely, and 'infection incidence' and 'attack-incidence' avoided where possible.

The foregoing considerations make it clear that a review of tuberculin surveys is a task of great difficulty.

(d) *Practical Value of Tuberculin Surveys.*

The study of the tuberculin reactions of the clinically non-tuber-

[1] For technique see Appendix 5, and for method of adjusting percentages see p. 52. A dilution of 1 : 10 is recommended as the upper limit.

culous members of the community has considerable practical importance. It was pointed out in Section V that the importance of the tuberculin test in clinical practice was due to the eliminating value of the negative reaction, rather than to the positive response; in consequence, the usefulness of the test in a case of suspected clinical tuberculosis depends upon the chance of finding a negative reaction if some other disease is the cause of the patient's symptoms. The age-frequency of tuberculin reactions among clinically non-tuberculous people belonging to the same class and locality as the patient concerned allows this chance, and consequently the utility of the test in negative diagnosis, to be estimated.

The incidence of positive tuberculin reactions in the healthy community, if accurately measured, is probably an index of the risk of exposure incurred by its members. This risk is determined by a number of factors: (1) the frequency of open tuberculosis;[1] (2) the measures taken to treat this condition, and to combat the spread of infection in tuberculous households; (3) the environmental conditions of the populace (e.g. standard of hygiene and degree of overcrowding); and (4) the infectivity of the milk supply. The influence of some of these factors has been discussed in the preceding section, and will be made clearer when the various investigations are described. Since, however, the degree of exposure to contagion is the resultant of the interplay of all the factors concerned, the relationship between any one of them and the incidence of positive reactions is likely to be inconstant.

Published observations of the tuberculin reactions of clinically non-tuberculous individuals are legion, practically every writer on the subject having provided the literature with a series of cases. All parts of the world are represented, but there appears to be only one recent English series. This was described in Section V, where it served as a control to the series of clinical tuberculosis, and was used also to find the utility of the intracutaneous test for the purpose of negative diagnosis at each age-period of life. In the present section the London results are compared with statistics obtained during the last quarter of a century in other parts of the world.[2]

2. Tuberculin Reactions in Various Sections of the Population.

(a) *Representative Series.*

Group i. Urban communities—hospital class (a) 20 *years ago* (*Chart* 5) *and* (b) *during the last* 10 *years* (*Chart* 6). This group of curves shows the incidence of positive reactions to skin tuberculin tests among members of the hospital class population (defined on p. 7) in various cities of 50,000 or more inhabitants. The majority of the investigations illustrated refer to clinically non-tuberculous

[1] Until such time as accurate case notifications enable a more direct estimate of tuberculosis morbidity to be made, the mortality from pulmonary tuberculosis, rather than the total tuberculous death-rate, is probably the best index of the prevalence of open tuberculosis in a community.

[2] The figures of Dow & Lloyd (1931), published while this report was in the press, and based upon observations of children attending the Brompton Chest Hospital, London, are slightly higher than those of the present writer's series.

individuals. A number of other series are omitted because they contain many clinically tuberculous patients without sufficient data to enable them to be subtracted from each age-group.

The results of investigations made between 1907 (when the first inquiry was carried out) and 1914 are depicted on Chart 5, while the curves shown on Chart 6 are based on inquiries made during the last ten years. The cities represented in the first sub-group are Vienna, Prague, Paris, Oslo, Geneva, St. Louis, and three German towns, namely, Charlottenburg, Heidelberg, and Rostock; the cities in the second sub-group are New York, San Francisco ((*a*) general population, and (*b*) Orientals), Madras, Graz (Austria), Charlottenburg, Rostock, Dortmund (Germany), and London. Hamburger & Monti's (1909) curve for Vienna is given instead of von Pirquet's (1909), for the reasons stated on p. 24.

Examination of Charts 5 and 6 brings out the following points:

(1) The poorer classes of Vienna, Paris, and Prague were extensively tuberculized twenty years ago, about half the children reacting positively by five years, about three-quarters at ten years, and nearly all by the time they reached the age of puberty. The explanation of this extensive tuberculization appears to lie in the great risk of exposure incurred by the children of the working classes. At the time of the early inquiries these cities were notorious for the overcrowding, the prevalence of open tuberculosis (indicated by the high phthisis death-rate[1]), the unhygienic home conditions, and the defective milk supply, all of which circumstances would tend to promote the spread of infection. The pulmonary tuberculosis death-rates per 10,000 persons in 1905 were: Prague 42, Paris 38, and Vienna 33, as compared with 14·5 in London (Appendix 4).

The high figures of these investigations, and of others with like results (von Pirquet, Calmette *et al.*, Nothmann—see below) have coloured the whole of the subsequent history of the subject. Reported by the protagonists of the tuberculin test, they were widely accepted as capable of universal application to urban communities, a view that is prevalent even at the present time. The lower figures of contemporary observations in other cities (see (2)) did not serve to dispel this idea, partly, reasonably enough, because the von Pirquet test was used in almost every instance (and uncertainty must always exist where this method yields a small percentage of positives), and partly perhaps because the workers were less prominent authorities. It has remained for recent investigations with the intracutaneous test to demonstrate the incorrectness of the belief that tuberculous infection is universal in later childhood.

(2) If the results of the von Pirquet test be believed, the sensitization incidence was considerably lower among the children of the poorer classes of some other European cities before the War, namely, Charlottenburg, Oslo, Heidelberg, Geneva, and Rostock, and also in St. Louis, less than half being positive reactors at puberty. In seeking for a possible explanation of these findings, other than faulty technique, the statements of Feer and of Synwoldt may be mentioned, namely, that Heidelberg and Rostock enjoyed unusually favourable climatic conditions, while a large agricultural element existed among the patients attending the clinics of these towns. It is possible, therefore, that, other things being equal, the risk of exposure to contagion was less than among the children of more densely populated cities. It should, however, be pointed out that an investigation made on public school-children in Oslo about the same period by Frölich (Chart 7) yielded more than twice the number of positives that were in Hellesen's group of hospital patients. Although the clinical material was not strictly comparable, Frölich considered that technique was partly responsible for this gross difference. Some of the other figures may be similarly at fault.

The phthisis death-rates in 1905 for these towns were: Charlottenburg 13, Rostock 14, St. Louis 19·5[2] (approx.), Oslo 28, Geneva 29 (approx.), Heidelberg 33[2] (Appendix 4).

(3) The more recent curves showing the tuberculin reactions of the hospital class of urban communities in Europe and North America have similar features (Chart 6). The New York and San Francisco series are included here—though

[1] See footnote 1, p. 76.

[2] Total tuberculosis mortality.

they contain some clinically tuberculous patients—because of the low figures obtained. Although the intracutaneous test was used in the majority of these investigations, the percentage of positive reactions was found to be smaller from infancy to adolescence than that reported in Paris, Prague, and Vienna before the War, the difference being greatest in early childhood. By five years of age about 20 per cent. of children reacted to tuberculin (as against 50 per cent.); by ten years the percentage was about 40 (as against 70–80); and about 60 or 70 per cent. were tuberculin-positive at fifteen years (as against about 80 per cent.). This difference does not necessarily imply a general decline in the sensitization incidence, because the cities in the two groups were not the same.

The lower figures of these, as compared with some of the earlier investigations, can in most cases be adequately accounted for by a smaller risk of exposure to contagion. Phthisis mortality statistics indicate that at a time when the disease was rife in Vienna, Paris, and Prague the incidence was significantly lower among the general population of the cities at present under consideration, with the exception of Graz; and the mortality has since become gradually further reduced, except during the period of the War. The pulmonary tuberculosis death-rates per 10,000 persons in 1905 were as follows: Dortmund 13, Charlottenburg 13, Rostock 14,[1] London 14·5, New York 21, San Francisco 23 (approx.), Graz 46,[1] as compared with Vienna 33, Paris 38, and Prague 42 (Appendix 4). Differences in the environmental conditions of the subjects tested also may have influenced the risk of exposure, and, in fact, may be more important than the prevalence of open tuberculosis.

On the other hand, the low incidence of positive reactions among Oriental children in San Francisco is difficult to explain, in view of their congested living quarters and high tuberculosis mortality, the mortality among the Chinese being at present five times that of the general population (45 as compared with 9). The educational campaign for improved personal hygiene is suggested tentatively as an explanation by the author of the paper quoted.

(4) The recent curves described above (Chart 6) show an incidence of positive reactions no lower than that reported for Charlottenburg, Oslo, Heidelberg, Geneva, Rostock, and St. Louis, before the War (Chart 5). Indeed, tuberculin reactions were stated to be slightly more frequent in Charlottenburg and Rostock at the end of the War, as compared with 1912–14 (but see later pp. 82, 85).

(5) A recent investigation in Madras revealed extensive tuberculization of children, but the latter, although stated to be clinically non-tuberculous, were all patients of a tuberculosis hospital. It may be noted that the phthisis death-rate in this city (26–31) is reported to be three times that in London (9). But so is the mortality of the Chinese in San Francisco, among whom positive tuberculin reactions appear to be much less frequent than among the native population of Madras.

(6) The comments made so far apply only to children, most of the curves not extending to persons over school age. The percentage of reactors among clinically non-tuberculous adults in urban communities twenty years ago is given as 95, 95, 82, and 50 by Freund (1908), Hamburger & Monti (1909), Mantoux (1910), and Stadelmann & Wolff-Eisner (1908), respectively. The few recent inquiries into the tuberculin reactions of clinically non-tuberculous adults of the urban hospital class indicate a sensitization incidence as high as that reported by earlier investigators. Ninety-five per cent. of the London group of 134 adults (but including 16 doctors, one of whom was negative) reacted positively; 100 per cent. of Opie & McPhedran's (1926) series in Philadelphia were positive, and 93 per cent. was the figure reported by Cummins (1929) in London and (1930) in Cardiff. All these observers used the intracutaneous test. Heimbeck (1929 b) found that 95 per cent. of a series of 576 adults of the working-class in Oslo gave positive von Pirquet reactions.

Group ii. Urban communities—public school-children of various classes (*Chart* 7). The county primary schools of London are composed very largely of members of what has been termed in this report the hospital class. In Germany and some other countries most of the public schools (in the sense used on the Continent and

[1] Total tuberculosis mortality.

in America—see p. 7) contain a varying proportion of children of the middle classes, while the school population of United States towns is a very fair cross-section of the general healthy community of the district served. It is for these reasons that the investigations on public school-children are placed in a separate group. The cities represented are Oslo, Bergen, Trondheim (Norway), and Odessa, before the War; and Oslo, Cologne (two series), Kiel, Stolp-i-Pom (Germany), The Hague, Minneapolis, Philadelphia, and Peking, during the last ten years. A series of healthy persons of all classes tested in Lille in 1910 is included in this group for convenience. It will be seen that the Lille and Oslo figures were higher, and the remaining pre-War figures lower, than those reported for the hospital class in Vienna, Prague, and Paris about the same time. The post-War curves show considerable variation, Peking yielding the lowest and Philadelphia the highest figures. The latter are little lower than those reported in Lille fifteen years before. On the other hand, the recent Oslo series shows lower figures than did the pre-War series in the same town. There is little correspondence between some of the curves (e.g. Philadelphia, Odessa, and Peking) and the phthisis mortalities in the cities concerned. In particular, the low incidence among children, many of them in extremely poor circumstances, in Peking is hard to reconcile with the great prevalence of tuberculosis in that city, as the author points out. The phthisis mortality rates in 1905 for the towns of this group were as follows: Odessa 31, Lille 31, Oslo 28, Trondheim 24, Philadelphia 21 (approx.), Cologne 20, Bergen 18, Stolp 16·5,[1] The Hague 13, Kiel 11, Minneapolis 11 (approx.); and in 1927 were as follows: Oslo 14, Stolp 12, Cologne 8, Kiel 8, Philadelphia 7, The Hague 6, Minneapolis 4. (Appendix 4.)

The majority of tuberculin surveys in the future will probably be undertaken on school-children, because of the convenience of this method of investigation. A small series, however, does not represent a town as a whole, as Armstrong (1919), Chadwick & Zacks (1927, 1929), and Hetherington *et al.* (1929) have shown. Chadwick & Zacks found 11 and 60 per cent. positive in schools from different districts in a city of 60,000 inhabitants investigated by them. Those inquiries in which large numbers of children from various districts are tested approach the ideal.

(*b*) *The Influence of Social Conditions on Tuberculous Infection.*

Group iii. Urban communities—wealthier classes (*Chart* 8). Very few figures are available for the richer classes. The curves showing the von Pirquet reactions of children in Brüning's private practice in Rostock from 1917 to 1920, and in Moro's and Volkmar's private practices in Heidelberg from 1911 to 1918, resemble those obtained for the hospital class of these two towns in 1917–19 and 1910, respectively (Charts 5 and 6). On the other hand, the incidence of positive von Pirquet reactions among the children of Schlossmann's richer clientele in Düsseldorf in 1908, and of Paraf's richer-class patients in Paris in 1928, was lower than that found in any other urban inquiry. This low incidence may be partly due to faulty technique. If not, it suggests that social and economic conditions influence the distribution of tuberculous infection. This point is again raised when considering the next two groups.

Few records have been published of the tuberculin reactions of well-to-do adults living in cities. Various series of medical students afford a poor second best, since the majority of these have begun their hospital course and, therefore, have had unusual opportunities to become infected. Kruse (1913) reported positive von Pirquet reactions in 84 per cent. of his students in Bonn, while 71 out of 72 medical students and laboratory workers tested by Selter (1921) in Königsberg with the cutaneous test, followed by the *stichreaktion*, were positive. On the other hand, Heimbeck (1927, 1928), in Oslo, found that only 47 per cent. of a group of medical students, and 55 per cent. of a series of probationer nurses, were Pirquet-positive, figures that were lower than those recorded by most workers, including Heimbeck himself, for adults of the hospital class. Krause (1928) states that many non-reactors are met with among adults in private practice. Finally, Lees & Myers (1930) found that only 32 per cent. of freshmen aged 16–22, entering Minnesota University, and 57 per cent. of those aged 23 and over, reacted positively to the

[1] Total tuberculosis mortality.

von Pirquet test. It is unfortunate that a more delicate method was not employed in this and the previous investigations.

Group iv. Urban communities—poorest class (*Chart* 9). The clinical material of these investigations was composed of children whose social and economic status was lower than that of the average hospital child, i.e. than the hospital class. The cities represented are Berlin (two series before, and one just after, the War), Düsseldorf, Edinburgh, New York, and Peking. Attention is drawn to the following points: (1) the intracutaneous test and *stichreaktion* yielded the highest figures, and the von Pirquet test the lowest; (2) a large percentage of reactors was found among the very poor in Düsseldorf, New York, and Berlin (Davidsohn), suggesting that bad social and economic conditions, which are known to be accompanied by a high tuberculosis death-rate (Fishberg, 1922 d; Biraud, 1930), also favour the spread of tuberculous infection; (3) on the other hand, a small percentage of positives was found in Peking, and by Küchenhoff in Berlin. The statement of the latter author that 'scrofulous children very often show negative von Pirquet reactions' raises the suspicion that technical error was partly responsible for his low figures, based on this method.

Group v. Rich compared with poor in the same city and at the same period (*Chart* 10). This chart indicates that two classes of the same community may show a difference in the percentage of positive reactors, fewer positive being found among the rich than among the poor. This difference can be accounted for by a smaller risk of exposure—due probably to a superior standard of hygiene, better housing, and a lower incidence of open tuberculosis. The three examples explain themselves. The differences found are probably significant, because the method and observer were the same in both groups of cases. Similar results were obtained by Keifetz (1915), Armstrong (1919), Asserson (1927), and Heimbeck (1929 b). It may also be mentioned that Schlossmann (1909) found that only 5 per cent. of the children of his richer clientele were tuberculin-positive at a time when the majority of his colleague Nothmann's hospital patients reacted positively. In contrast to these results, Korns (1925) found actually a smaller percentage (26 per cent.) positive among destitute but healthy native children in Peking, than among middle-class children (37 per cent.), an observation that he was unable to explain.

(c) *The Influence of Urbanization.*

Group vi. Civilized rural communities (*Chart* 11). Seven surveys of civilized white communities are illustrated on this chart, and the results of an investigation of the reactions of the Indians of the Great Canadian Plains are also shown. The Saskatchewan figures include 12 per cent. of Indians with 93 per cent. positive, but when these are omitted the total incidence is still over 50 per cent. With the exception of this series the curves of white rural communities show a low incidence of positive reactions, especially in adolescence, when compared with most urban figures. Similarly, Overland (1913 b) found a low incidence in Norwegian villages, Baker (1925) in Alberta (intracutaneous test), and Fenger *et al.* (1930) and Frost (1930) in Minnesota; while Hillenberg (1909), in a series drawn from a small town of 3,000 inhabitants in a prosperous agricultural district, found an unusually small percentage of positive reactors to the von Pirquet test even in adults (53 per cent.). Finally, 23 per cent. of a large series (569) of adults tested by Scheel (1929) in Norway were Pirquet-negative.

It is unfortunate that more reliable tests were not employed in all these rural surveys, in view of the comparatively low incidence of positives recorded. The continued use of the von Pirquet test in the series of inquiries at present being carried out in Minnesota is particularly regrettable. If not due to defective technique, the comparative infrequency of positive reactions indicates a low sensitization incidence. The latter may be due to a small amount of infection occurring in the rural districts investigated, on account of a relatively small risk of exposure to contagion; or possibly it may be due to commonly occurring loss of reactivity after extinction of infection.

Investigations of the tuberculin reactions of uncivilized primitive tribes are omitted from this section, as they have been well reviewed elsewhere (consult Cummins, 1912, 1920, 1928, 1930; Schlesinger, 1916; Bushnell, 1920; Cobbett,

1925; Ferguson, 1928; Vassal, 1928). It may be stated briefly that primitive races in their native habitat show positive reactions much less frequently than do civilized peoples, but the nearer they approach civilized communities the more they become tuberculized. Tuberculization may become extreme, at least for a time, when the disease becomes endemic in a native population, as has occurred among the Indians of the Canadian Plains, whose tuberculin sensitiveness is illustrated in the present chart. These facts can be simply explained by differences in the amount of exposure to open tuberculosis. Exposure is at first non-existent, but increases as the country becomes developed, first on account of contact with white adults, and soon from the rapidly increasing frequency of open tuberculosis and inadequate sanitation. Thus, an epidemic of tuberculosis began among the Canadian Indians in 1880, when they were forced to submit to white civilization. At the same time living conditions became very bad, but have shown gradual improvement, although they are still far from satisfactory. Even now the tuberculosis death-rate is twenty times that of the surrounding white community (Ferguson, 1928).

Group vii. Town compared with country at the same period (no chart). Simultaneous surveys of the tuberculin reactions of people living in town and country districts, using identical technique and as far as possible similar clinical material, have given conflicting results.

Elliott (1925) compared the intracutaneous tuberculin reactions of a series of children in Dundas, Ontario (population 5,000) with those of a series living in surrounding rural districts. The percentage of positives in the urban group (36 per cent.) significantly exceeded that in the country group (22 per cent.), the disparity being greatest in adolescence. Baker (1925) reported similar findings in Alberta. Rathbun (1927) found that 41 per cent. of children reacted positively to tuberculin in the largest city, and 25 per cent. in the smaller towns, of the American districts investigated by him. Heimbeck (1927) classed 397 probationer nurses entering Oslo municipal hospital according as to whether they came from large towns, small towns, or from country districts. The percentage of reactors to the von Pirquet test in each group was 55, 48, and 39, respectively. Among the children of another Norwegian town, Bergen, Overland (1913 b) had previously found a larger percentage than in the surrounding villages.

On the other hand, little difference in the percentage of positives was noted by Chadwick & Zacks (1927) among the school-children of rural and of urban Massachusetts (towns of over and under 2,500 inhabitants), and by Lees & Myers (1930) among freshmen in the University of Minnesota who came from large towns and from country districts; while Bruns, quoted by Bushnell (1920), found that a group of American soldiers gave approximately the same percentage of positives (90 to 97 per cent.), whether hailing from town or from country.

It is not easy to explain these conflicting results, for comparison of conditions of life and tuberculosis mortality in the urban and rural populations of the same country suggest that the latter run a somewhat smaller risk of exposure to contagion. Thus, the density of population in country districts is less than in towns, although it is true that the standard of hygiene may be no better; and the phthisis death-rate in rural districts is generally lower than in the urban districts of the same state (see Fishberg, 1922 c; Wolff, 1926; Registrar-General, 1929 b; League of Nations, 1931). According to Biraud (1930), however, this difference in mortality, once conspicuous, is gradually becoming effaced with the advance of industrialization: moreover, the difference is possibly exaggerated by defective notifications in country districts (Wolff, 1926; League of Nations, 1931).

(d) *The Influence of War.*

Group viii. War compared with peace in the same city (no chart). It has been suggested that the fall in the standard of living and rise in the tuberculosis mortality that occurred in many countries, including some neutral states, during the War (Fishberg, 1922 f; Wolff, 1926) would cause an increased spread of tuberculous infection, accompanied by a rise in the frequency of positive reactors. It is probable, however, that the increased mortality from this disease was due to the death of cases already present when the War broke out, rather than to fatality

among new sources of infection (Cobbett, 1930). It was pointed out when considering Group i that the figures of tuberculin surveys carried out during or just after the War in Charlottenburg and Rostock by Umber (1921) and Synwoldt (1920), respectively, were slightly higher than those obtained in the same cities before the War by Umber (1921) and Kessler (1920). A slight increase in the percentage of positive Pirquet reactions in a Berlin orphanage was also noted by Davidsohn (1920) in 1919, as compared with a similar series tested in 1913; and Bartschmid (1920) reported that a group of children under 15 tested by him during the War in Munich contained more Pirquet-positive individuals than did a similar group in 1912–14 (48 per cent. as against 36 per cent.). Pape (1921) kept records of von Pirquet reactions in children of Frankfurt each year from 1910 to 1920. The percentage of positives fell until 1916, and then rose. A paper by Rominger (1920) illustrates the necessity of caution in interpreting these differences, mostly slight, as significant. This author published the von Pirquet reactions of similar series of individuals tested in 1913, 1917, 1918, and 1919, in Freiburg. A fall in the percentage positive was noted at first, but when in 1919 the *stichreaktion* was performed, in addition to the von Pirquet test, twice as many positives were revealed, making the final figure higher than in 1913.

(*e*) *The Influence of Contagion in the Home.*

Group ix. Non-contacts compared with home-contacts of tuberculosis (*Chart* 3). The contrast between these two sets of figures formed the subject of Section VI, where the influence of exposure to contagion in the home in producing tuberculous infection was fully discussed. The group is included here for completeness. If the figures published for the tuberculin reactions of children of the poorer classes living in tuberculous households in cities with populations of 50,000 or more are plotted, the curves, like those of the Philadelphia and London series (shown in the present chart), are found to rise rapidly at first and then to assume a more gradual slope. (Cohn, 1910; Fishberg, 1914; Delthloff, 1916; Schram, 1922; Parisot & Saleur, 1925.) The majority of the curves show as many as 60 per cent. of positives by 3 years, and about 80 per cent. by about 8 years of age. (Some of these series include a number of cases of clinical tuberculosis.) On the other hand, most of the curves for urban non-contacts of tuberculosis, like those illustrated on the present chart, are low and approximately rectilinear in shape from birth to manhood. By 3 years of age about 10 per cent., and by 8 years only about 30 per cent., of the children reacted positively. (Delthloff, 1916; Sill, 1918; Parisot & Saleur, 1925.) A series published by Fishberg (1915) is an exception.

Since social and economic conditions influence the spread of tuberculous infection, it might be hoped that their amelioration would compensate to a certain extent for the presence of an infective adult in the household, and according to Varrier-Jones positive tuberculin reactions are rarely found among the children in the Papworth Settlement (p. 69).

(*f*) *The Influence of Race.*

Group x. Tuberculin reactions of different peoples living in the same district under conditions of civilization (*no chart*). In this review the variations in incidence of positive tuberculin reactions among healthy groups of people have been attributed either to the methods employed or to variations in the risk of exposure to contagion, without reference to fixed racial or geographical peculiarities. It is now necessary to consider whether a racial diathesis towards tuberculous infection exists, or whether all peoples are equally susceptible. The distinction between tuberculous infection and tuberculous disease (clinical tuberculosis) is again emphasized. Comparisons of the tuberculin reactions of different peoples, living in proximity under civilized conditions, might be expected to give information about racial susceptibility. It is, however, difficult to exclude extrinsic differences that may influence contagion. The potency of these extrinsic factors has already been shown by the fact that two sections of the same race living in the same city, but of different social status, may show a distinct difference in the degree of their tuberculization (Group v).

Ferguson (1922) reported a considerably higher frequency of tuberculin re-

actions (93 per cent.) among Saskatchewan Indian-born school-children, than among school-children of white parentage in the same district (54 per cent.). McCain (1929) found that 27 per cent. of 2,500 coloured (negro) school-children of North Carolina reacted, as against 22 per cent. of 22,500 whites. Hetherington, McPhedran, Landis & Opie (1929) noted that positive reactions were more frequent among negro school-children in Philadelphia than among the white children investigated. Dickey (1928 a) found a slight but definite excess in the percentage of positive reactions among Oriental children in San Francisco over the percentage among the general population of that city (see Chart 6). At first sight these results appear to support the existence of inherent racial susceptibility to tuberculous infection. But although two races are born and reared in the same district, their opportunities for contagion are not necessarily the same. Overcrowding, an inferior standard of hygiene, and the greater frequency of cases of open tuberculosis, all increase the risk of infection, and these extrinsic differences alone seem sufficient to account for the findings of these authors. This view is supported by the report by Asserson (1927) of the tuberculin reactions of 3,000 coloured (negro) and 600 white infants living in New York City under similar conditions and with, she suggests, probably much the same opportunities for exposure. The percentages of positive reactions were approximately equal (11·3 and 11·6 per cent., respectively).

Asserson (1927) also reported the tuberculin reactions of 500 Jewish infants in New York City; 13 per cent. reacted positively, a figure not significantly different from that found among the other races studied (negroes, Irish, Italians, 'Americans'). Similarly, Hetherington and his co-workers found that the percentage of positive reactions among Jewish school-children in Philadelphia was approximately the same as among the other white children investigated.

Dickey (1929) states that he found that children of Oriental parentage gave considerably larger (in size) intracutaneous tuberculin reactions than other races. This greater sensitiveness to tuberculin must not be confused with susceptibility to infection, and may quite well be dependent upon some local difference, such as the texture of the skin. If a relatively insensitive method, e.g. the von Pirquet test, be applied to Orientals and Europeans, a larger percentage may react among the former than among the latter, on account of their greater sensitiveness to tuberculin, but without there being necessarily any difference in the number of persons infected in the two groups.

The most exhaustive study of tuberculous infection in different peoples living in the same district has been made by Chadwick & Zacks (1929) on 42,000 Massachusetts school-children, composed of the following nationalities: English and Scottish, Italians, Russians and Poles, 'Americans', Irish, Canadians, Scandinavians, Latins, and Teutons. There was no marked variation in the percentage of reactors to the von Pirquet test among children of different nationalities when there was no history of contact. Approximately twice the number of children reacted to tuberculin when there was a history of exposure to phthisis, with the exception of the Teuton group, which showed reactions three times as commonly. The variations between the percentage of positive reactions among children of different nationalities, when the whole series (non-contacts and contacts) was considered, was found to depend chiefly on the relative proportion of contacts in each group. These authors conclude that 'the tubercle bacillus infects the children of different nationalities to an equal degree when similar conditions for contact with carriers are present'. The differences in tuberculization that may be found can be usually accounted for by contagion and chance without reference to racial diathesis. *This does not mean that the latter may not play an important part in determining whether tuberculous infection, once it has taken place, pursues a benign course, or develops into manifest and perhaps fatal disease.*

(*g*) *The Influence of Infected Milk.*

Tuberculin surveys have afforded little information as to how far tubercle-infected milk contributes to the infection of the community, because of the difficulty of separating its effect from those of other factors. Information on this point has been gained mainly from bacteriological examination of milk and of

pathological specimens obtained from patients with tuberculous disease. In view of the differing frequency of human tuberculosis of bovine origin in various countries, noted by Griffith (1930) and others, the similarity of many of the recent tuberculin reaction incidence curves is of some interest.

(*h*) *Summary.*

After due consideration is given to the fact that deficiencies of technique make it difficult to deduce the sensitization incidence of a community from the frequency of positive reactors to the tuberculin test, certain conclusions may be tentatively drawn from the results of tuberculin surveys: (1) the sensitization incidence among home-contacts is greater than among non-contacts, (2) and is greater among the poor than among the rich; (3) it is usually high among the poorer classes of densely populated cities where pulmonary tuberculosis is rife, the standard of hygiene low, and the milk supply defective, but tends to be lower where living conditions are better and phthisis less common; (4) it tends to be low in white rural communities; (5) the sensitization incidence among the children of the poorer classes, or general population of the majority of cities recently investigated with the intracutaneous test (e.g. London), is lower than it was twenty years ago in some of the (different) continental cities where the most prominent of the earliest inquiries were carried out (e.g. in Vienna); in certain instances, however, e.g. the Philadelphia survey, high figures have been noted recently. These findings do not in themselves prove that a fall in the sensitization incidence has taken place. Adults of the urban working class appear, from the few recent investigations, to have as high a sensitization incidence as that indicated by earlier reports.

3. Discussion.

(*a*) *Is the Tuberculin Sensitization Incidence Falling?*

The circumstances that determine the intensity of contagion in a community were enumerated on p. 76. If the tuberculization of a population depends upon the risk of exposure to contagion incurred by its children, a reduction in the sensitization incidence would be expected to follow any marked and continued improvement of these circumstances. During the twenty years that have elapsed since the first tuberculin surveys were made the following changes can be detected in the majority of European and North American cities: (*a*) decrease in the prevalence of open tuberculosis, reflected by the decline—interrupted by the War—of the pulmonary tuberculosis death-rate;[1] (*b*) rise in the standard of living among the working classes; (*c*) more intensive anti-tuberculosis measures; (*d*) an improved milk service. Has a corresponding reduction taken place in the sensitization incidence of these populations?

Comparison of tuberculin reactions in one group of cities at one period with those in another group of different cities at an earlier

[1] See Biraud (1925, 1930), Wolff (1926), Greenwood & Wolff (1928), Cobbett (1930), Dow & Lloyd (1930), Registrar-General (1929 b), League of Nations (1931), and Appendix 4. See also footnote 1, p. 76.

period affords only indirect evidence for or against a reduction. Direct evidence is, however, obtainable from tuberculin surveys carried out on comparable samples of the population of the same town at sufficiently wide intervals. Up to the present very few such investigations have been described, and the majority were performed before, and either during or just after, the War in towns that suffered severely from shortage of food. These investigations have been already discussed (p. 81). The fact that in the majority a rise in percentage of positives was recorded does not preclude the possibility that a fall would have occurred under ordinary conditions. Three other important inquiries must now be considered; two of them were carried out in America, the third in Norway. In each a distinctly lowered frequency of positive reactors was noted.

Chadwick & Zacks (1929) found that the frequency of Pirquet-positive reactors among school-children in the town of Framingham (Mass.) showed a reduction of 23 per cent. between 1917 and 1926 for the same age-group. They considered that this reduction indicated a significant decrease of tuberculous infection, and pointed out that during the same decade the tuberculosis death-rate in Massachusetts fell from 14·4 to 7·3 per 10,000 persons, and that an intensive anti-tuberculosis health campaign had been carried out. Investigations in Baltimore in 1916 and 1926 gave similar results, according to Krause (1928), but details are not yet available. Ustvedt (1930) investigated the von Pirquet reactions of 5,000 children attending the state schools of Trondheim. He chose clinical material similar to that of Arfinsen (1919) in 1914, and used an identical technique. He found that 23·5 per cent. of his series reacted positively, as against 38 per cent. in the earlier investigation. The percentage rose uniformly from 8 at the age of 7 to 40·5 at 15, while in Arfinsen's series the corresponding figures were 24 and 60·5. This meant that the number of positive reactors that entered and left school was one-third and two-thirds, respectively, of the number in 1914. Ustvedt concludes that there has been a definite reduction in the incidence of positive reactions and postponement of the average date of infection during the last fifteen years, and attributes this change to the energetic measures taken to withdraw infectious cases from contact with children.

The postponement of first infection, reported by this author, suggests that it is becoming more and more the result of chance contact outside the home as the child grows up, rather than arising from exposure in the home environment in early life.

There would, therefore, seem to be some direct evidence of a decline in sensitization incidence among children, but further information is required, particularly in regard to large cities, such as Vienna, where extensive tuberculization is known to have been present formerly. There is no evidence that adults of the poorer classes have as yet been affected to any significant extent by a declining tuberculization, the few recent urban figures showing a large percentage of positives.

In a previous paragraph (p. 81) the writer referred to the Indians of the Canadian Plains. Ferguson (1928) reports that the tuberculosis

death-rate of this people has declined from the huge figure of 900 per 10,000 persons in 1886, to 80 in 1926, and that housing and sanitation have shown some improvement during this period, although still far from satisfactory. Nevertheless, 95 per cent. of the children are tuberculin-sensitive by the time they reach 11 years. These observations suggest that the frequency of tuberculosis must fall below a certain level before a reduction in the sensitization incidence begins. This level probably depends upon environmental factors, the important influence of which upon the spread of infection has been already pointed out.

(b) The Optimal Tuberculization of the Community.

'It is clear that an infected people is much safer than an uninfected people when brought into contact with tuberculosis, but what price does it pay for this protection? The question is important, for on it depends the justification of public-health measures designed to limit infection, e.g. by stamping out bovine tuberculosis' (Cobbett, 1931).

That primary tuberculous infection in man can confer some degree of protection against subsequent active disease has been inferred from the findings at human necropsies (Opie, 1917, 1924), from analogy with animals, and from the study of the evolution of tuberculosis among primitive peoples and in isolated European village communities (see Biraud, 1930). The ideal method of protection for the members of a community in which contact with open tuberculosis is unavoidable would be for every infant to be infected in such a way as to produce immunity against reinfection without risk of tuberculous disease resulting from the primary immunizing dose. Such appears to be the aim of Calmette's method of prophylactic immunization with B.C.G. vaccine (see Calmette, 1928, 1931; Chaussinand, 1931).[1] Until indisputable evidence of the efficacy of a method of this type has been obtained, it would seem best that individuals should receive their first infection naturally and under the most favourable conditions for regression of the focus and development of immunity, and that reinfection should take place under similar conditions as soon as this immunity wanes. It is important to consider how far the fulfilment of these requirements is possible.

The fact that natural tuberculous infection has a greater fatality in infancy than at other ages, at least among the poor (Section IV), provides theoretical justification for deferring, if possible, the date of first infection until later. The Norwegian town of Trondheim is a case in point, for, as stated above, postponement of the average date of primary infection appears to have occurred there during the last fifteen years. Statistics for this town show that so far no harm has arisen from this change, both the incidence of tuberculous meningitis and the total morbidity from tuberculosis having fallen during the period in question (Ustvedt, 1930).

It is obvious that prevention of exposure to human contagion in early life (Section VI) and pasteurization of milk are two measures

[1] For a summary of recent criticism of this method see Topley & Wilson, 1929 b, and Brit. Med. J., 1931, i. 1080.

that tend to defer first infection. Winslow & Gray (1924) have presented evidence suggesting that in the United States pasteurization has had a marked effect on non-pulmonary tuberculosis in children aged 1–4. The question whether sterilization of milk, while removing a source of danger in childhood, at the same time removes a source of protection against disease in adult life cannot yet be answered.

Further considerations are largely speculative. Krause (1925 a) believes that infants, when infected, are liable to succumb to tuberculosis more readily than older children because of peculiar opportunities for such infection to be massive and repeated, owing to intimate contact with a tuberculous parent, relative, or nurse, or because of the large consumption of milk. In older children, on the other hand, contacts with tuberculosis are likely to be slighter and more transient than in infancy, especially if they take place outside the home, and the resulting infection is possibly more likely to be mild and to regress. In support of this view, the estimated tuberculosis death-rate among sensitized children of school age (6–15) is lower than in earlier life (Section IV). Consequently, protection against reinfection should be more safely attained during this period than in early childhood, and, having been more recently acquired, may possibly last farther into adult life.

From these considerations one might suggest that a tuberculized race would be safer when its first infection is received in middle and later childhood and outside the home, than when this occurs in infancy and early childhood and in the home environment. Moreover, it would seem advantageous for first infection to take place before adult life is reached, because of the partial immunity that may result, although only too often the latter appears insufficient to prevent active tuberculous disease ultimately developing from reinfection.

Before leaving this subject two important papers by Heimbeck (1927, 1928) must be considered. This worker followed up a series of 185 Pirquet-negative healthy young adults who came, the majority (126) from country districts or small towns, to work as nurses at Oslo municipal hospital in 1924, 1925, and 1926. Almost all of these girls became tuberculin-positive within one or two years of entering the hospital, and as many as 24 per cent. developed clinical tuberculosis;[1] whereas of 152 nurses who were Pirquet-positive on arrival only 3 per cent. contracted manifest disease. Heimbeck believed that childhood infection, and with it immunity, had become extinct in the Pirquet-negative group, although it seems as likely that infection had never occurred. His observations suggest that the outlook of healthy adults entering tuberculous surroundings is better if they have been previously infected and show positive von Pirquet reactions, than if they give negative tests. This is what would be expected from animal experiments. The work, however, requires confirmation with a

[1] This number, however, includes 13 cases of erythema nodosum, which were regarded as tuberculous because they were all found to give strong positive tuberculin reactions after the diagnosis was made, and because 4 of them developed other tuberculous manifestations later. If the other 9 cases are excluded, the percentage of this group that developed clinical tuberculosis is reduced from 24 to 19.

more delicate test, for a negative von Pirquet skin reaction does not necessarily exclude tuberculin sensitivity. Moreover, the findings are opposed to those of Ustvedt (1930), who reported little difference in the fate of school-children living in tuberculous surroundings, according to whether they were Pirquet-positive or negative at a given date. If Heimbeck's views prove correct, the tuberculin test will assume a new role of importance in distinguishing partially immune from non-immune healthy subjects, as for example in deciding the advisability of exposing nurses and students to the risk of repeated and massive infection during their hospital training.[1]

(c) *The Present Mode of Infection of London Children.*

The lower incidence of positive tuberculin reactions among clinically non-tuberculous London children, as compared with some other towns, has been alluded to. Assuming that the figures are representative of the hospital class of London, certain inferences as to the usual mode of infection of the children can be made. On reference to Chart 6 it will be noted that by school age only one-fifth, and by 10 years about two-fifths, are positive reactors, but that practically all are tuberculin-sensitive when adult life is reached. In other words, most of the tuberculization appears to take place in later childhood and adolescence, i.e. at a period when the principal activities of the individual are away from the home, and when exposure is likely to be chance and diffuse.[2] This mode of infection resembles that of children in non-tuberculous homes, but contrasts with that of children living in tuberculous households, most of whom appear to receive their infection in infancy and early childhood, and in the home environment, where exposure is likely to be frequent and intense (pp. 63, 65, 82). The first is possibly the better mode of infection for the reasons given in the preceding sub-section. The present extensive tuberculization of clinically non-tuberculous adults of the hospital class also is possibly fortunate, because of the partial protection conferred by their childhood infection.

Attempts have been made to apply mathematical formulae to tuberculin reaction frequency curves. Brunn plotted the logarithms of the percentage of children negative at each age in his Kiel series against the age, and found that the points lay approximately on a straight line, suggesting that a constant fraction of the negative reactors remaining became positive each year. The London curve, on the other hand, is itself approximately rectilinear, the percentage positive increasing steadily from birth to manhood. The rectilinear shape may be due to the large age-groups examined. If not, it suggests that the percentage of the community (hospital class) that

[1] In a more recent paper the number of probationer nurses examined by Heimbeck (1929 a) is increased to 299 with negative von Pirquet reactions on entry, and 282 who gave positive tests. Twenty-two per cent. of the first, and 1·5 per cent. of the second group are stated to have developed clinical tuberculosis since their entry to hospital in 1924 or later, up to the date of publication.

[2] In the series of Dow & Lloyd (see footnote 2, p. 76), from the Brompton Chest Hospital, 50 per cent. of the children were positive by 10 years of age.

changes from negative to positive each year, i.e. the attack-rate (p. 6), is the same from birth to adult life; and that the proportion of negatives remaining that becomes positive each year increases with age with growing rapidity according to a rectangular hyperbola. This is in keeping with the probable present mode of exposure in London, for the great majority of the urban population make their contacts with tuberculosis outside their homes and, therefore, with a frequency that increases as they grow up to manhood. The reduction of natural curves to a mathematical basis is, however, admittedly dangerous, and the above interpretation may be fallacious.

The London figures do not permit a judgement of the relative importance of bovine and human infection. According to Griffith (1930), tuberculosis of bovine origin is proportionately more frequent in Great Britain than on the Continent or in North America. The recent wholesale pasteurization of the London milk combines cannot be expected to have had any effect as yet on the incidence of positive tuberculin reactions.

(d) Is the Tuberculization of the Community Symptomless?

The majority of the inhabitants of large towns receive their first tuberculous infection in childhood or during adolescence without being aware that this has taken place. There is, however, some evidence to show that even in these individuals first infection may be attended by slight and transitory symptoms, which are liable to be mistaken for influenza, chill, or other minor ailments. The most serious study of this subject has been made by Léon Bernard and his associates at the Laënnec Hospital Crèche, Paris. Some of their interesting results are given by Debré & Leplane (1924) and by Bernard (1927). These workers found that in a series of children observed by them the period between natural primary infection and the appearance of tuberculin hypersensitiveness was in most cases absolutely symptomless, but that its termination, as indicated by the elicitation of a positive tuberculin skin reaction, was in some instances attended by unobtrusive symptoms, such as fever, slight loss of weight, malaise, cough, digestive disturbance, and occasionally by radiographical changes. Fever was the commonest symptom noted. In the majority of their cases these symptoms subsided after a week or so, and the child recovered its previous health. In only the minority were the symptoms persistent and progressive, with the ultimate development of florid tuberculosis. The occurrence of this transient and benign illness accompanying primary infection demonstrates the faultiness of the definition of clinical tuberculosis adopted in this report (p. 6), but it would seem advisable to exclude the condition from that category until its incidence among the general population has been ascertained.

(e) The Future Trend of Tuberculous Infection.

Periodical surveys of the tuberculin reactions of the general population of large cities, by acting as records of the changing tuberculization of the community, would provide indications of the efficacy

of measures taken by the State to reduce the spread of infection, such as the control of open tuberculosis, the protection of young children from exposure to the disease, the pasteurization of milk, and the improvement of environmental conditions. Furthermore, such surveys would make it possible to construct charts showing the ratio of the tuberculosis death-rate to the tuberculin sensitization incidence at each age, as indicated in Section IV. The comparison of charts showing this ratio in various places, and in the same place from time to time, should yield evidence as to the optimal age-distribution of tuberculous infection.

What the future trend of tuberculous infection in healthy civilized urban communities will actually be is open to speculation. The probability of a reduction in the amount of infection, should the risk of exposure to contagion continue to decrease, raises the question whether or not this process can go too far. Postponement of the age of natural first infection from early to later childhood is likely to be beneficial by freeing the first years of life from danger. Should, however, a time come when tuberculous infection is rare at all ages, we may find ourselves in the precarious unprotected state of a non-tuberculized race; but before such an event can occur it is to be hoped that an altogether satisfactory method of prophylactic immunization will have been developed.

General Conclusions.[1]

1. The intracutaneous method is the most satisfactory tuberculin test in man, and should take the place of the subcutaneous and cutaneous (von Pirquet) methods for all purposes.

2. A positive tuberculin reaction indicates that the individual has at some time been infected by the tubercle bacillus; a negative reaction excludes the presence of infection with an accuracy depending upon the method and dosage used.

3. A positive response in infancy, more than at any other age, suggests that the tuberculous infection is progressive, that clinical tuberculosis is—or soon will be—present, and that the outlook is unfavourable. While its absolute value in these respects appears to be less at the present time than it was formerly believed to be, tuberculous disease should be suspected, and the prognosis guarded, in such cases, especially if obscure and persistent symptoms are present or the conditions of living are bad.

A positive reaction in a young child aged two to five, and showing symptoms, should suggest that these symptoms are tuberculous in origin.

4. In patients over five years of age the value of the positive test in the diagnosis of clinical tuberculosis is practically limited to observations made with weak dilutions of tuberculin, administered by the intracutaneous route. The interpretation of these observations requires considerable experience, and until wider use has been made of the methods they are not recommended for routine practice.

[1] Summaries will also be found in certain sections.

5. Tuberculin tests are of doubtful value in estimating the prognosis in clinical tuberculosis. According to some workers, however, useful information may be obtained from repeated intracutaneous tests.

6. When the conditions under which infection protects against, rather than produces, active disease are more fully understood, tuberculin tests may assume an important role in distinguishing immune from non-immune healthy subjects.

7. Conclusions must be drawn with caution from a negative von Pirquet test, owing to the relative insensitiveness of this method. A negative tuberculin reaction, obtained by the intracutaneous method when used in the manner recommended, excludes clinical tuberculosis with a very small error, except in patients tested a few days before death. The widely held opinion that the usefulness of tuberculin tests for the negative diagnosis of clinical tuberculosis is limited to early childhood is incorrect. At the present time the intracutaneous test can be profitably applied for this purpose at all ages up to adult life in large cities, and possibly even in adults who belong to the wealthier classes or who live in the country.

An increase in the advantage derived from using the intracutaneous test to exclude clinical tuberculosis will accompany any fall in the incidence of positive reactions among the apparently healthy members of the population, because this fall will increase the chance that a suspect patient whose clinical condition is really non-tuberculous is free of all tuberculous infection and will, therefore, give a negative reaction.

8. The diagnosis of tuberculosis in childhood should never be made without performing a tuberculin test, unless there is clear bacteriological or pathological evidence of the disease.

9. Tuberculin tests have a special application in certain skin conditions, e.g. lupus pernio and the sarcoids, which, although believed to be tuberculous in origin, appear to be associated with loss of tuberculin reactivity. Here a positive reaction is evidence against the diagnosis, while a negative response, demonstrated by the intracutaneous method in maximal doses, strongly supports it.

10. Estimations of the tuberculosis death-rate in the tuberculin-sensitized population at each age-period at the present time indicate that this is higher in infancy than in older children (cf. Conclusion 3). It is to be hoped that these rates will fall in the future. Should this fall principally affect infants, tuberculous infection may cease to be most fatal in early life.

11. Positive tuberculin reactions are more frequent among children of tuberculous households than among children whose homes are free of clinical tuberculosis. Children who have tuberculous relatives not living with them also run the risk of infection, depending on the intimacy of their contact with these relatives. Non-pulmonary and closed pulmonary tuberculosis do not appear to be sources of infection.

12. It is suggested that steps should be taken to improve the present methods of preventing tuberculosis in children of tuberculous parents. The formation of further settlements of the Papworth type might possibly be considered, together with a voluntary system for the

separation of infants from their parents before infection, as shown by a positive tuberculin reaction, has taken place. These infants should be placed in healthy families for a period of several years. If infection has already occurred, the advantage of separation is less certain.

Home conditions are an important factor in the fate of children living in tuberculous families, and their amelioration may compensate to a certain extent for the presence of an infective adult. Whether this result is due more to prevention or limitation of infection, than to an effect on its subsequent course in the body, is not yet clear.

13. Variations in the incidence of positive reactions in different sections of the community, when not due to technique, can be usually accounted for by differences in the opportunities for exposure to contagion.

14. Correlation between the various tuberculin surveys is made difficult by the diversity of methods used. Until workers adopt a uniform technique, and use a tuberculin whose potency is known in terms of the International Standard Tuberculin, little further progress is likely to be made. Meanwhile, greater importance must be attached to the results obtained by using the intracutaneous method or the *stichreaktion*, than to those based on the von Pirquet test.

15. The belief, based on the results of some of the prominent earliest tuberculin surveys, that tuberculous infection is universal in later childhood among the poorer classes or general population of urban communities does not appear to be true at the present time of London and some other European and American cities, where a third or more of the clinically non-tuberculous children react negatively to the intracutaneous test at the age of puberty. In certain instances, however, extensive tuberculization has been noted recently.

16. A reduction in the incidence of tuberculin sensitization in the community may be expected to follow a further decline in the phthisis death-rate, improvement in the standard of living, and public measures directed to the limitation of exposure, e.g. by pasteurization of milk. There is already some evidence of a reduction among children in a few cities during the last twenty years, but further information is needed before the significance of this change can be assessed. The reduction is believed to be the manifestation of postponement of first infection.

17. Tuberculous infection in London children of the poorer classes appears at the present time to be acquired for the most part in later childhood and adolescence, i.e. during a period when the principal activities of the individual are away from the home. Almost all are sensitive to tuberculin when adult life is reached.

18. Systematic tuberculin tests on children may throw light on a new clinical entity—a transient and benign illness accompanying tuberculous first infection. Owing to the unobtrusive and temporary nature of its symptoms, it is likely that the tuberculous origin of this illness usually escapes recognition. The detection of a positive skin reaction in a previously tuberculin-negative individual may assist in establishing the tuberculous nature of the symptoms.

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The 1,030 cases composing the series of clinical tuberculosis used in the London investigation described in this report were drawn from the following sources: Alexandra Hospital, Swanley, Kent (Mr. A. L. Moreton); Colindale Hospital (Dr. Marcus Paterson); Highwood Hospital, Brentwood, Essex (Dr. C. D. S. Agassiz); Holborn & Finsbury Hospital (Dr. T. Evans); Lewisham Hospital (Dr. H. Nockolds); Lord Mayor Treloar's Hospital, Alton, Hants (Sir Henry J. Gauvain); North Islington Tuberculosis Dispensary (Dr. W. E. Snell); Queen Mary's Hospital for Children, Carshalton, Surrey (Dr. W. T. G. Pugh); Royal National Orthopaedic Hospital, London; Royal National Orthopaedic Hospital, Stanmore, Middlesex (Mr. J. B. Barnett); Royal Northern Hospital; St. Columba's Hospital, Swiss Cottage (Dr. J. Clark Wilson); St. Mary's Hospital, Islington (Dr. W. R. M. Turtle); Hospital for Sick Children, Great Ormond Street; and University College Hospital.

The majority of the 751 clinically non-tuberculous subjects who formed the material for the survey of the incidence of tuberculous infection in London were patients in the wards and out-patient department of the Royal Northern Hospital, and children under the care of Dr. Bernard Schlesinger in the out-patient department of the Hospital for Sick Children. The latter hospital derives its patients from the whole of London. The remainder of this series were drawn from the wards of the Royal National Orthopaedic Hospital, London and Stanmore (33 children), the Heart Home, West Wickham (20 children), and Lord Mayor Treloar's Hospital, Alton (16 adolescents).

Many of the 118 contact children who formed the material for the London investigation of the effect of contagion in tuberculous households were drawn from the contact clinic of the North Islington Tuberculosis Dispensary, under the charge of Dr. W. E. Snell. The remainder were patients under the care of Dr. Bernard Schlesinger in the Children's Department of the Royal Northern Hospital and in the out-patient department of the Hospital for Sick Children. The controls were drawn from the latter two sources.

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TABLE 2.

Error of the von Pirquet Test in the Negative Diagnosis of Clinical Tuberculosis.

(*From Literature, 1907–28: forty-two authors.*) (See p. 42.)

	Number tested.	*Number negative.*	*Percentage negative = error.*
Total	4,787	766	16·0
Total adults and adolescents only .	2,168	212	9·8
Total children only	1,933	445	23·0
Total children, excluding series composed entirely of miliary Tb. or Tb. meningitis	1,326	218	16·5
Total series composed entirely of miliary Tb. or Tb. meningitis . . .	607	227	37·5

NOTE. Details of each series are given in Appendix 1.

TABLE 3.

Error of the Mantoux Intracutaneous Test in the Negative Diagnosis of Clinical Tuberculosis.

(*From Literature, 1909–31: fourteen authors.*) (See p. 44.)

	Number tested.	*Number negative.*	*Percentage negative = error.*
Total	1,061	103	9·7
Total children only . . .	589	68	11·5

NOTE. Details of each series are given in Appendix 2.

TABLE 4.

Conditions that may produce Non-reactivity to the von Pirquet Test in Clinical Tuberculosis or in Subliminal Tuberculous Infection.[1]

(*From Literature.*) (See p. 43.)

1. Acute tuberculosis. Very active tuberculosis. Very heavily infected cases.
2. Miliary tuberculosis. Tuberculous meningitis. (Happ & Casparis, 1922; Loewenstein, 1928.)
3. Advanced pulmonary tuberculosis with marked toxaemia.
4. Cachexia. Moribund patients. Patients a week or less before death. (von Pirquet, 1908 b.)
5. Tuberculosis of serous membranes. (Kolmer, 1923 b.)
6. Lupus vulgaris confined to mucous membranes.
7. Certain skin conditions, believed to be tuberculous—lupus pernio, lupus miliaris, and the sarcoids. (Schaumann, 1924; Jadassohn, 1929.)
8. During tuberculin therapy. (Hamman & Wolman, 1912 a; Bandelier & Roepke, 1922.)
9. After ultra-violet ray treatment. (Morabito, 1926.)
10. After cure of once active clinical tuberculosis. (Hamburger, 1912 c; Krause, 1925 a.) After infection has become extinct. (Austrian, 1924; and see p. 21.)
11. Tuberculosis complicated by measles (von Pirquet, 1908 b, 1908 c; Lesné & Coffin, 1926); *pneumonia*; *pertussis* (Nobécourt & Forgeron, 1922); *scarlet fever*

[1] The conditions in italics have been reported responsible for non-reactivity only in individuals with subliminal infection (p. 6). The other conditions are said to produce non-reactivity in clinical tuberculosis as well.

(Rolly, 1910); *diphtheria*; *typhoid*; *influenza* (Bloomfield & Mateer, 1919); *secondary syphilis* (Duval, 1923); vaccinia, &c. For a variable period subsequent to these complications.

12. *During the incubation or latent period following tuberculous infection.* (Debré & Jacquet, 1920; and see p. 20.)
13. *General debility. Malnutrition.* (Rominger, 1920.)
14. *Pregnancy and lactation.* (Nobécourt & Paraf, 1920.) *Menstruation; after ovariotomy.* (Coulaud, 1921.)
15. *After general anaesthetics.* (Decressac & Jacquelin, 1924.)
16. *Old Age.* (Heimbeck, 1927.)
17. *During typhoid immunization.* (Mayer, 1919.)

Table 5.

Distribution of Cases of Clinical Tuberculosis according to Age and Type.

(*London Investigation, 1929.*) (See p. 47.)

Age-groups.	*Pulmonary.*	*Bone or Joint.*	*Lupus vulgaris.*	*Miliary with Meningitis.*	*Pleurisy and/or Peritonitis.*	*Adenitis.*	*Genito-urinary cases, unclassified cases, and cases with disease in more than one system.*	*Total.*
0–5	4 (3)	139	3	3	4	9	5	167
6–10	15 (10)	176	7		11	11	2	222
11–20	126[1] (117)	123	22	2	9	8	10	300
21–	291 (280)	16	7		3	4	20	341
All ages	436 (410)	454	39	5	27	32	37	1,030

[1] 77 (72) aged 11–15.

Note. Bracketed figures refer to patients known to have had positive sputum at some period.

TABLE 6.

General results of Intracutaneous Test on 1,030 Cases of Clinical Tuberculosis, all Types and Stages, grouped according to Age and Dilution of Tuberculin used.

(*London Investigation, 1929.*) (See p. 48.)

Type of Tuberculosis.	Age-group.	Dilution.	*Number of cases in the series.*	*Number*[1] *of cases tested.*	*Number negative of cases tested.*	*Percentage negative of cases tested.*	Adjusted[2] percentage negative of series = error.	Adjusted[2] percentage positive of series.
All types and stages.	0–5	1 : 10,000	167	158	25	16	16	84
		1 : 1,000	,,	167	10	6	6	94
		1 : 100	,,	10	7	70	4	96
		1 : 10	,,	7	7	100	4	96
		1 : 1	,,	7	6	86	3·5	96·5
	6–10	1 : 10,000	222	216	23	11	11	89
		1 : 1,000	,,	222	8	3·5	3·5	96·5
		1 : 100	,,	8	7	87	3	97
		1 : 10	,,	7	7	100	3	97
		1 : 1	,,	7	7	100	3	97
	11–20	1 : 10,000	300	250	26	10	10	90
		1 : 1,000	,,	300	8	3	3	97
		1 : 100	,,	8	5	62	2	98
		1 : 10	,,	5	3	60	1	99
		1 : 1	,,	3	3	100	1	99
	21–	1 : 10,000	341	142	19	13	13	87
		1 : 1,000	,,	341	12	3·5	3·5	96·5
		1 : 100	,,	12	9	75	2·5	97·5
		1 : 10	,,	4	2	50	1·5	98·5
		1 : 1	,,	2	2	100	1·5	98·5
	All ages.	1 : 10,000	1,030	766	93	12	12	88
		1 : 1,000	,,	1,030	38	3·7	3·7	96·3
		1 : 100	,,	38	28	74	2·75	97·3
		1 : 10	,,	23	19	83	2·3	97·7
		1 : 1	,,	19	18	95	2·2	97·8

[1] Figures for 1 : 10,000 refer to patients selected at random from cases in the series.
,, ,, 1 : 1,000 comprise all the cases in the series.
,, ,, 1 : 100, 1 : 10, and 1 : 1 consist of all the cases negative to the previous dilution, except in the adult age-group, where only 4 of the 9 patients negative to 1 : 100 were tested with 1 : 10.

[2] This percentage is only adjusted for the higher strengths of the adult age-group, where it is adjusted on the assumption that the 5 patients negative to 1 : 100 who were not tested with 1 : 10 tuberculin would have behaved like those who were tested.

TABLE 7.

Intracutaneous Test in Pulmonary Tuberculosis.

(*London Investigation, 1929.*) (See p. 49.)

Type of Tuberculosis.	Age-group.	Dilution.	*Number[1] of cases in the series.*	*Number[2] of cases tested.*	*Number negative of cases tested.*	*Percentage negative of cases tested.*	Adjusted[3] percentage negative of series = error.	Adjusted[3] percentage positive of series.
Pulmonary, all stages	0–15	1 : 10,000	96 (85)	96	11	11	11	89
		1 : 1,000	,,	96	4	4	4	96
		1 : 100	,,	4	3	75	3	97
		1 : 10	,,	3	3	100	3	97
		1 : 1	,,	3	3	100	3	97
	16–	1 : 10,000	340 (325)	122	18	15	15	85
		1 : 1,000	,,	340	14	4	4	96
		1 : 100	,,	14	9	64	2·5	97·5
		1 : 10	,,	5	2	40	1	99
		1 : 1	,,	2	2	100	1	99
	All ages	1 : 10,000	436 (410)	218	29	13	13	87
		1 : 1,000	,,	436	18	4	4	96
		1 : 100	,,	18	12	67	2·7	97·3
		1 : 10	,,	8	5	62·5	1·7	98·3
		1 : 1	,,	5	5	100	1·7	98·3
Pulmonary, advanced with marked toxaemia	All[4] ages	1 : 1,000	93	93	14	15	15	85
		1 : 100	,,	14	8	57	8·5	91·5
		1 : 10	,,	5	2	40	3·5	96·5
		1 : 1	,,	2	2	100	3·5	96·5

[1] 77 (72) of the 96 cases in age-group 0–15 were aged 11–15, and 49 (45) in the age-group 16– were aged 16–20. The figures in brackets refer to patients known to have had positive sputum at some period.

[2] Figures for 1 : 10,000 refer to patients selected at random from cases in the series.
,, ,, 1 : 1,000 comprise all the cases in the series.
,, ,, 1 : 100, 1 : 10, and 1 : 1 consist of all the cases negative to the previous dilution, except in the adult age-group, and in the advanced cases with severe toxaemia, where only 5 and 5 of the 9 and 8 patients, respectively, negative to 1 : 100 were tested with 1 : 10.

[3] This percentage is only adjusted for the higher strengths of the adult age-group and of the advanced toxaemic cases, where it is adjusted on the assumption that the patients (see footnote 2) negative to 1 : 100 who were not tested with 1 : 10 tuberculin would have behaved like those who were tested.

[4] 3 under 11 years, 19 aged 11–20, and remainder adults.

TABLE 8.

Intracutaneous Test in Bone or Joint Tuberculosis.

(*London Investigation, 1929.*) (See p. 49.)

Type of Tuberculosis.	Age-group.	Dilution.	*Number[1] of cases in the series.*	*Number[2] of cases tested.*	*Number negative of cases tested.*	*Percentage negative of cases tested.*	Adjusted[3] percentage negative of series = error.	Adjusted[3] percentage positive of series.
Bone and/or Joint	0–5	1 : 10,000	139	136	19	14	14	86
		1 : 1,000	,,	139	6	4	4	96
		1 : 100	,,	6	4	67	3	97
		1 : 10	,,	4	4	100	3	97
		1 : 1	,,	4	3	75	2	98
	6–10	1 : 10,000	176	169	17	10	10	90
		1 : 1,000	,,	176	4	2	2	98
		1 : 100	,,	4	4	100	2	98
		1 : 10	,,	4	4	100	2	98
		1 : 1	,,	4	4	100	2	98
	11–	1 : 10,000	139	127	13	10	10	90
		1 : 1,000	,,	139	2	1·5	1·5	98·5
		1 : 100	,,	2	2	100	1·5	98·5
		1 : 10	,,	1	1	100	1·5	98·5
		1 : 1	,,	1	1	100	1·5	98·5
	All ages	1 : 10,000	454	432	49	11	11	89
		1 : 1,000	,,	454	12	2·6	2·6	97·4
		1 : 100	,,	12	10	83	2·2	97·8
		1 : 10	,,	9	9	100	2·2	97·8
		1 : 1	,,	9	8	90	2·0	98·0

[1] 123 of the 139 cases in the age-group 11– were aged 11–20.

[2] Figures for 1 : 10,000 refer to patients selected at random from cases in the series.
,, ,, 1 : 1,000 comprise all the cases in the series.
,, ,, 1 : 100, 1 : 10, and 1 : 1 consist of all the cases negative to the previous dilution, except in the upper age-group, where only 1 of the 2 patients negative to 1 : 100 was tested with 1 : 10.

[3] This percentage is only adjusted for the higher strengths of the upper age-group, where it is adjusted on the assumption that the patient negative to 1 : 100 who was not tested with 1 : 10 tuberculin would have behaved like the other patient who was tested.

Table 9.

The Intracutaneous Test in Various Forms of Tuberculosis.

(*London Investigation, 1929.*) (See p. 49.)

Type of tuberculosis.	Age-group.	Dilution.	*Number[1] of cases in the series.*	*Number[2] of cases tested.*	*Number negative of cases tested.*	*Percentage negative of cases tested.*	Percentage negative of series = error.	Percentage positive of series.
(a) Lupus vulgaris	all ages	1 : 10,000	39	36	3	8	8	92
		1 : 1,000	,,	39	1	3·5	3·5	96·5
		1 : 100	,,	1	0	0	0	100
(b) Pleurisy and/or Peritonitis	all ages	1 : 1,000	27	27	2	7	7	93
		1 : 1	,,	2	2	100	7	93
(c) Adenitis	all ages	1 : 10,000	32	24	3	13	13	87
		1 : 1,000	,,	32	1	3	3	97
		1 : 1	,,	1	1	100	3	97
(d) Miliary with Meningitis	all ages	1 : 1,000	5	5	1	20	20	80
		1 : 100	,,	1	0	0	0	100
(e) Genito-urinary Tb., unclassified Tb., and cases with disease localized in more than one system	all ages	1 : 1,000	37	37	3	8	8	92
		1 : 100	,,	3	3	100	8	92
		1 : 10	,,	3	2	67	5	95
		1 : 1	,,	2	2	100	5	95

[1] See Table 5 for age distribution.

[2] Figures for 1 : 10,000 refer to patients selected at random from cases in the series.
,, ,, 1 : 1,000 comprise all the cases in the series.
,, ,, 1 : 100, 1 : 10, and 1 : 1 consist of all the cases negative to the previous dilution.

TABLE 10.

Comparison of Results of Intracutaneous Test in Active and Quiescent Bone or Joint Tuberculosis.

(*London Investigation, 1929.*) (See p. 49.)

Bone and/or Joint Tuberculosis.	Age-group.	Dilution.	*Number[1] of cases in the series.*	*Number[2] of cases tested.*	*Number negative of cases tested.*	*Percentage negative of cases tested.*	Adjusted[3] percentage negative of series = error.	Adjusted[3] percentage positive of series.
Active	0–10	1 : 10,000	202	197	27	14	14	86
		1 : 1,000	,,	202	8	4	4	96
		1 : 100	,,	8	7	87	3·5	96·5
		1 : 10	,,	7	7	100	3·5	96·5
		1 : 1	,,	7	6	86	3	97
	11–	1 : 10,000	55	55	6	11	11	89
		1 : 1,000	,,	55	2	3·6	3·6	96·4
		1 : 100	,,	2	2	100	3·6	96·4
		1 : 10	,,	1	1	100	3·6	96·4
		1 : 1	,,	1	1	100	3·6	96·4
Quiescent or approaching quiescence	0–10	1 : 10,000	66	60	4	6·7	6·7	93·3
		1 : 1,000	,,	66	2	3	3	97
		1 : 100	,,	2	1	50	1·5	98·5
		1 : 10	,,	1	1	100	1·5	98·5
		1 : 1	,,	1	1	100	1·5	98·5
	11–	1 : 10,000	59	53	5	9·4	9·4	90·6
		1 : 1,000	,,	59	0	0	0	100

[1] 48 of the 55 cases in the 'Active' age-group 11– were aged 11–20.
56 of the 59 cases in the 'Quiescent' age-group 11– were aged 11–20.

[2] Figures for 1 : 10,000 refer to patients selected at random from cases in the series.
,, ,, 1 : 1,000 comprise all the cases in the series.
,, ,, 1 : 100, 1 : 10, and 1 : 1 consist of all the cases negative to the previous dilution, except in the 'Active' upper age-group, where only 1 of the 2 patients negative to 1 : 100 was tested with 1 : 10.

[3] This percentage is only adjusted for the higher strengths of the 'Active' upper age-group, where it is adjusted on the assumption that the patient negative to 1 : 100 who was not tested with 1 : 10 tuberculin would have behaved like the other patient who was tested.

Table 11.

Results of Intracutaneous Test on 751 Clinically Non-tuberculous Controls, grouped according to Age and Dilution of Tuberculin used.

(*London Investigation, 1929.*) (See p. 52.)

Age last birth-day.	Dilution.	*Number of cases in the series.*	*Number*[1] *of cases tested.*	*Number negative of cases tested.*	*Percentage negative of cases tested.*	Adjusted[2] percentage negative of series.	Adjusted[2] percentage positive of series.
0–2	1 : 10,000	121	20	18	—	—	—
	1 : 1,000	,,	121	113	93·5	93·5	6·5
	1 : 100	,,	83	83	100	93·5	6·5
	1 : 10	,,	8	8	100	93·5	6·5
	1 : 1	,,	6	6	100	93·5	6·5
3–5	1 : 10,000	124	36	32	89	89	11
	1 : 1,000	,,	124	103	83	83	17
	1 : 100	,,	69	68	99	82	18
	1 : 10	,,	7	7	100	82	18
	1 : 1	,,	6	6	100	82	18
6–10	1 : 10,000	245	79	62	78	78	22
	1 : 1,000	,,	245	170	69·5	69·5	30·5
	1 : 100	,,	117	113	97	67	33
	1 : 10	,,	13	12	92	62	38
	1 : 1	,,	8	8	100	62	38
11–20	1 : 10,000	127	58	34	59	59	41
	1 : 1,000	,,	127	50[3]	39·5	39·5	60·5
	1 : 100	,,	37	33	89	35	65
	1 : 10	,,	15	14	93	33	67
	1 : 1	,,	13	12	92	30[4]	70
21–	1 : 10,000	134	102	38	37	37	63
	1 : 1,000	,,	134	16	12	12	88
	1 : 100	,,	12	7	58	7	93
	1 : 10	,,	6	4	67	5	95
	1 : 1	,,	2	2	100	5	95

[1] Figures for 1 : 10,000 refer to individuals selected at random from cases in the series.
,, ,, 1 : 1,000 comprise all the cases in the series.
,, ,, 1 : 100, 1 : 10, and 1 : 1 consist of individuals selected at random from cases negative to the previous dilution.

[2] Adjusted on the assumption that the individuals negative to 1 : 1,000, 1 : 100, or 1 : 10 who were not tested with 1 : 100, 1 : 10, or 1 : 1 tuberculin, respectively, would have behaved like the individuals who were so tested.

[3] 42 negative of 97 aged 11–15, i.e. 43 per cent.

[4] Percentage negative (adjusted) of the 97 aged 11–15 also = 30.

Table 12.

Summarized Results of Intracutaneous Tuberculin Test in 1,030 cases of Clinical Tuberculosis, and in 751 Clinically Non-tuberculous Controls.

(*London Investigation, 1929.*)

	Table.	Type of tuberculosis.	Age-group.	% positive to dilution of tuberculin. 1 : 10,000	1 : 1,000	1 : 100	1 : 10	1 : 1
Clinically non-tuberculous controls.	11	——	0–2	—	6·5	6·5	6·5	6·5
			3–5	11	17	18	18	18
			6–10	22	30·5	33	38	38
			11–20	41	60·5	65	67	70
			21–	63	88	93	95	95
Clinical tuberculosis.	6	All types and stages.	0–5	84	94	96	96	96·5
			6–10	89	96·5	97	97	97
			11–20	90	97	98	99	99
			21–	87	96·5	97·5	98·5	98·5
			All ages.	88	96·3	97·3	97·7	97·8
	7	Pulmonary—all stages.	0–15	89	96	97	97	97
			16–	85	96	97·5	99	99
			All ages.	87	96	97·3	98·3	98·3
	7	Pulmonary—advanced with marked toxaemia.	All ages.	—	85	91·5	96·5	96·5
	8	Bone and/or joint.	0–5	86	96	97	97	98
			6–10	90	98	98	98	98
			11–	90	98·5	98·5	98·5	98·5
			All ages.	89	97·4	97·8	97·8	98·0
	9	Lupus vulgaris.	All ages.	92	96·5	100	100	100
	9	Pleura and/or peritoneum.	All ages.	—	93	93	93	93
	9	Glands.	All ages.	87	97	97	97	97
	9	Miliary with meningitis.	All ages.	—	80	100	100	100
	10	Active bone or joint Tb.	0–10	86	96	96·5	96·5	97
			11–	89	96·5	96·5	96·5	96·5
	10	Bone or joint Tb.—quiescent or approaching quiescence.	0–10	93·3	97	98·5	98·5	98·5
			11–	90·5	100	—	—	—

Table 13.

London Investigation of Contagion, 1929: Comparison of Tuberculin Reactions in Home-contacts of Pulmonary Tuberculosis and Non-contact Controls.

(*From Schlesinger & Hart, 1930.*) (See p. 65.)

Age last birthday.	*Home-contacts[1] of pulmonary tuberculosis.*			*Non-contact[1] controls.*		
	Number tested.	*Number positive.*	*Percentage positive.*	*Number tested.*	*Number positive.*	*Percentage positive.*
0–2	11	4	36	109	5	4·5
3–5	21	8	38	112	17	15
6–10	36	28	78	217	58	27
11–15	19	14	74	75	44	59
16–20	2	2	—	28	20	71
21–	14	13	93	118	106	90
0–10	68	40	59	438	80	18
0–15	87	54	62	513	124	24

[1] See definition, p. 7.

NOTE. Results are shown graphically on Charts 3 and 4.

Table 14.

London Investigation of Contagion, 1929: Tuberculin Reactions in Home-contacts of Various Types compared with those in Non-contact Controls.

(*From Schlesinger & Hart, 1930.*) (See p. 66.)

Group.	*Number tested aged 0–10.*	*Number positive.*	*Percentage positive.*
Home-contacts of pulmonary tuberculosis .	68	40	59
Home-contacts of open pulmonary tuberculosis (bacilli in sputum)	42	29	69
Home-contacts of pulmonary tuberculosis where sputum result was negative or unknown	26	11	42
Home-contacts of non-pulmonary tuberculosis.	26	4	15
Non-contact controls	438	80	18
Bedroom-contacts of open pulmonary tuberculosis (bacilli in sputum)	34	24	71

TABLE 15.

London Investigation of Contagion, 1929: Tuberculin Reactions in Various Types of Non-contact Controls.

(*From Schlesinger & Hart, 1930.*) (See p. 66.)

Children who are not home-contacts but have—	*Number tested aged 0–10.*	*Number positive.*	*Percentage positive.*
Two or more tuberculous relatives[1] [2] . .	35	16	46
One tuberculous relative[1] [3]	50	11	22
No tuberculous relatives	353	53	15
Total = non-contact controls[4] . . .	438	80	18

[1] Sibling, grandparent, uncle, aunt, great-uncle, great-aunt, great-grandparent, first cousin,—living or dead.

[2] In 26 cases one or more of the relatives was stated to have had phthisis; in the remainder the type of disease was not known. Eleven of the 26 children reacted positively.

[3] In 32 cases the relative was stated to have had phthisis; in the remainder the type of disease was not known. Eight of the 32 children reacted positively.

[4] See definition, p. 7.

CHARTS

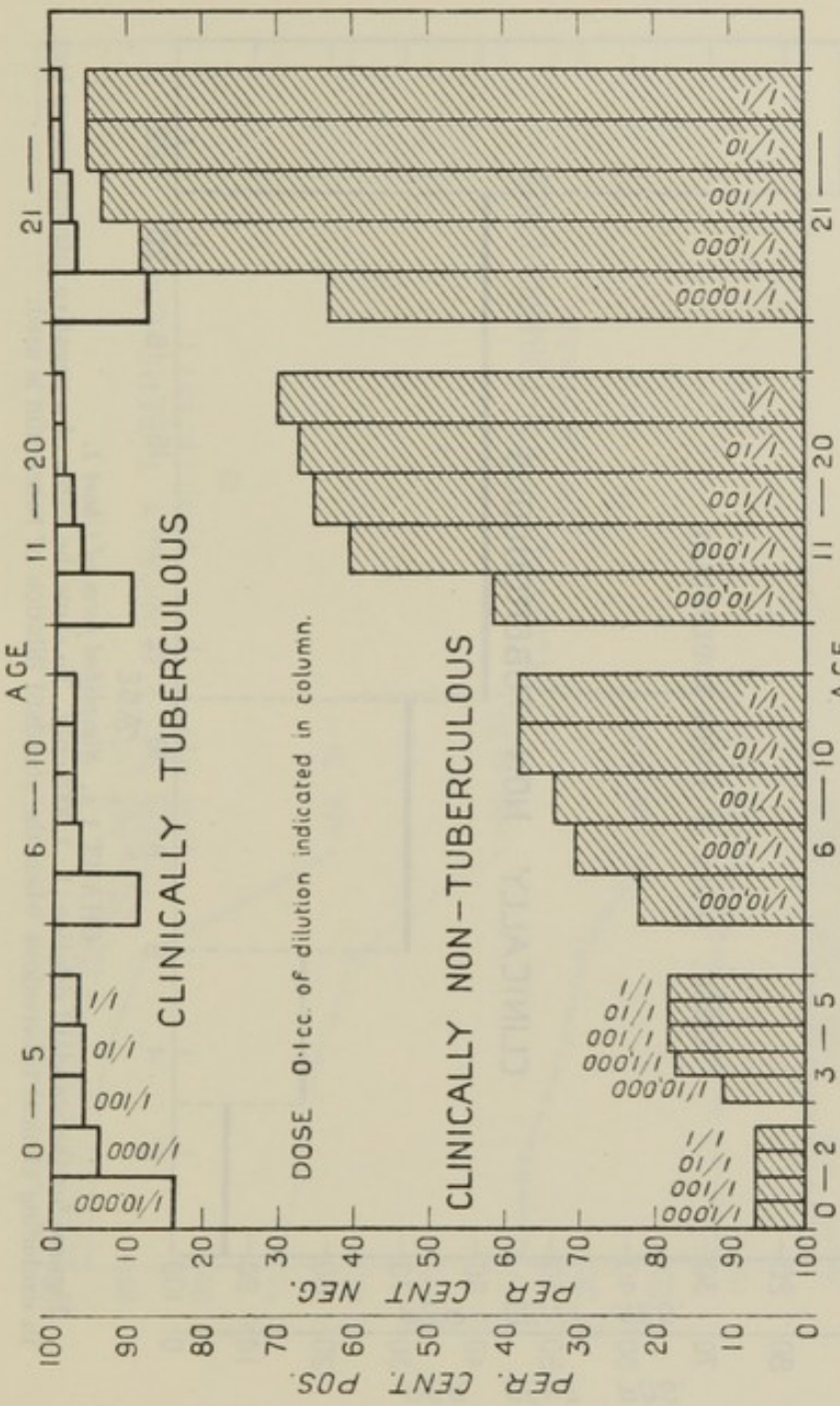


CHART 1. *Intracutaneous tuberculin reactions in (1) the clinically tuberculous (1,030 cases—all types and stages together); (2) the clinically non-tuberculous (751 cases).* (London Investigation, 1929.)

To show the value of a negative test in excluding clinical tuberculosis when various dilutions of tuberculin are used.

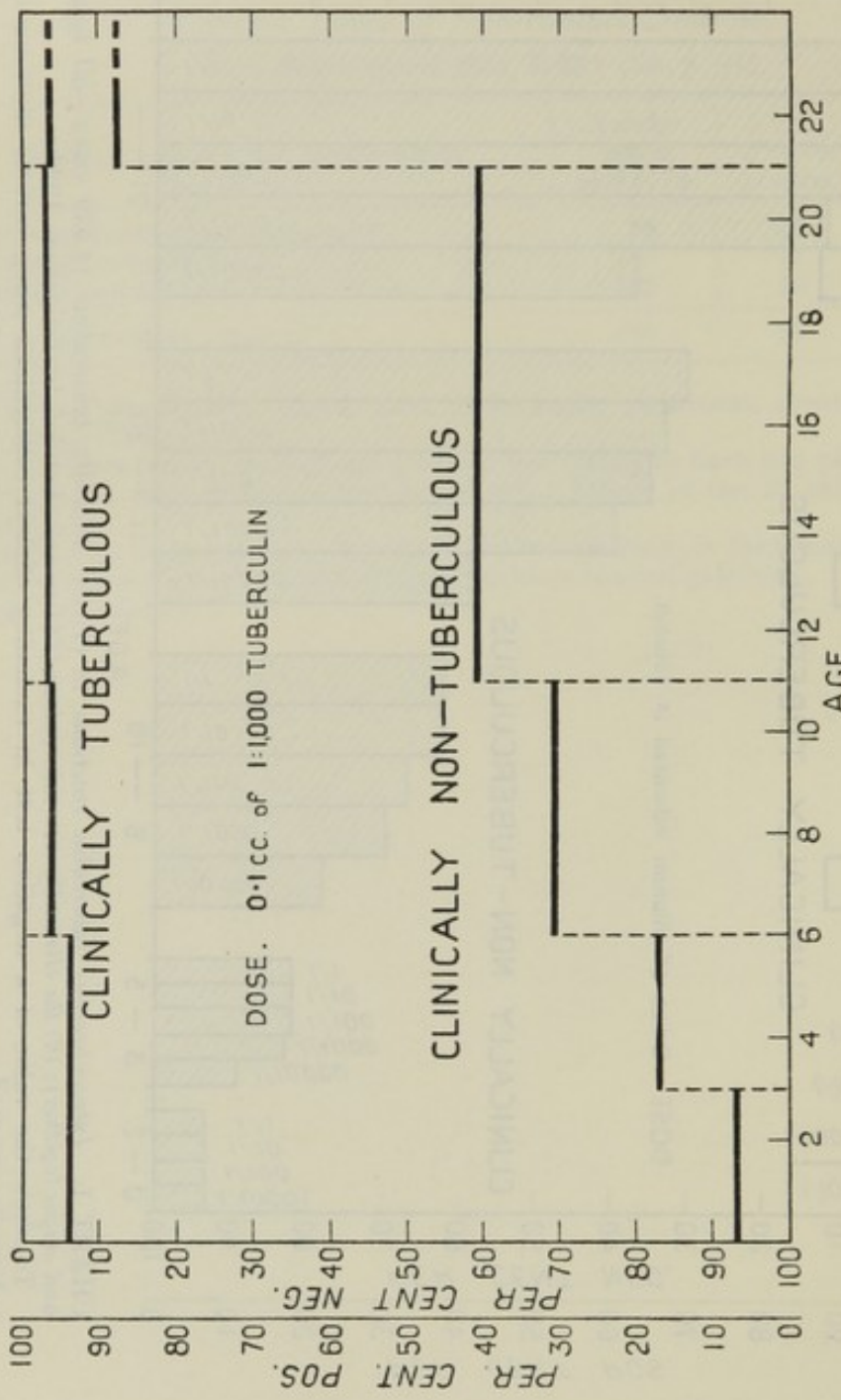


CHART 1 A. *Simplified form of Chart 1.*

Figures for the standard dilution (1 : 1,000) only. To show the value of a negative intracutaneous test in excluding clinical tuberculosis when the standard dilution of tuberculin is used.

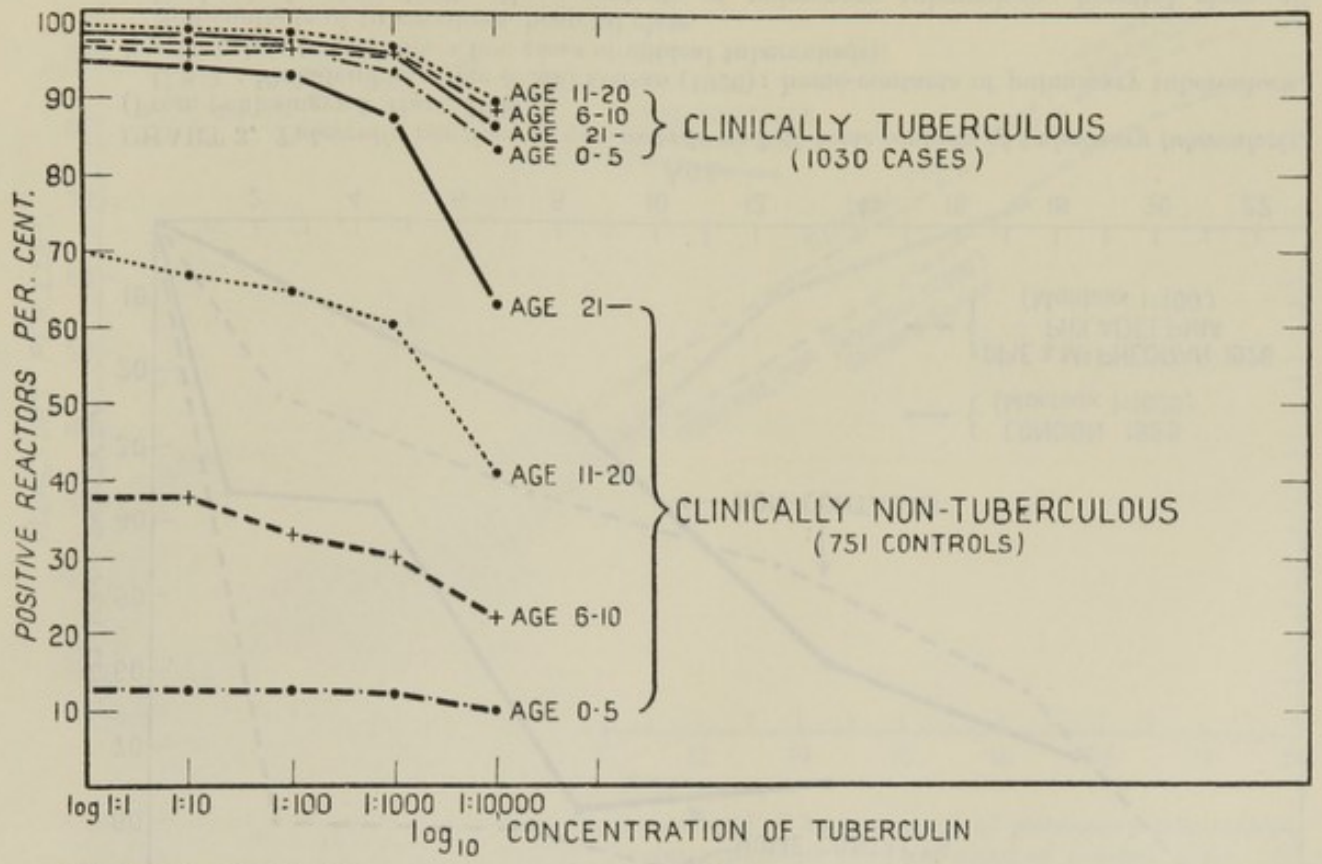


CHART 2. *Relation between the percentage of positive reactors to the intracutaneous test among the clinically tuberculous (all types and stages) and non-tuberculous and the concentration of tuberculin used.* (London Investigation, 1929.)

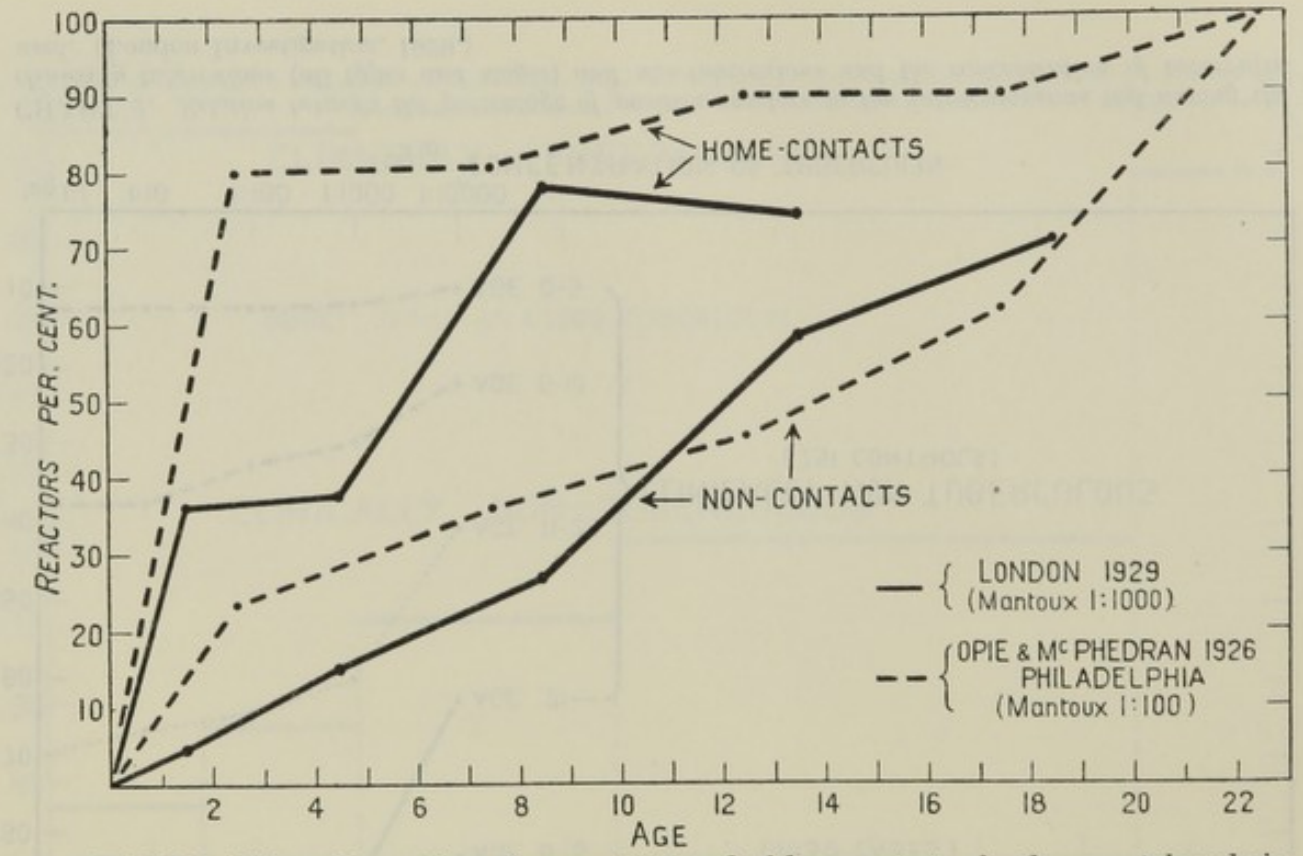


CHART 3. *Tuberculin reactions of non-contacts and of home-contacts of pulmonary tuberculosis.* (From Schlesinger & Hart, 1930.)

U.S.A.: Philadelphia. Opie & McPhedran (1926): home-contacts of pulmonary tuberculosis, hospital class (? includes a few cases of clinical tuberculosis).

Non-contacts of tuberculosis, hospital class.

England: London, 1929. Home-contacts of pulmonary tuberculosis, hospital class, all clinically non-tuberculous.

Non-contacts of tuberculosis, hospital class, all clinically non-tuberculous.

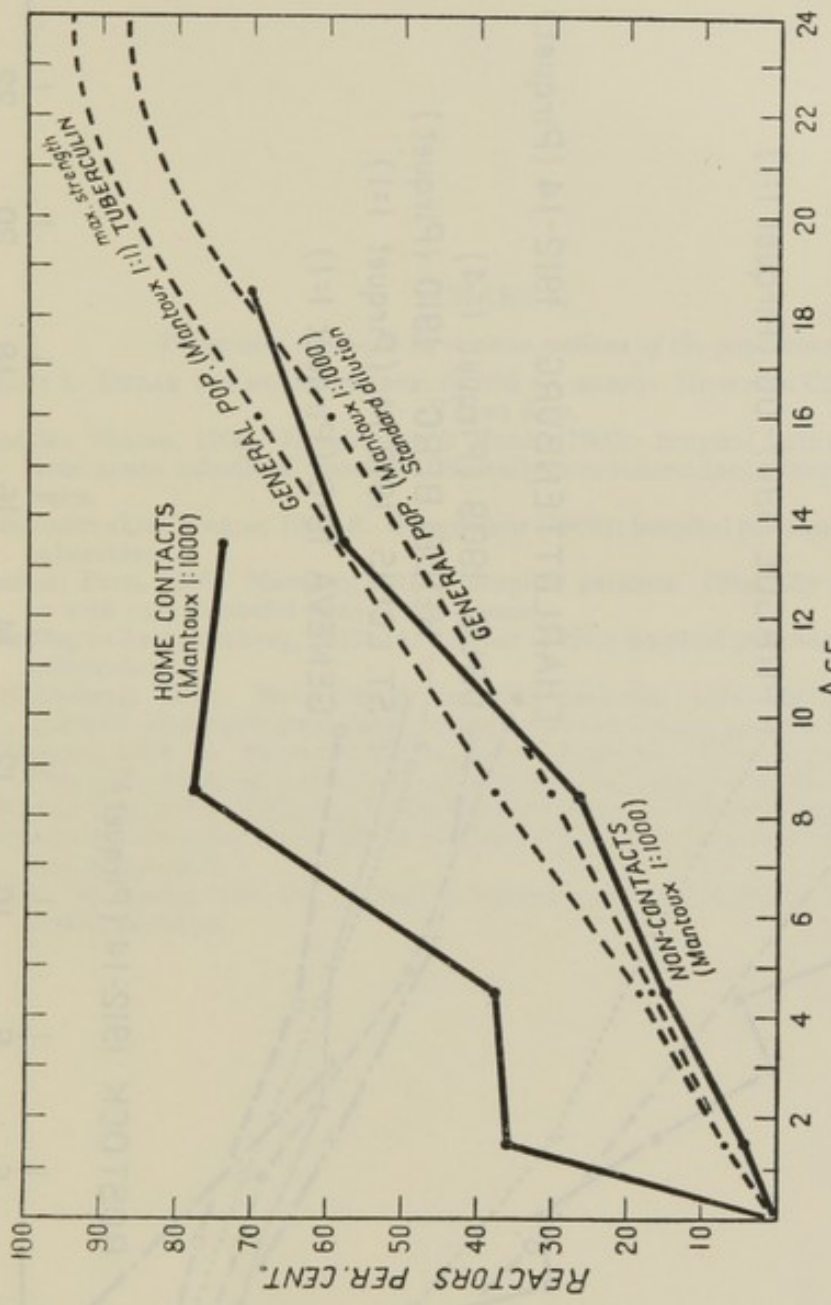


CHART 4. *Tuberculin reactions of non-contacts and of home-contacts of pulmonary tuberculosis, compared with tuberculin reactions of the general population (hospital class) of London, 1929. All clinically non-tuberculous.* (From Schlesinger & Hart, 1930.)

CHART 5

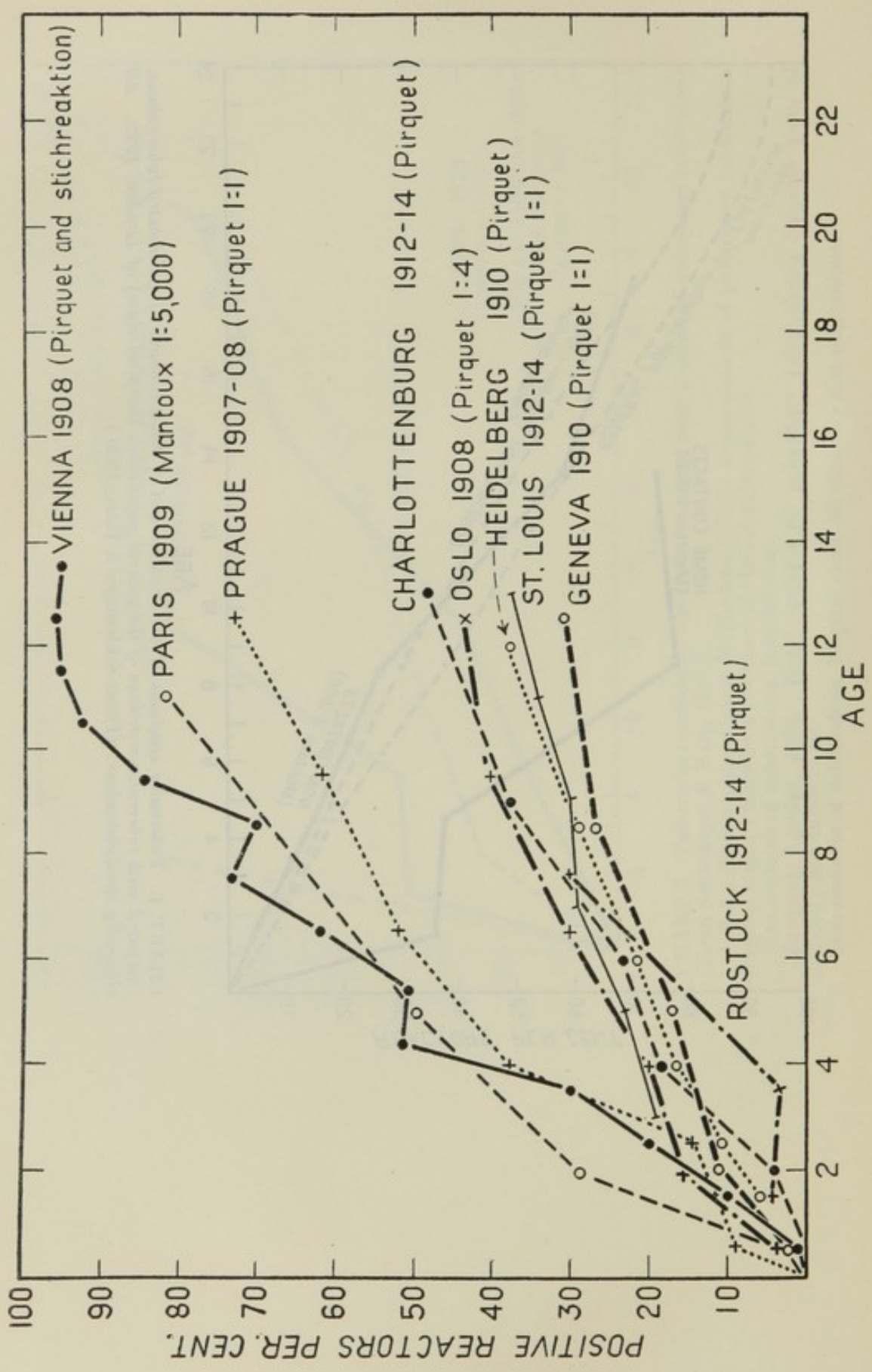

VIENNA 1908 (Pirquet and stichreaktion)
PARIS 1909 (Mantoux 1:5,000)
PRAGUE 1907-08 (Pirquet 1:1)
CHARLOTTENBURG 1912-14 (Pirquet)
OSLO 1908 (Pirquet 1:4)
HEIDELBERG 1910 (Pirquet)
ST. LOUIS 1912-14 (Pirquet 1:1)
GENEVA 1910 (Pirquet 1:1)
ROSTOCK 1912-14 (Pirquet)
POSITIVE REACTORS PER. CENT.
AGE
100
90
80
70
60
50
40
30
20
10
2
4
6
8
10
12
14
16
18
20
22

CHART 5.

Tuberculin reactions in various sections of the population.

GROUP i. URBAN COMMUNITIES (POP. 50,000 OR MORE)—HOSPITAL CLASS (A) TWENTY YEARS AGO.

Austria: Vienna, 1908. Hamburger & Monti (1909): hospital patients convalescent from acute infectious diseases. Clinically non-tuberculous except for a very few cases.

Czechoslovakia: Prague, 1907–8. Ganghofner (1908): hospital patients. Clinically non-tuberculous.

France: Paris, 1909. Mantoux (1910): hospital patients. Clinically non-tuberculous, or with only doubtful signs of the disease.

Germany: Charlottenburg, 1912–14. Umber (1921): hospital patients. Clinically non-tuberculous.

Heidelberg, 1910. Feer (1910): hospital patients. Clinically non-tuberculous. Climatic conditions good, large country element among patients.

Rostock, 1912–14. Synwoldt (1920): hospital patients. Clinically non-tuberculous. Climatic conditions good, large country element among patients.

Norway: Oslo, 1908. Hellesen (1909): hospital patients. Clinically non-tuberculous.

Switzerland: Geneva, 1910. Méroz & Khalatoff (1911): hospital patients. Clinically non-tuberculous.

U.S.A.: St. Louis, 1912–14. Veeder & Johnston (1915): hospital patients. Clinically non-tuberculous.

CHART 6

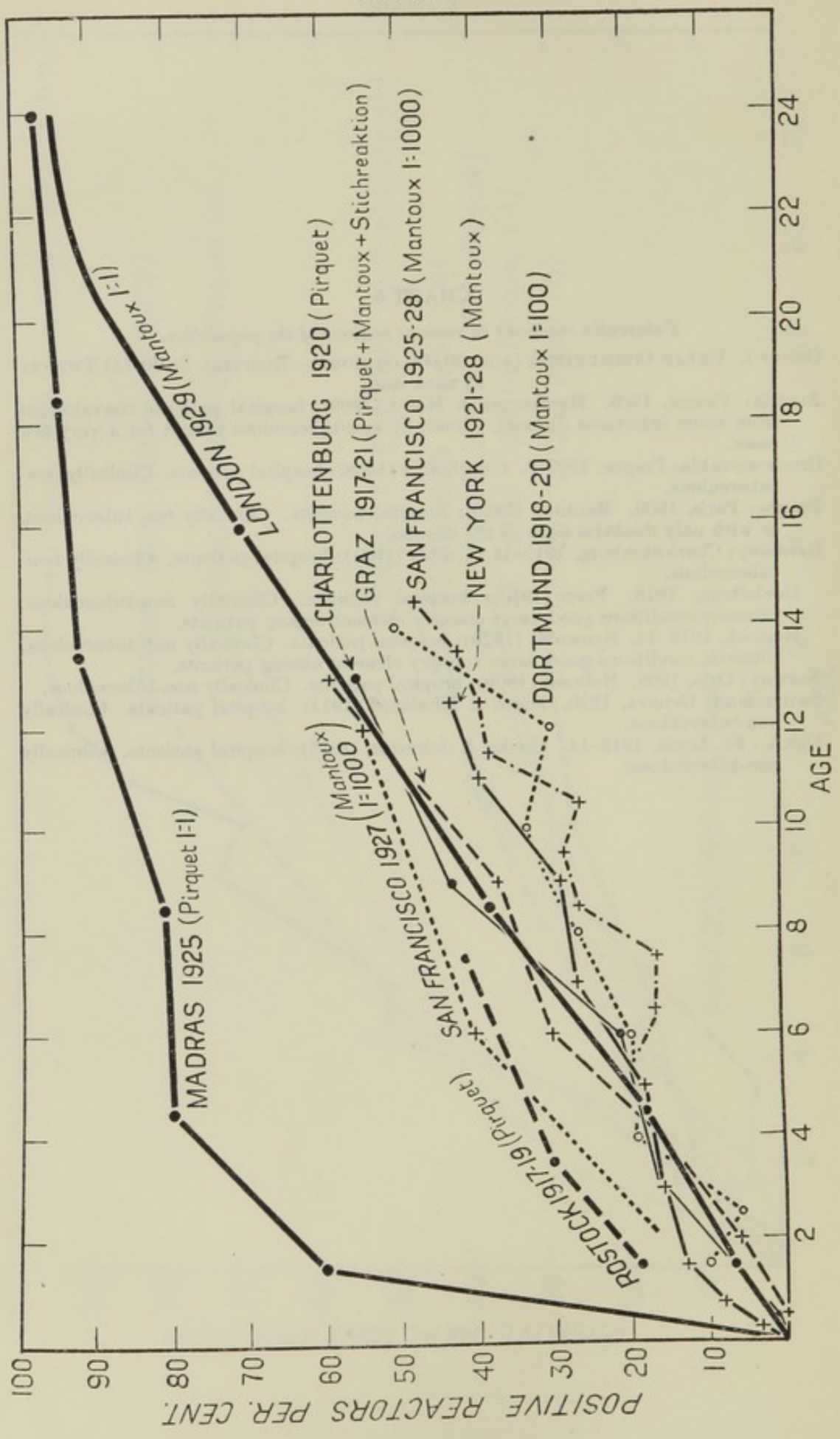

POSITIVE REACTORS PER. CENT.
100
90
80
70
60
50
40
30
20
10
2
4
6
8
10
12
14
16
18
20
22
24
AGE
MADRAS 1925 (Pirquet 1:1)
LONDON 1929 (Mantoux 1:1)
CHARLOTTENBURG 1920 (Pirquet)
GRAZ 1917-21 (Pirquet + Mantoux + Stichreaktion)
SAN FRANCISCO 1925-28 (Mantoux 1:1000)
NEW YORK 1921-28 (Mantoux)
DORTMUND 1918-20 (Mantoux 1:100)
SAN FRANCISCO 1927 (Mantoux 1:1000)
ROSTOCK 1917-21 (Pirquet)

CHART 6.

Tuberculin reactions in various sections of the population.

GROUP ii. URBAN COMMUNITIES (POP. 50,000 OR MORE)—HOSPITAL CLASS (B) DURING LAST TEN YEARS.

Austria: Graz, 1917–21. Barchetti (1922): hospital patients. Clinically non-tuberculous.
England: London, 1929. Hospital patients. Clinically non-tuberculous.
Germany: Charlottenburg, 1920. Umber (1921): hospital patients. Clinically non-tuberculous.
Dortmund, 1918–20. Sander (1921): mainly hospital patients, remainder orphans? Clinically non-tuberculous.
Rostock, 1917–19. Kessler (1920): hospital patients. Clinically non-tuberculous. Climatic conditions good, large country element among patients.
India: Madras, 1925. Pai & Venugopal (1926): native patients of tuberculosis hospital, but stated to be clinically non-tuberculous.
U.S.A.: New York, 1921–8. Smith (1929): hospital population (includes clinically tuberculous patients).
San Francisco, 1925–8. Dickey & Seitz (1931): (α) patients of children's clinic (includes some clinically tuberculous children), drawn from general population.
San Francisco, 1927. Dickey (1928 a): (β) children of Oriental parentage attending clinics.

CHART 7

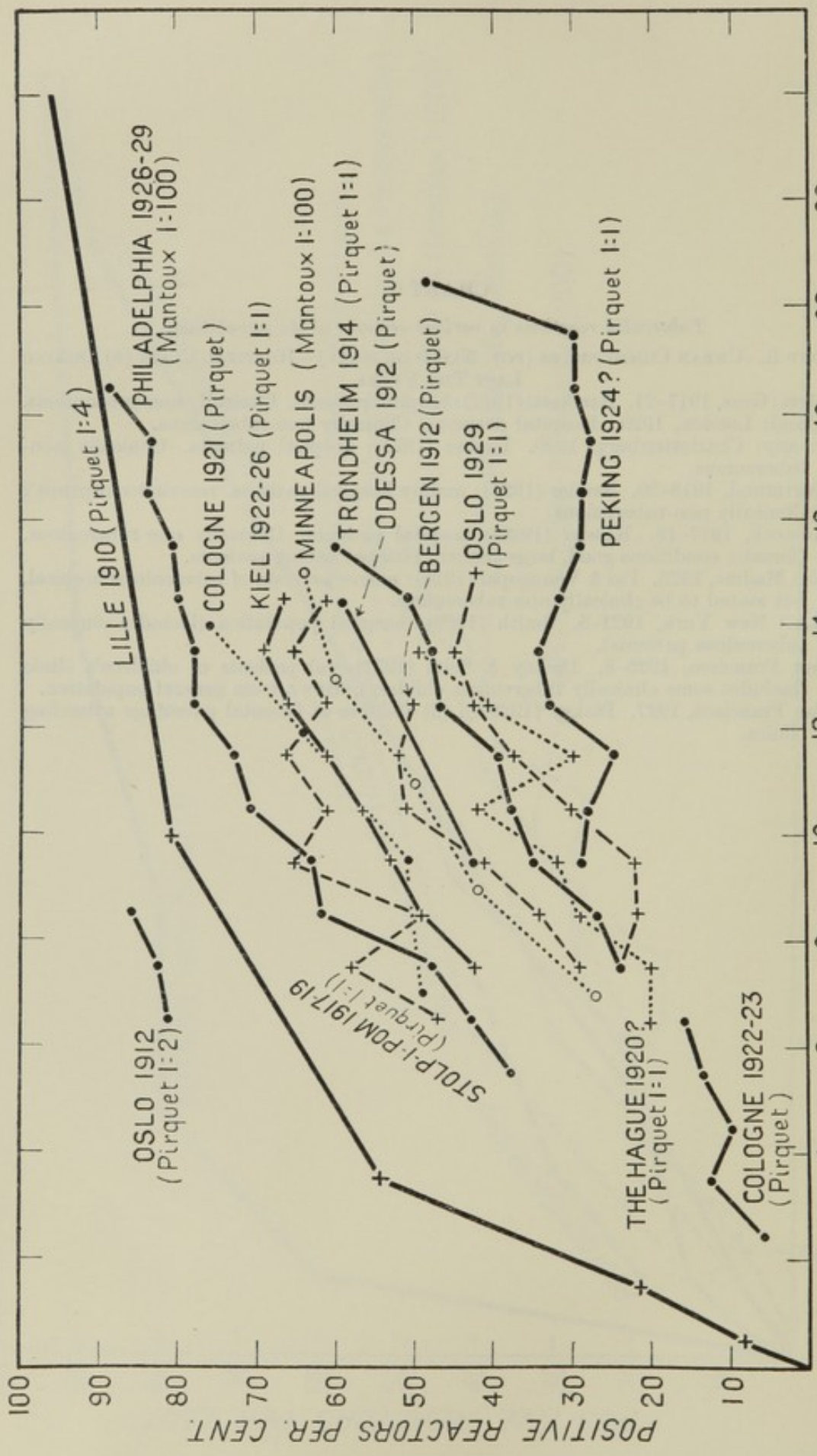

POSITIVE REACTORS PER CENT.
100
90
80
70
60
50
40
30
20
10
2
4
6
8
10
12
14
16
18
20
22
AGE
LILLE 1910 (Pirquet 1:4)
PHILADELPHIA 1926-29 (Mantoux 1:100)
COLOGNE 1921 (Pirquet)
KIEL 1922-26 (Pirquet 1:1)
MINNEAPOLIS (Mantoux 1:100)
TRONDHEIM 1914 (Pirquet 1:1)
ODESSA 1912 (Pirquet)
BERGEN 1912 (Pirquet)
OSLO 1929 (Pirquet 1:1)
PEKING 1924? (Pirquet 1:1)
OSLO 1912 (Pirquet 1:2)
STOLP-I-POM 1917-19 (Pirquet 1:1)
THE HAGUE 1920? (Pirquet 1:1)
COLOGNE 1922-23 (Pirquet)

CHART 7.

Tuberculin reactions in various sections of the population.

GROUP ii. URBAN COMMUNITIES (POP. 50,000 OR MORE)—PUBLIC SCHOOL-CHILDREN OF VARIOUS CLASSES: (A) TWENTY YEARS AGO, (B) DURING LAST TEN YEARS.

(a) France: Lille, 1910. Calmette *et al.* (1911): all classes, apparently healthy people.
Norway: Oslo, 1912. Frölich (1914): children from 'volkschulen'.
Trondheim, 1914. Arfinsen (1919): children from state schools.
Bergen, 1912. Overland (1913 b): children from state schools.
Russia: Odessa, 1912. Stawsky (1913): elementary school-children.

(b) Germany: Cologne, 1921. Vonessen (1922): children from one 'volkschule' in best part of town; includes a proportion of middle-class children.
Cologne, 1922–3. Loewy (1924): children attending kindergarten and welfare stations; includes a proportion of middle-class children.
Kiel, 1922–6. Brunn (1926): children from 'volkschulen', 'mittelschulen', 'förderschulen', and 'hilftschulen', chiefly the first; includes middle-class children.
Stolp-i-pom, 1917–19. Thinius (1924): children from 'gemeindeschulen' (chiefly), 'gymnasium', 'lyzeum', 'mittelschulen', 'catholicschulen', and 'hilftschulen'; includes middle-class children. Housing conditions stated to be very bad in this town.
Holland: The Hague, 1920? Furstner-Risselada (1921): children from 'volkschulen'.
Norway: Oslo, 1929. Heimbeck (1929 b): children from a 'folkeskole'.
U.S.A.: Minneapolis. Harrington & Myers (1926): school-children.
Philadelphia, 1926–9. Hetherington *et al.* (1929): public school-children.
China: Peking, 1924? Korns (1925): native school-children from charity, mission, and government schools: includes both middle-class and destitute children.

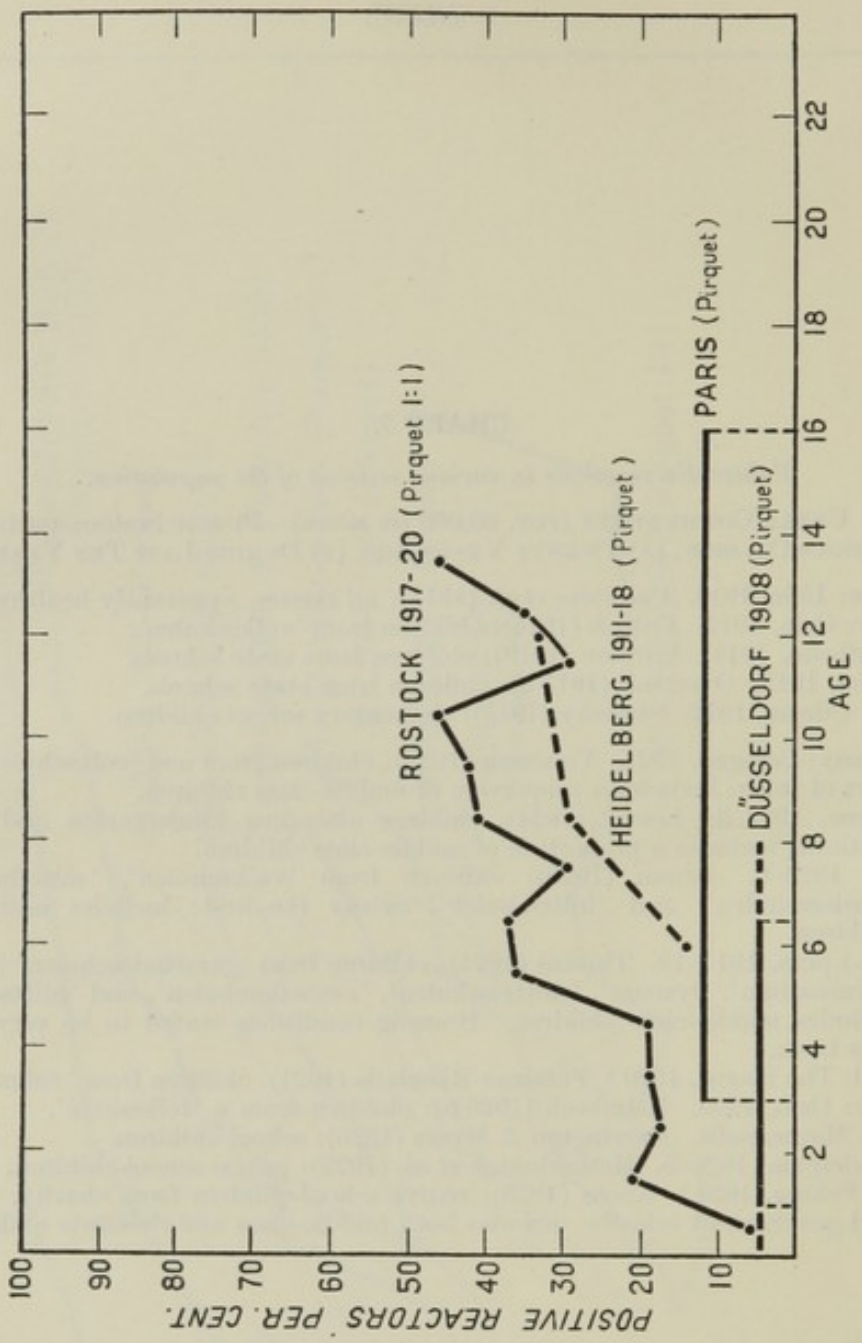


CHART 8.

Tuberculin reactions in various sections of the population.

GROUP iii. URBAN COMMUNITIES (POP. 50,000 OR MORE)—WEALTHIER CLASSES.

France: Paris. Paraf (1928): 64 children of richer classes. Clinically non-tuberculous.
Germany: Heidelberg, 1911–18. Moro & Volkmar (1918): children in private practice.
Rostock, 1917–20. Brüning (1921): children in private practice. Clinically non-tuberculous.
Düsseldorf, 1908. Schlossmann (1909): children in private practice.

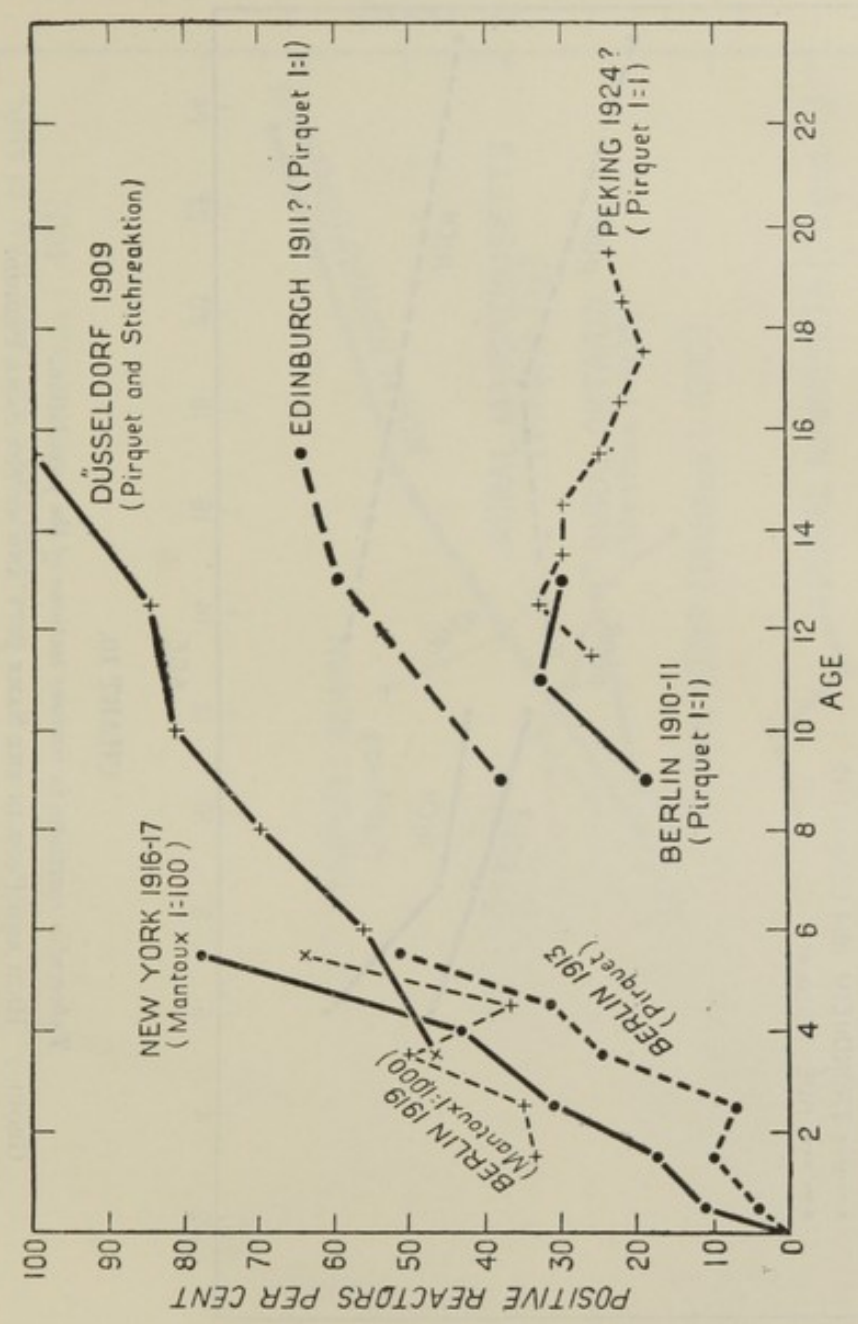


CHART 9.

Tuberculin reactions in various sections of the population.

GROUP iv. URBAN COMMUNITIES (POP. 50,000 OR MORE)—POOREST CLASS.

Germany: Berlin, 1910–11. Küchenhoff (1914): delicate children of lowest class of North Berlin, in a holiday colony.

Berlin, 1913. Davidsohn (1920): inmates of an orphanage; poorest class and lowest social status.

Berlin, 1919. Davidsohn (1920): ditto.

Düsseldorf, 1909. Nothmann (1910): inmates of a state 'pflegehaus'; of lowest social status.

Scotland: Edinburgh, 1911? McNeil (1912): lowest class, from an industrial school.

U.S.A.: New York, 1916–17. Bass (1918): Jewish children of poorest class, from an infant asylum.

China: Peking, 1924? Korns (1925): destitute children in charitable institutions.

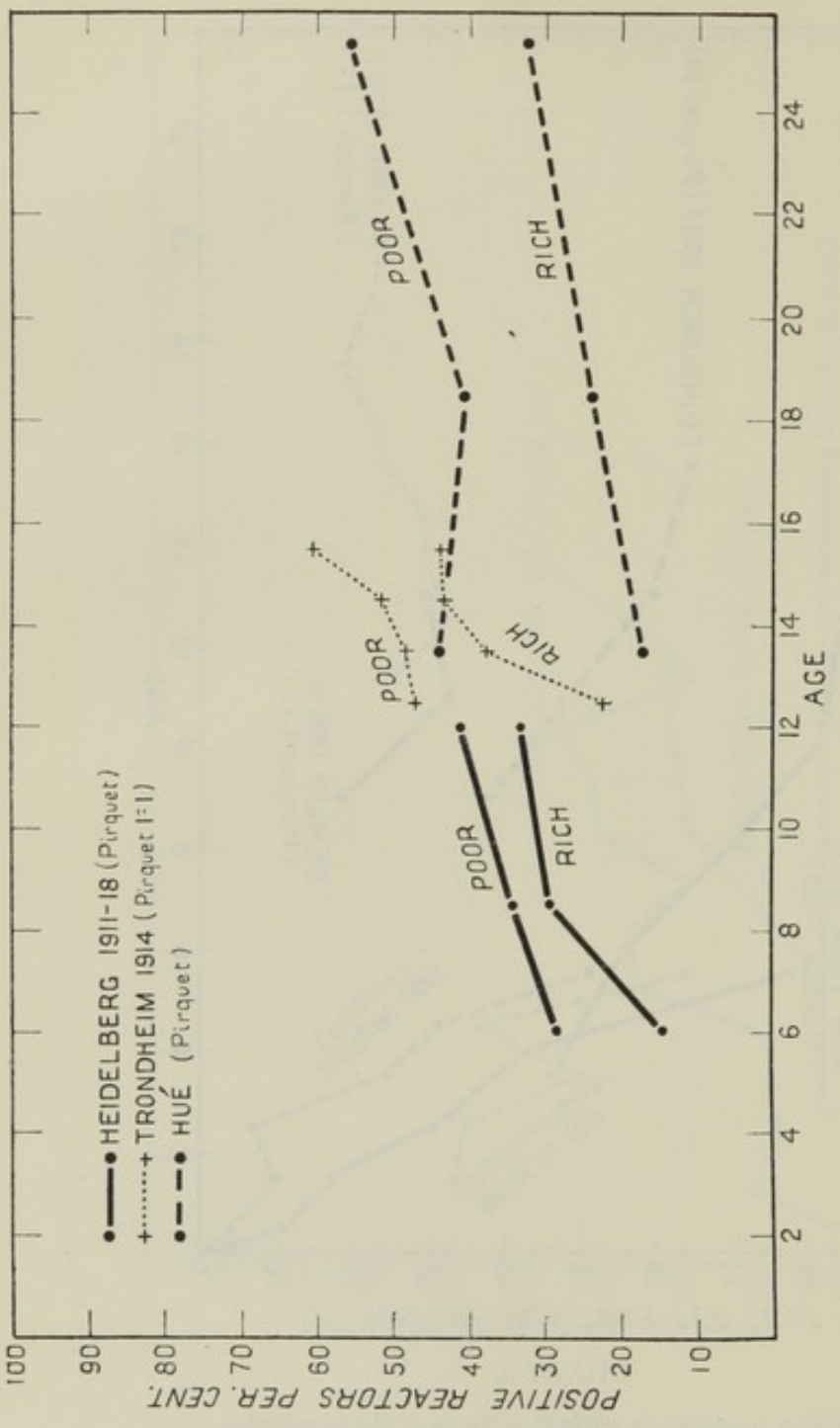


CHART 10.

Tuberculin reactions in various sections of the population.

Group v. Rich and Poor in the Same City and at the Same Period.

Annam: Hué. Bernard *et al.* (1911, 1912): civilized natives: poorer classes compared with richer inhabitants.
Germany: Heidelberg, 1911–18. Moro & Volkmar (1918): hospital clinic compared with private practice.
Norway: Trondheim, 1914. Arfinsen (1919): school-children: state schools compared with private schools.

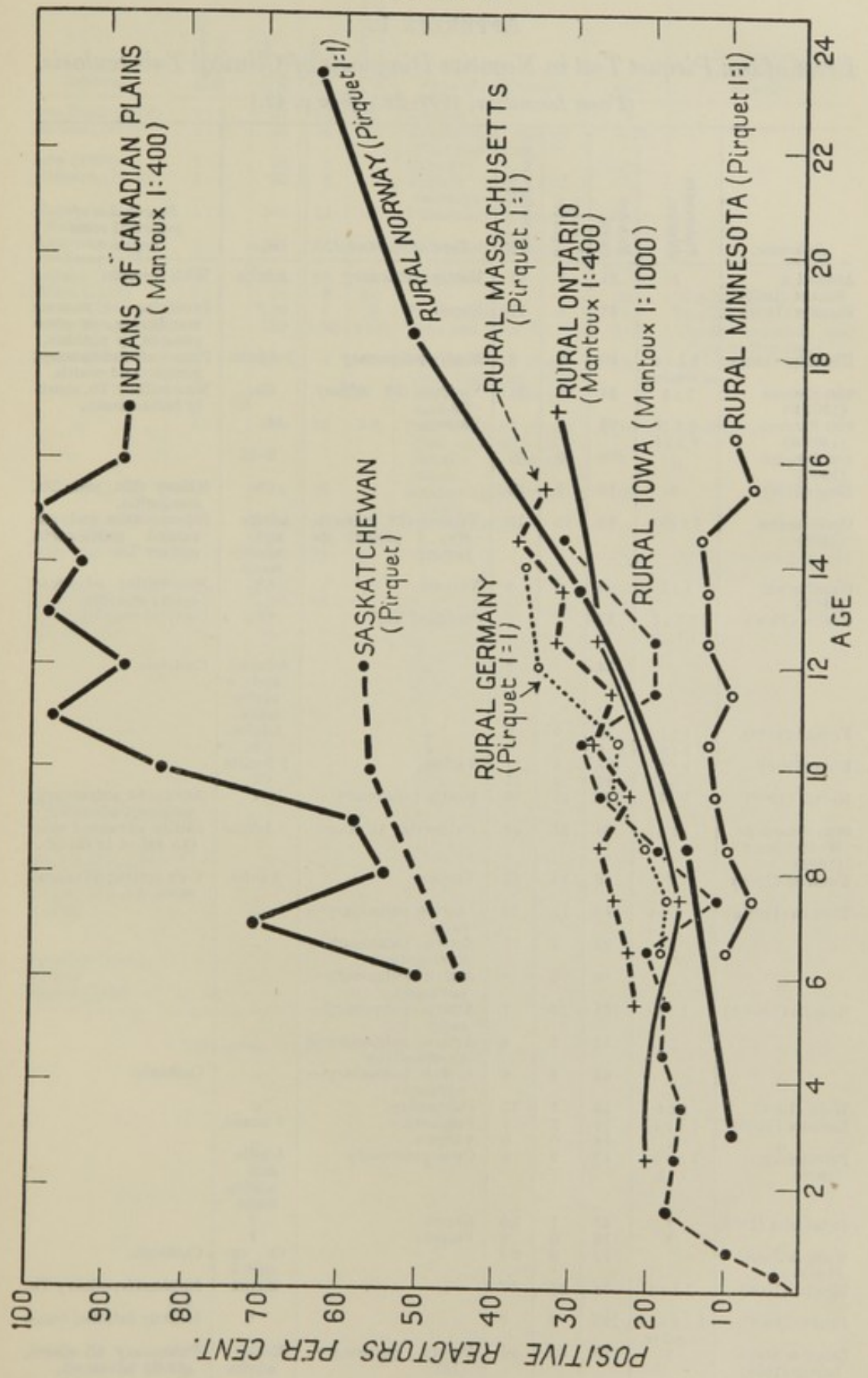
CHART 11.

Tuberculin reactions in various sections of the population.

Group vi. Civilized Rural Communities.

Canada. Elliott (1925): rural Ontario: school-children. Volume of tuberculin injected unusually small (0·02 c.c.).
Ferguson (1922): small cities and rural districts of Saskatchewan: school-children.
Ferguson (1928): Indians of Great Canadian Plains: school-children.
Germany. Hillenberg (1911): rural German districts, agricultural and prosperous.
Norway. Scheel (1929): rural district in Norway. Including 13 per cent. with previous tuberculous history.
U.S.A. Slater (1924): rural Minnesota: wealthy farming community with widely separated homes.
Chadwick & Zacks (1927): rural Massachusetts: school-children.
Taylor (1930): rural Iowa: hospital patients, clinically non-tuberculous.

CHART 11


INDIANS OF CANADIAN PLAINS
(Mantoux 1:400)
SASKATCHEWAN
(Pirquet)
RURAL NORWAY (Pirquet 1:1)
RURAL MASSACHUSETTS
(Pirquet 1:1)
RURAL ONTARIO
(Mantoux 1:400)
RURAL GERMANY
(Pirquet 1:1)
RURAL IOWA (Mantoux 1:1000)
RURAL MINNESOTA (Pirquet 1:1)
POSITIVE REACTORS PER CENT.
AGE
100
90
80
70
60
50
40
30
20
10
2
4
6
8
10
12
14
16
18
20
22
24

Appendices.

Appendix 1.

Error of von Pirquet Test in Negative Diagnosis of Clinical Tuberculosis.

(*From Literature, 1907–28.*) (See p. 42.)

Reference.	*Tuberculin dilution.*	*Number tested.*	*Number negative.*	*Percentage negative = error.*	*Type of case tested.*	*Age.*	*Description of principal cases negative.*
Abrami & Burnet (1907)	?	21	7	33	Mostly pulmonary	Adults	With cavities.
Bandler (1907)	?	38	4	11	Lupus	?	Involving mucous membranes, or complicated by phthisis.
Hirschler (1907)	? 1 : 4 or weaker	50	3	6	Mostly pulmonary	? Adults	Progressive pulmonary; gonitis; and coxitis.
von Pirquet (1907 b)	1 : 4	34	13	38	Various (23 miliary Tb.)	Ch.	Nine miliary Tb. shortly before death.
von Pirquet (1907 d)	? 1 :4 ? 1 : 1	75	1	1	Various	14–	
von Pirquet (1909)	,,	209	20	10	,,	0–13	
Bing (1908)	?	19	5	26	,,	Ch.	Miliary Tb. and Tb. meningitis.
Curschmann (1908)	1 : 100	53	10	19	Various (36 pulmonary, 6 pleural effusion)	Adults and adolescents	Intermediate and advanced pulmonary; miliary Tb.
Ganghofner (1908)	1 : 1	90	8	9	Various	Ch.	Meningitis; advanced cases; adenitis.
Goebel (1908)	? 1 : 1 ? 1 : 4	17	1	6	'Surgical Tb.'	Ch.	Cervical adenitis.
,,	,,	54	1	2	,,	Adults and adolescents	Cachexia.
Freund (1908)	1 : 1	10	0	0	,,	Adults	—
,,	1 : 4	8	0	0	,,	Ch.	—
Entz (1908)	1 : 4	35	6	17	Various	? Adults	
,,	,,	19	6	31	,,	Ch.	
Morelli (1908)	1 : 4	136	22	16	Mostly pulmonary	?	Advanced pulmonary; quiescent adenitis.
Stadelmann & Wolff-Eisner (1908)	1 : 4	64	27	42	Pulmonary all stages	? Adults	Mostly advanced cases (15 out of 18 failed).
Kentzler (1908)	?	98	23	23	Various	Adults	Very active; advanced cases, &c.
Roepke (1908 a)	1 : 4	144	17	12	Active pulmonary—early	,,	
,,	,,	82	9	11	Active pulmonary—intermediate	,,	
,,	,,	88	6	8	Active pulmonary—advanced	,,	
Roepke (1908 b)	1 : 1	138	10	7	Active pulmonary—early	,,	
,,	,,	81	3	4	Active pulmonary—intermediate	,,	
,,	,,	62	4	6	Active pulmonary—advanced	,,	Cachexia.
Wolff (1908)	1 : 4	19	7	37	Pulmonary	,,	
Lemaire (1908)	? 1 : 10	38	2	5	Pulmonary	? mixed	
,,	,,	18	0	0	Various	,,	
Petruschky (1908)	1 : 5 or 1 : 1	43	2	5	Open pulmonary	Adults and adolescents	
Jadassohn (1908)	?	43	1	2·5	Lupus	?	
,,	?	16	0	0	Various	?	—
Heim & John (1908)		77	2	2·5	,,	Ch. except 5	Cachexia.
Ziesche (1908)	? 1 : 4	36	13	36	,,	Mixed	Meningitis; miliary Tb. &c.
Junker (1908)	1 : 4 and weaker	121	5	4	,,	?	Heavily infected cases.
Leber & Steinharter (1908)	?	185	53	29	Mostly pulmonary (165)	Mostly adults and adolescents	Pulmonary all stages, chiefly advanced.

Reference.	*Tuberculin dilution.*	*Number tested.*	*Number negative.*	*Percentage negative = error.*	*Type of case tested.*	*Age.*	*Description of principal cases negative.*
von Ruck (1909)	1 : 2	67	19	28	Various	?	
McNeil (1909)	1 : 1	20	10	50	Meningitis; miliary Tb.; peritonitis (?)	0–12	
Low (1909)	1 : 4	16	0	0	Skin Tb.	Adults	—
Hellesen (1909)	1 : 4	58	2	3	Various (50% pulmonary)	Ch.	
Hamman & Wolman (1909)	1 : 1	200	13	6·5	Pulmonary all stages	Mainly adults	
Hamman & Wolman (1910)	1 : 1	303	20	7	Pulmonary all stages	Mainly adults	
Lapage (1912)	1 : 4	191	35	18	Various	0–14	
Monti (1912)	? 1 : 1	58	2	3·5	,,	Ch.	Meningitis.
Brown (1913)	1 : 1	100	5	5	Tb. meningitis	Ch.	Moribund.
Robertson (1914)	1 : 1	182	8 (6)	4 (3)	Bone and joint Tb.	0–12	Chiefly toxaemic and suppurating cases.
Ritter (1914)	1 : 1	105	8	8	Pulmonary all stages (but excluding very heavily infected cases)	Ch.	All stages.
,,	1 : 1	396	22	5·5	Pulmonary all stages (but excluding very heavily infected cases)	Adults	All stages.
Ramsey (1915)	?	58	30	52	Bone and joint tuberculosis	Ch.	
Meyers (1915)	?	105	37	35	Tb. meningitis	,,	
Hess (1918)		12	10	83	,,	,,	
Muir (1920)	1 : 1	58	19	33	Pulmonary	Ch.	Reasons for failure not apparent.
,,	,,	42	4	9·5	,,	Adults	
Dunn & Cohen (1921)	?	120	49	41	Tb. meningitis; and miliary Tb.	Ch.	
Peters (1921)[1]	1 : 1	105	1	1	Open pulmonary, excluding heavily infected cases	?	
,,	,,	56	2	3·5	'Closed pulmonary Tb.' All stages	Ch.	Healed cases, &c.
,,	,,	1,909	29	1·5	,,	Adults and adolescents (14–)	,, ,,
Happ & Casparis (1922)	1 : 1	143	64	45	Miliary Tb. with meningitis	Ch.	
,,	1 : 1	40	25	62	Miliary Tb. without meningitis	Ch.	
Wahl & Gerstenberger (1923)	1 : 1	33	13	40	Meningitis; and pulmonary miliary Tb.	0–4	
Krogsgaard (1925)	1 : 1	24	7	29	50% miliary Tb. and meningitis	?	Miliary Tb. (1); miliary Tb. and meningitis (2); pulmonary or bronchial adenitis (4).
Tisdall & Brown (1926)	,,	177	74	42	Various	Ch.	
Gunter (1928)	1 : 100	58	0	0	Active pulmonary	Adults	

[1] This author's series is not included in Table 2; see text, Section V.

Appendix 2.

Error of Mantoux Intracutaneous Test in Negative Diagnosis of Clinical Tuberculosis.

(*From Literature, 1909–31.*) (See p. 44.)

Reference.	*Upper limit of intracutaneous dose.*	*Number tested.*	*Number negative to upper limit of dose.*	*Percentage negative to upper limit of dose = error*	*Type of case tested.*	*Age.*	*Description of principal cases negative.*
de Beurmann & Laroche (1909)	?	20	2	10	Skin Tb. (15 lupus)	Not stated	Cachexia. All lupus reacted.
Mantoux (1910)	0·05 c.c. of 1 : 5,000 = 0·01 mg.	148	8	5	Various	,,	Cachexia. Miliary Tb. (Meningitis.)
Monti (1912)	0·1 c.c. of 1 : 10,000 = 0·01 mg.	58	2	3·5	,,	Ch.	Meningitis.
Jeanneret (1913)	0·1 c.c. of 1 : 1,000 = 0·1 mg.	150	6	4	,,	,,	Cachexia. Adenitis, &c.
Happ & Casparis (1922)	0·1 c.c. of > 1 : 100 = > 1 mg.	72	0	0	Miliary Tb. and meningitis	,,	Miliary Tb. and meningitis.
Wahl & Gerstenberger (1923)	0·1 c.c. of 1 : 100 = 1 mg.	11	1	9	Meningitis and pulmonary miliary Tb.	Under 5	Pulmonary miliary Tb.
Krogsgaard (1925)	0·1 c.c. of 1 : 100 = 1 mg.	24	2	8·5	Half miliary Tb. and meningitis	Not stated	Miliary Tb. and meningitis.
de Potter (1926)	0·1 c.c. of 1 : 100 = 1 mg.	28	0	0	Various	,,	Not stated.
Tisdall & Brown (1926)	0·1 c.c. of 1 : 400 = 0·25 mg.	177	27	15	,,	Ch.	Advanced cases.
Cummins (1929)	0·1 c.c. of 1 : 500 = 0·2 mg.	91	—	28[1]	,,	,,	Not stated.
,,	0·1 c.c. of 1 : 500 = 0·2 mg.	33	—	15[1]	,,	Adults	,,
Preston (1929)	0·1 c.c. of 1 : 1,000 = 0·1 mg.	32	10	31	,,	Mostly adults	Chronic cases approaching quiescence.
Lobban (1930)	0·2 c.c. of 1 : 1,000 = 0·2 mg.	108	0	0	Various (55% pulmonary)	Not stated	
Maxwell (1930)	0·1 c.c. of 1 : 1,000 = 0·1 mg.	79	8	10	Various	,,	
Moncrieff (1931)	0·1 mg.	30	7	23	,,	Ch.	
London Investigation, 1929[2]	0·1 c.c. of 1 : 1 = 100 mg.	389	1,030	3·3	Various. See text for analysis as to types	0–10	See Appendix 3.
,,	,,	300		1	,,	11–20	,, ,,
,,	,,	341		1·5	,,	21–	,, ,,
,,	,,	93		3·5	Advanced pulmonary with marked toxaemia	All ages	,, ,,

[1] Twenty-eight per cent. of pulmonary all ages were negative, and 22 per cent. of non-pulmonary cases.
[2] Not included in Table 3; see text, Section V.

Appendix 3.

Clinical Summary of the 38 cases of Clinical Tuberculosis, out of 1,030 cases tested, that were Negative to 0·1 c.c. of 1 : 1,000 (= 0·1 mg.) Old Tuberculin, Intracutaneously, with Details of their reactions to larger Doses.

(*London Investigation, 1929.*) (See p. 49.)

No.	*Initial.*	*Age.*	*Clinical Summary.*	*Concentrations.*	*Reaction.*	*Table reference.*
1	L.V.	1½	Pulmonary miliary Tb. Tb. meningitis. Sputum positive. Toxaemia marked. Died 3 weeks later.	1 : 100	+	9 d
2	T.W.	2	Tb. spine. Very active. T. 98–100°. X-ray = partial collapse of bodies of D. 12 and L. 1. Alive a year later.	1 : 1	—	8
3	R.	2	Tb. knee. Active. X-ray fairly definite.	1 : 1	—	8
4	L.G.	3	Tb. spine. Very active. X-ray = diminished space between bodies of L. 2 and 3, D. 7 and 8, D. 9 and 10. Alive a year later.	1 : 10 1 : 1	— +	8
5	S.B.	4	Tb. ulcer of caecum and Tb. mesenteric glands. Diagnosed at operation. No section taken. No toxaemia.	1 : 1	—	9 e
6	N.	4	Tb. peritonitis. Quiescent. Rickets. No masses felt in abdomen.[1]	1 : 1	—	9 b
7	W.	4	Tb. hip. Approaching quiescence.	1 : 100	+	8
8	R.J.	4	Synovial Tb. of knee. Active.[2]	1 : 1	—	8
9	A.S.	5	Pulmonary Tb. Quiescent. Early. Sputum positive once six months previously. Sputum negative at other examinations. X-ray fairly definite.	1 : 1	—	7
10	O.	5	Tb. hip. Active.	1 : 100	+	8
11	L.G.	6	Tb. hip. Very active. X-ray = destruction of acetabulum, neck of femur, and part of head of femur. Femur displaced upwards. Area of rarefaction in shaft, just below lesser trochanter. Alive a year later.	1 : 1	—	8
12	A.	6	Lupus vulgaris of face. Active.	1 : 100	+	9 a
13	D.N.	7	Old Tb. pleural effusion. Now quiescent, or possibly arrested. X-ray now = nil. Tb. not found in fluid.	1 : 1	—	9 b
14	W.	7	Tb. knee. Active.	1 : 1	—	8
15	L.	7	Tb. iliac bone. Sinus. Scrofuloderma. Approaching quiescence.[1]	1 : 1	—	9 e
16	J.H.	8	Tb. hip. Active. X-ray supported clinical diagnosis.	1 : 1	—	8
17	C.W.	9	Pulmonary tuberculosis. Active. Early. X-ray = indefinite. Toxaemia not marked. Sputum positive once.	1 : 1	—	7
18	B.P.	10	Tb. hip. Quiescent.	1 : 1	—	8
19	M.H.	11	Tb. tracheo-bronchial glands. Quiescent. X-ray = calcified glands.	1 : 1	—	9 c
20	A.S.	11	Tb. hip and sacro-iliac bones. Sinus. Active. Very chronic. No toxaemia. X-ray = destruction upper part of femoral head and acetabulum. Joint space absent. Alive a year later.	1 : 1	—	8

[1] In reply to questionnaire sent a year later it was stated that this case might quite possibly have been non-tuberculous. [2] In reply to questionnaire sent a year later it was stated that this case was no longer considered to be tuberculous.

No.	*Initial.*	*Age.*	*Clinical Summary.*	*Concentrations.*	*Reaction.*	*Table reference.*
21	B.	11	Pulmonary tuberculosis. Active. Advanced. Toxaemia very marked. Sputum positive.	1 : 100	+	7
22	R.P.	13	Pulmonary tuberculosis. Quiescent. Early. X-ray = indefinite. Sputum positive once, 6 months ago. Negative 7 other times.	1 : 1	—	7
23	O.L.	16	Pulmonary tuberculosis. Active. Advanced. Tb. laryngitis. Toxaemia marked. Sputum positive. Died 2 months later.	1 : 100 1 : 10	— +	7
24	B.	17	Pulmonary tuberculosis. Active. Advanced. Toxaemia marked. Sputum positive. Died a week later.	1: 100	+	7
25	E.	20	Pulmonary tuberculosis. Active. Advanced. Toxaemia very marked. Sputum positive.	1 : 100 1 : 10	— +	7
26	M.	20	Pulmonary tuberculosis. Active. Advanced. Toxaemia marked. Sputum positive. Alive a month later.	1 : 100	+	7
27	D.D.	21	Tb. spine. Psoas abscess. Active. Toxaemia marked. Pus positive. Died 1 week later. P.M. confirmed.	1 : 100	—[1]	8
28	H.M.	22	Tb. colitis and fistula. Active. Toxaemia marked. Biopsy of fistula supported diagnosis.	1 : 100 1 : 10	— +	9 e
29	C.	23	Pulmonary tuberculosis. Active. Advanced. Toxaemia marked. Temp. 101°. Sputum positive. Died 1 month later.	1 : 100	+	7
30	P.	28	Pulmonary tuberculosis. Active. Intermediate. Toxaemia not marked. X-ray definite. Sputum positive several times and recently. Alive 4 months later.	1 : 100	—[1]	7
31	W.	30	Pulmonary tuberculosis. Active. Advanced. Toxaemia very marked. Sputum positive. Died 3 days later.	1 : 100	—[1]	7
32	A.	39	Pulmonary tuberculosis. Active. Advanced. Toxaemia marked. T. 99°. Sputum positive. Died 3 months later.	1 : 100	+	7
33	L.	47	Pulmonary tuberculosis. Active. Advanced. Toxaemia marked. Sputum positive. Died 1 week later.	1 : 100	+	7
34	B.	48	Pulmonary tuberculosis. Active. Advanced. Toxaemia marked. Sputum positive. Died 4 days later.	1 : 100	—[1]	7
35	P.	50	Pulmonary tuberculosis. Active. Advanced. Toxaemia marked. T. 102°. Sputum positive. Died 3 days later.	1 : 100	—[1]	7
36	H.	52	Pulmonary tuberculosis. Active. Advanced. Toxaemia very marked. Sputum positive. Died 1 week later.	1 : 1	—	7
37	G.C.	56	Pulmonary tuberculosis. Active. Advanced. Toxaemia marked. Sputum positive. Died 3 weeks later.	1 : 1	—	7
38	B.	59	Pulmonary tuberculosis. Active. Advanced. Toxaemia marked. T. 101°. Sputum positive. Died 5 days later.	1 : 100 1 : 10	— +	7

[1] Refused further tests, or died before these could be made.

Appendix 4.

Respiratory or Pulmonary, and Total Tuberculosis death-rates[1] (per 10,000 persons) in some of the Towns whose Tuberculin Surveys are mentioned in this Report.

(See pp. 76–9.)

(Chief sources: Wolff, 1926; Fishberg, 1922 b; League of Nations, 1929, 1931; U.S. Treasury Dept., 1926, 1927, 1928; Office Internat. d'Hygiène Publique, 1912, 1913, 1915; Statistiches Reichsamt, 1931.)

	1900–4.	*1901–5.*	*1905–9.*	*1906–10.*	*1905.*	*1909.*	*1927.*	*1928.*
Baltimore	24		23		(27)	—	9 (10)	9
Bergen					18	19	13 (13)	12 (12)
Berlin				19	21·5 (28)	18 (23)	9 (11)[2]	9 (10)[2]
Buenos Aires					19	16	(18)	(18)
Charlottenburg					13	12	8	7·5
Cologne					20	16	8 (10)	8 (10)
Dortmund					13	12	8	7
Düsseldorf					15	12	6	6
Edinburgh		16		11	15 (23)	12 (18)	9 (12)	8 (10·5)
Geneva[3]		31 (41)		27	—	—	(19)	(16·5)
Graz					(46)	(43)	—	—
The Hague		13	13	12	13	11	6 (8)	6 (8)
Heidelberg					(33)	(29·5)	6	5·5
Kiel					11	9·5	8	7·5
Lille					31	29·5	(24)	(23)
London[4]		16		13	14·5 (19)	14 (17)	9 (10·5)	9 (10)
Madras					—	—	31	26
Minneapolis	11·5	(13)	11		(11)		4 (4)	3 (4)
New York[5]	22	(25)	20		21 (24)	19 (21)	7 (8)	7 (8)
Odessa					31	—	—	—
Oslo		23			28 (34)	20 (24·5)	14 (16)	13 (16)
Paris		39 (46)		38 (44)	38 (44·5)	36 (42)	20 (24)	19 (23)
Philadelphia	21·5	(24)	21		(24)	—	7 (8·5)	7 (8)
Prague		52·5		(39)	42 (44)	39 (40)	(18)	(17)
Rostock					(14)	(16)	10	6
St. Louis	20		19		(25)	(18)	6 (7)	7 (8)
San Francisco	28		19		—	—	9 (11)	9 (11)
,, (Chinese)					—	—	45?	—
Stolp-i-Pom					(16·5)	(15)	12	8
Trondheim					24	26	(19)	(21)
Vienna		34 (40)	27	(35)	33	27	17 (20·5)	15 (19)

[1] Bracketed figures are total rates.
[2] Greater Berlin.
[3] Agglomeration commune.
[4] Administrative County.
[5] Greater New York.

Appendix 5.

Suggested Routine Technique for the Intracutaneous Test.

Tuberculin: Human Old Tuberculin is recommended. The bacillary broth culture should have been concentrated ten times by heat by the manufacturer. The tuberculin must have been standardized satisfactorily, and its potency should be known in terms of an easily accessible standard tuberculin, e.g. the International Standard (see p. 42). (The product supplied by Burroughs Wellcome & Co. conforms to these requirements.) Dilutions of 1 : 10,000, 1 : 1,000, 1 : 100, and 1 : 10 are prepared. They should not be used more than a fortnight after dilution. The diluent recommended is 0·5 per cent. phenol-saline.

Syringe: A well-fitting two-piece 1 c.c. (graduated in 20ths) all-glass tuberculin syringe and short-bevelled intradermal needle are required. (These are obtainable from B. W. & Co., the Hospital & Laboratory Supplies Ltd., &c. Summit No. 20 'short point' needles fit the latter firm's syringes.) The syringe, once filled, holds fluid for ten tests, the needle being kept in position and merely flamed between each injection. It is well to have a separate syringe for each dilution.

Injection: The front of the forearm or upper arm is cleaned with methylated spirit or ether before the test, and an injection of 0·1 c.c. of the dilution is made with the bevel of the needle upwards. In making the injection the skin should not be pinched but should be held taut with the left hand, the needle being inserted at the smallest angle possible until the bevel is just completely covered. In this way subcutaneous injection is avoided. The injection must raise a white bleb, on the surface of which the hair follicles should be clearly visible. No dressing or further sterilization of the skin is required, nor need special directions be given, but adults may be told to dab on calamine lotion should the irritation be troublesome. The lotion must be washed off before reading the test.

Controls: Control solutions are unnecessary as a routine when solutions of 1 : 100 tuberculin or weaker dilutions are used, but are advisable with 1 : 10 or more concentrated tuberculin in order to prevent confusion with delayed atypical non-specific reactions, which follow these large doses with comparative frequency (see p. 46). The controls must consist of glycerinated veal peptone broth of the same strength and composition as that contained in the tuberculin, and preferably obtained from the actual medium from which the particular sample of Old Tuberculin has been prepared.

Reading: It is suggested that reactions be read at 48 and 96 hours or, if only one observation is possible, at 72 hours. (Reading at 24 hours is discouraged, as atypical non-specific reactions may not yet have disappeared.) The test should be recorded as positive or negative to the given dilution. A reaction should be regarded as positive if it consists of an area of erythema or erythematous infiltration the greatest diameter of which equals or exceeds 5 mm. If a control test is made, an area definitely greater than that on the control arm constitutes a positive response.

Feeble positive reactions are liable to cause difficulty. Doubt may generally be removed by re-testing with ten times the strength of tuberculin. This procedure should, if possible, be carried out within a week of the previous test, or else after an interval of several months, on account of the possibility of sensitization causing a violent reaction. (For ill-effects of the intracutaneous test see p. 54.)

Initial dose: The initial injection should be 0·1 c.c. of 1 : 10,000 tuberculin. When, however, experience and confidence in the test have been gained, the initial dose may be 0·1 c.c. of 1 : 1,000 tuberculin, except in patients (*a*) suspected of having bone, joint, ophthalmic, or skin tuberculosis, or (*b*) who have had a recent haemoptysis, or (*c*) who are home-contacts of tuberculosis, and (*d*) in other patients when attendance for further tests can be guaranteed. The weaker solution is naturally less likely to produce discomfort or severe reactions than is the 1 : 1,000 solution, when given as initial injection. The latter solution, on the other hand, gives a clearer reading and saves time. The initial concentration must never be greater than 1 : 1,000.

Re-tests: All patients negative to 1 : 10,000 tuberculin must be re-tested with a 1 : 1,000 solution, which can be given without fear under these conditions. A dose of 0·1 c.c. of a 1 : 1,000 solution of a tuberculin whose potency is approximately equal to that of the International Standard Tuberculin (see p. 42) is in general sufficient for routine purposes. (This dilution is called the standard dilution, a term that should not be confused with standard tuberculin.) To make more certain, however, patients negative to this dose should, where possible, be re-tested with 0·1 c.c. of 1 : 100 tuberculin. In any event this procedure should be carried out if there is still the slightest doubt as to the diagnosis from the clinical standpoint, or if reduced reactivity is to be expected; and in such cases the patient, if still negative, should be further tested with a 1 : 10 dilution. The latter is the practical upper limit, because stronger solutions are difficult to inject and cause pain, rarely yield additional positives, and give atypical non-specific reactions with comparative frequency. Re-tests are conveniently made at the time of reading the previous reactions. The above procedure ensures that there is not more than a ten times difference of strength between the graded dilutions used for re-testing negative reactors; in this way the lowest dilutions can be given with safety.

For technique of the test in the positive diagnosis of clinical tuberculosis see also pp. 33, 34, 40.

For technique of the test in the negative diagnosis of clinical tuberculosis see also pp. 54, 55, 57.

For technique in the contact clinic see also pp. 70, 71.

For technique of the test in tuberculin surveys of the community see also pp. 74, 75.

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STUDIES IN NUTRITION

AN INQUIRY INTO THE DIET OF FAMILIES IN CARDIFF AND READING

By E. P. CATHCART and A. M. T. MURRAY

Assisted by M. SHANKS

30 MAY 1932

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PREFACE

LAST year the Medical Research Council published a report (No. 151 in this Series) giving the results of an inquiry into the diet of 154 families in St. Andrews. The present report describes two further studies of the same kind and by the same investigators. These deal with the diet of 56 families in Cardiff and 57 families in Reading.

The purpose of the whole inquiry has been to obtain more accurate quantitative knowledge of the normal diets actually used by inhabitants of this country. In the first study, at St. Andrews, information was obtained with regard to a vertical section of a comparatively well-to-do population; in this more recent work the data relate to horizontal samples of two working-class communities living under divergent industrial and environmental conditions.

The inquiry deals with the quantitative or 'energy' aspect of nutrition, as distinguished from the qualitative aspect which is concerned chiefly with the quality, source, and distribution of the various constituents that go to form the complete diet. Its method has been to determine the calorie value of the diets used, and the proportions of this for which the three main principles of food—protein, fat, and carbohydrate—are respectively responsible. Variations in the diets in relation to economic circumstances are also considered.

As at St. Andrews, the figures for Cardiff and Reading have shown substantial divergence from those that have been generally accepted in the past as representing an average standard. In particular, an unexpectedly high consumption of fat has again been disclosed. Accurate knowledge on points such as these is obviously essential for the proper application of the results of nutritional science to the practical problems of diet in health and disease.

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STUDIES IN NUTRITION

AN INQUIRY INTO THE DIET OF FAMILIES IN CARDIFF AND READING

BY

E. P. Cathcart, F.R.S. and A. M. T. Murray, Ph.D

ASSISTED BY

Miss M. Shanks

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I. Methods. The Problem and the Population Studied.

One of the very real difficulties which those who have to devise diets for the use of specialized communities, e.g. inmates of a Poor Law Institution, have to contend with is the absence of accurate data regarding the food actually consumed by people who have the purchasing of their foodstuffs within their own control. It is perfectly easy to devise a diet which will agree with the accepted canons of dietetics, but will such a diet be close to or diverge from the composition of the freely selected diet? It is obviously impossible in institutional dieting to pay attention to the vagaries of appetite of each inmate although perhaps the neglect of, what may be called, the psychic aspect of diet has been rather overdone. Except when the amount of money available for the purchase of food is very limited it may be taken that the nature of the diet consumed is, for the most part, dictated by the appetite rather than the immediate physiological needs of the purchaser. It has been contended that the deductions drawn from the dietary habits of communities may be quite fallacious, but we favour the view put forward long ago by Sir William Roberts that long established food customs cannot be ignored as they represent 'the fruit of colossal experience accumulated by countless millions of men through successive generations'.

The present study is the second of what we had intended to be a comprehensive series. It had been our intention to extend our investigations to the study of one London Borough, one large South Midland, and one large North Midland town, and to one or more strictly rural communities. Unfortunately, owing to circumstances over which we had no control, this comprehensive investigation had

to be, for the moment, given up. If, as we believe, knowledge of the national diet is desirable it is only by the accumulation of data of this order that reasonably accurate information can be obtained.

In our first study we dealt with a more or less vertical slice of a fairly well-to-do population in St. Andrews. A critical examination of our results confirmed us in the belief that for all practical purposes continued investigation of the diets of those financially comparatively well off, although interesting and suggestive enough, was unnecessary. These people, with incomes sufficiently large, have abundant choice in the selection of their diet, yet they, as our figures show, do not consume inordinately large quantities of food. We, in the studies now to be considered, reverted to a fairly thick horizontal sample of two very divergent, so-called working-class, populations living respectively in Cardiff and Reading.

In these two studies carried out with the assistance of the same investigator, Miss M. Shanks, we attempted, using the same methods and basal data as in the St. Andrews study, to obtain two more or less identical studies of the nature of the diet consumed by the industrial class in the two towns in question. In Cardiff 56 accurate family studies, comprising 378 individuals, and in Reading 57 accurate studies, comprising 370 individuals, were carried out. The Cardiff study was made in May, June, and July of 1928 and the Reading one in September, October, and November of the same year. Any seasonal variations due to difference in the time of collection would

TABLE I.

Nature of Occupations.

	Cardiff.	*Reading.*
Labourers	11	13
Dock workers	9	—
Unemployed	7	13
Carters	3	4
Railway workers	3	5
Metal workers	2	—
Corporation workers	6	3
Boiler makers	2	—
Police	1	—
Painters	1	1
Gardeners	1	1
Hairdressers	1	—
Tailors	1	1
Clerks	1	3
Boot repairers	1	—
Widows or women with husbands away at time of study	7	7
Woodworkers	—	3
Stable workers	—	2
Clay workers	—	1

seem, from our St. Andrews data, to be of little moment. As Table I shows, the two collections of families are on the whole very similar, except that obviously the dock workers of Cardiff are replaced by other trades in Reading and that more of unemployed families are present in the Reading set.

II. General Summary of Results.

In Tables II and III are given the general summaries of our results for the two studies. It will be noted that there are certain quite well-marked differences between the two sets of results. The Cardiff families, although their percentage yield of energy from the various proximate principles resembles very closely the yield in Reading, have a diet of higher calorie value. This is obtained by a very slight increase in the protein and a definite increase in the fat and carbohydrate intakes. Although the Cardiff living accommodation is a shade more cramped than in Reading, the families not only spend more on rent, but their weekly expenditure on food is 1*s.* 2*d.* per man higher, and this in spite of the fact that the man value is definitely higher.

Table II.

CARDIFF

General Summary of Means and Standard Deviations. 56 Families.

Man Value.			*Diet per Man per Day.*				*Expend. on Food per Man* (Weekly).*	*Calories per 1d.*	*No. of Rooms.*	*Rent.*
Family.	*Diet.*		*Protein (g.).*	*Fat (g.).*	*Carbo-hydrate (g.).*	*Calories.*				
4·55	4·54		78·7	113·6	440·9	3,174	8/6	225·0	3·96	13/3
—	—	%T.C.†	10·1	33·2	56·7	—	—	—	scullery, &c.	—
—	—	S.D.‡	16·68	32·19	78·72	579·94	29·27	45·38	—	—

* Per Man = in this and all subsequent tables 'per man' values are the mean of the sum of the individual family per man values.
† %T.C. = percentage of total calories.
‡ S.D. = standard deviation. In the case of money the standard deviation is stated in pence.

Table III a.

READING

General Summary of Means and Standard Deviations. 57 Families.

Man Value.			*Diet per Man per Day.*				*Expend. on Food per Man (Weekly).*	*Calories per 1d.*	*No. of Rooms.*	*Rent.*
Family.	*Diet.*		*Protein (g.).*	*Fat (g.).*	*Carbo-hydrate (g.).*	*Calories.*				
4·35	4·30		75·0	100·8	408·2	2,906	7/3¾	242·2	4·07	9/9
—	—	%T.C.	10·5	32·1	57·4	—	—	—	scullery &c.	—
—	—	S.D.	15·39	24·53	82·26	513·54	26·53	43·42	—	—

Table III b.

Income stated and considered Reliable. Means and Standard Deviations. 49 Families.

Man Value.			*Diet per Man per Day.*				*Expend. on Food per Man (Weekly).*	*Calories per 1d.*	*Income per Man (Weekly).*
Family.	*Diet.*		*Protein (g.).*	*Fat (g.).*	*Carbo-hydrate (g.).*	*Calories.*			
4·22	4·15	—	74·5	99·6	409·2	2,896	7/2¼	245·9	12/8¾
—	—	%T.C.	10·5	31·8	57·7	—	—	—	—
—	—	S.D.	16·04	24·62	85·45	531·38	26·72	43·51	—

Still, the figures, for Cardiff at least, are not so very different (Table IV) from those derived from the mixed population of St. Andrews.

TABLE IV.

Study.	Man Value.		Diet per Man per Day in g.			Calories.		Expend. on Food per Man (Weekly).
	Family.	Diet.	Protein.	Fat.	Carbo-hydrate.	Total.	Per 1d.	
St. Andrews	3·37	3·51	83·8	118·6	410·7	3,119	159·6	12/1¾
Cardiff	4·55	4·54	78·7	113·6	440·9	3,174	225·0	8/6
Reading	4·35	4·30	75·0	100·8	408·2	2,906	242·2	7/3¾

If, however, a fair comparison is to be made between the Cardiff and Reading diets, which for the most part are those of families belonging to the less well-to-do members of the community, and other existing diets, it would seem to be more reasonable to compare the values not with those obtained from a mixed population like St. Andrews, but with other similar studies such as those made earlier in Scotland. In this comparison it must be remembered that values are not strictly comparable, as firstly, slightly different man values were employed and, secondly, the Scottish population studied belonged on the whole to a much poorer class. Still, the comparison (Table V) is of

TABLE V.

Study.	Diet per Man per Day (g.).			Calories.		Mean Income per Man per Week.
	Protein.	Fat.	Carbohydrate.	Total.	Per 1d. spent.	
Glasgow, 1921*	72·9	77·6	352·5	2,466	174	12/-
" "	64·1	73·3	380·4	2,505	220	6/10½
Glasgow, 1922†	78·4	87·7	358·0	2,605	237	8/9¾
" "	62·6	61·3	330·7	2,182	302	4/11¾
Glasgow, 1924‡	70·5	78·3	389·9	2,617	206	11/1
Glasgow, 1924§ (artisans)	87·9	96·8	441·1	3,070	177	Unknown
Dundee, 1923‖	63·1	59·6	337·4	2,197	225	12/9
" "	56·2	55·0	336·8	2,064	265	6/6
Cardiff	78·7	113·6	440·9	3,174	225	14/11¾
Reading	75·0	100·8	408·2	2,906	242	12/8¾

* Tully, *Lancet*, 1921, ii. 57.
† Tully and Urie, *Glas. Med. Journal*, 1922, **16**, 353.
‡ *Med. Res. Council Report*, No. 101, p. 162.
§ Tully, *Glas. Med. Journal*, 1924, **19**, 1.
‖ *Med. Res. Council Report*, No. 101, p. 165.

interest, as it goes to show that the Cardiff and Reading values are more nearly comparable with the Glasgow artisan class, a group with a fairly high income, than with any of the other groups in Glasgow or Dundee. The total energy value of the Cardiff diet is slightly above the Glasgow artisan value, but with the protein content a little below, the fat content definitely above, and a practically identical value for carbohydrate. In the case of Reading, it, too, more nearly approaches the artisan values than any of the others. It is curious to note how definitely the fat content of the two English diets is above that of any of these Scottish diets. This may be in part due to the change in the

cost of living in the seven or eight years which separate the two assessments, but racial idiosyncrasy also possibly plays a part. It certainly cannot wholly be ascribed to marked difference in income between the different communities.

III. Nature of Diets related to Income Level.

As regards the amount of the income spent on food, it is interesting to note (Table VI) that of the Scottish families the Glasgow families spend a much greater proportion of their income on food, whereas the Dundee families spend even less than the English families. Although change in the cost of living may play some part, the divergences, in our opinion, cannot be entirely or even mainly ascribed to this cause.

Table VI.

	Aver. Income per Man per Week.	*Percentage of Income spent on Food.*
Glasgow, 1921	12/-	78·7
	6/10½	91·3
,, 1922	8/9¾	69·5
	4/11¾	82·8
,, 1924	11/1	71·5
Dundee, 1923	12/9	44·3
	6/6	46·5
Cardiff	14/11¼	56·9
Reading	12/8¾	56·5

When we turn to the consideration of the Cardiff and Reading families grouped with regard to total incomes, identical groups being employed in the two studies (see Tables VII A and B), it is made very clear that the Reading families are economically on a definitely lower level than those of Cardiff. The tables (p. 10) show that practically only 53 per cent. of the Cardiff families have incomes below £3 per week, and just over 18 per cent. have incomes over £4, whereas in Reading 72 per cent. have incomes under £3 and just below 9 per cent. incomes over £4. This economic difference is reflected in their respective diets. Both series of diets show in the most unmistakable fashion that with rising income there is a definite rise in the consumption of fat, a rise which is not necessarily accompanied by an increase in the protein intake. The inference therefore may, we believe, be drawn that this increase in the fat intake is not primarily due to increased consumption of meat. As a matter of fact, as reference to Tables VII A and B shows, the intake of protein, although somewhat variable, exhibits no definite relation to income. It is true that in the Cardiff study the families with the largest incomes consumed most protein, but this is not true as regards Reading, where although the actual number of families is small, so also is the standard deviation. So far as Reading is concerned, the yield of calories from protein sources shows almost a steady fall from the families with the smallest incomes to those with the largest, whereas in the Cardiff study the protein calorie yield remains practically constant. As regards the

Table VII a.

CARDIFF

Grouping: Total Incomes. Means and Standard Deviations.

55 Families.

Group I: £1 to £1/19/11.
,, II: £2 to £2/19/11.
,, III: £3 to £3/19/11.
,, IV: £4 upwards.

Group.	No. of Families.	Man Value. Family.	Man Value. Diet.		Diet per Man per Day. Protein (g.).	Fat (g.).	Carbohydrate (g.).	Calories.	Expenditure on Food per Man (Weekly).	Calories per Id.
I. £1 to £1/19/11	9	3·22	3·15	—	73·7	101·4	453·9	-3,091	7/11	229·7
(Mean = £1/13/7¼)	—	—	—	%T.C.	9·7	30·4	59·9	—	—	—
	—	—	—	S.D.	13·35	24·44	84·72	534·63	16·11	31·59
II. £2 to £2/19/11	20	4·43	4·39	—	78·7	105·7	447·7	3,128	7/7½	252·4
(Mean = £2/10/7¼)	—	—	—	%T.C.	10·3	31·3	58·4	—	—	—
	—	—	—	S.D.	21·11	28·62	84·90	606·10	30·22	46·07
III. £3 to £3/19/11	16	5·02	5·01	—	77·6	114·4	425·4	3,113	8/9½	213·9
(Mean = £3/7/2¾)	—	—	—	%T.C.	10·2	34·0	55·8	—	—	—
	—	—	—	S.D.	11·38	24·89	69·62	479·06	28·72	32·22
IV. £4 upwards	10	5·04	5·11	—	86·2	140·2	450·7	3,494	10/3¼	189·3
(Mean=£4/17/10¾)	—	—	—	%T.C.	10·1	37·2	52·7	—	—	—
	—	—	—	S.D.	14·34	40·31	65·18	608·51	25·16	37·41

Table VII b.

READING

Grouping: Total Incomes. Means and Standard Deviations.

57 Families.

Group I: £1 to £1/19/11.
,, II: £2 to £2/19/11.
,, III: £3 to £3/19/11.
,, IV: £4 upwards.

Group.	No. of Families.	Man Value. Family.	Man Value. Diet.		Diet per Man per Day. Protein (g.).	Fat (g.).	Carbohydrate (g.).	Calories.	Expenditure on Food per Man (Weekly).	Calories per Id.
I. £1 to £1/19/11	11	3·54	3·49	—	70·9	91·3	368·6	2,640	6/6¼	246·9
(Mean = £1/12/0½)	—	—	—	%T.C.	11·0	32·0	57·0	—	—	—
	—	—	—	S.D.	17·20	27·90	84·54	601·26	26·44	38·58
II. £2 to £2/19/11	30	4·17	4·09	—	74·9	97·5	417·3	2,912	7/0¼	251·1
(Mean = £2/7/5)	—	—	—	%T.C.	10·5	31·0	58·5	—	—	—
	—	—	—	S.D.	16·09	19·40	88·70	499·12	23·33	44·18
III. £3 to £3/19/11	11	4·44	4·36	—	78·9	110·1	418·8	3,051	8/9½	212·4
(Mean = £3/6/4¼)	—	—	—	%T.C.	10·6	33·4	56·0	—	—	—
	—	—	—	S.D.	12·84	29·38	61·42	417·24	30·43	39·27
IV. £4 upwards	5	7·08	7·15	—	75·9	120·8	417·2	3,132	7/7	244·1
(Mean = £5/7/9)	—	—	—	%T.C.	9·9	35·7	54·4	—	—	—
	—	—	—	S.D.	7·94	11·89	40·97	282·50	14·29	27·17

sources of the protein, the Cardiff families derived a slightly greater proportion from animal sources. This constancy in the case of Cardiff also holds good for the total calorie intake so far as the first three groups are concerned, but the fourth group is about 12 per cent. higher than the one immediately below it, a rise from 3,113 to 3,494 cals. In the case of Reading, despite the similarity of mean incomes, and, on the whole, smaller man value, the calorie intake in all groups is definitely below that of Cardiff. The difference between the two populations lies apparently in the fact that the Reading people for one reason or another spend less on food. Examination of the column 'Expenditure on Food per Man' in Tables VII A and B shows the marked difference in expenditure which exists between the two populations.

Further, from these tables (VII A and B) one might deduce that the yield of calories per penny spent was not closely related to the expenditure on food. If, however, this return per penny spent is examined in greater detail in Tables VIII A and B, where the grouping is carried out in terms of this return, the relation to income and expenditure becomes very obvious. In the case of Cardiff it will be noted that with the rising return in calories per penny there is practically a steady fall in the amount both of protein and fat in the diet

TABLE VIII A.

CARDIFF

Grouping: Calories per 1d. spent.

GROUP I: 100 to 150 Calories per 1*d.*
,, II: 150 to 200 ,, ,,
,, III: 200 to 250 ,, ,,
,, IV: 250 to 300 ,, ,,
,, V: 300 upwards ,, ,,

Group.	*No. of Families.*	*Man Value.*			*Diet per Man per Day.*				*Expenditure on Food per Man (Weekly).*	*Income.*	
		Family.	*Diet.*		*Protein (g.).*	*Fat (g.).*	*Carbohydrate (g.).*	*Calories.*		*Per Man (Weekly).*	*No. of Families.*
I. 100–50	2	4·15	4·15		91·6	132·0	451·3	3,440	11/10	£1/4/5¼	2
(Mean = 119·9)	—	—	—	%T.C.	10·9	35·5	53·6	—	—	—	—
	—	—	—	S.D.	7·43	9·00	46·10	96·40	34·95	—	—
II. 150–200	15	3·51	3·54	—	84·8	132·4	424·9	3,308	10/6	£1/1/3¾	14
(Mean = 184·3)	—	—	—	%T.C.	10·5	37·1	52·4	—	—	—	—
	—	—	—	S.D.	16·39	29·36	73·61	549·06	21·22	—	—
III. 200–50	24	4·75	4·73	—	76·6	113·4	444·3	3,177	8/4½	13/6	24
(Mean = 222·7)	—	—	—	%T.C.	9·8	33·1	57·1	—	—	—	—
	—	—	—	S.D.	15·86	31·73	79·48	621·21	21·51	—	—
IV. 250–300	10	5·09	5·01	—	77·7	100·5	461·6	3,134	6/10¼	10/11	10
(Mean = 267·9)	—	—	—	%T.C.	10·1	29·7	60·2	—	—	—	—
	—	—	—	S.D.	17·74	23·17	95·68	565·73	16·33	—	—
V. 300 upwards	5	5·82	5·80	—	67·5	77·3	427·3	2,736	5/1	8/4½	5
(Mean = 314·5)	—	—	—	%T.C.	10·1	26·2	63·7	—	—	—	—
	—	—	—	S.D.	10·87	12·19	38·91	242·12	6·08	—	—

TABLE VIII B.

READING

Grouping: Calories per 1d. spent.

Means and Standard Deviations.

GROUP I: 150 to 200 Calories per 1*d*.
„ II: 200 to 250 „ „
„ III: 250 to 300 „ „
„ IV: 300 to 350 „ „

Group.	*No. of Families.*	*Man Value.*			*Diet per Man per Day.*				*Expenditure on Food per Man (Weekly).*	*Income.*	
		Family.	*Diet.*		*Protein (g.).*	*Fat (g.).*	*Carbohydrate (g.).*	*Calories.*		*Per Man (Weekly).*	*No. of Families.*
I. 150–200	13	3·09	3·02	—	87·1	118·1	427·0	3,194	10/2	16/4¾	10
(Mean = 184·7)	—	—	—	%T.C.	11·1	34·3	54·6	—	—	—	—
	—	—	—	S.D.	16·41	23·47	87·32	443·26	21·30	—	—
II. 200–50	21	4·32	4·28	—	74·6	102·3	406·0	2,909	7/5	13/8½	17
(Mean = 229·9)	—	—	—	%T.C.	10·5	32·5	57·0	—	—	—	—
	—	—	—	S.D.	13·38	21·90	72·20	468·65	16·42	—	—
III. 250–300	18	5·08	5·00	—	69·6	92·1	400·9	2,772	5/10¼	10/8½	17
(Mean = 275·8)	—	—	—	%T.C.	10·2	30·8	59·0	—	—	—	—
	—	—	—	S.D.	12·50	22·36	86·85	523·29	12·16	—	—
IV. 300–50	5	5·20	5·14	—	64·8	80·9	394·6	2,623	4/8¾	8/10¾	5
(Mean = 322·4)	—	—	—	%T.C.	10·1	28·5	61·4	—	—	—	—
	—	—	—	S.D.	9·82	12·61	83·75	475·95	10·05	—	—

and also of the total calorie yield, whereas the carbohydrate content remains relatively constant with a variation of only about 8 per cent. Hence it follows that the yield from carbohydrate sources steadily rises. In the case of Reading, with the rise in the return per penny spent, there is a steady fall not only in the total calorie yield, but in the amounts of all three proximate principles. Although the income per man is about 6*d*. lower in Cardiff than in Reading, in the group with the highest return per penny spent the expenditure on food is about 5*d*. greater.

In Tables IX A and B the two sets of data have been grouped in respect to the expenditure per man on food. As regards Cardiff where only some 7 per cent. of the families have a per man per week expenditure below 5*s*., about 25 per cent. below 7*s*., about 42 per cent. below 10*s*., and 25 per cent. below 15*s*., there is noted a steady rise in the intake of protein, fat, carbohydrate, and total calories with rising expenditure. In Reading some 33 per cent. spend less than 6*s*. per man per week on food, about 35 per cent. less than 8*s*., about 26 per cent. under 11*s*., and 5 per cent. below 15*s*. The same rise in all values is to be noted. It will be observed that the composition of the Reading diets differs in absolute amounts from the Cardiff set, tending to be richer in protein and fat. This discrepancy is only in

Table IX A. CARDIFF

Grouping: Expenditure on Food per Man (Weekly).
Means and Standard Deviations.

Group I: 4/- to 5/-.
„ II: 5/- to 7/-.
„ III: 7/- to 10/-.
„ IV: 10/- to 15/-.

Group.	No. of Families.	Man Value.			Diet per Man per Day.				Calories per Id.	Income (Weekly).	
		Family.	Diet.		Protein (g.).	Fat (g.).	Carbohydrate (g.).	Calories.		Per Man.	No. of Families.
I. 4/- to 5/-	4	6·11	6·09	—	56·4	67·8	389·2	2,444	309·2	8/7¼	4
(Mean = 4/7¼)	—	—	—	%T.C.	9·4	25·7	64·9	—	—	—	—
	—	—	—	S.D.	5·72	12·79	24·34	200·10	15·81	—	—
II. 5/- to 7/-	14	4·96	4·87	—	65·3	89·6	381·6	2,654	249·6	11/4¾	14
(Mean = 6/3)	—	—	—	%T.C.	10·0	31·3	58·7	—	—	—	—
	—	—	—	S.D.	8·51	15·47	49·23	242·58	32·93	—	—
III. 7/- to 10/	24	4·51	4·57	—	79·4	111·3	457·3	3,222	217·6	13/11¾	23
(Mean = 8/6)	—	—	—	%T.C.	10·1	32·0	57·9	—	—	—	—
	—	—	—	S.D.	10·98	15·64	85·15	433·03	38·39	—	—
IV. 10/- to 15/	14	3·76	3·71	—	97·3	154·8	486·9	3,821	189·1	£1/1/10¾	14
(Mean = 11/10¼)	—	—	—	%T.C.	10·4	37·5	52·1	—	—	—	—
	—	—	—	S.D.	12·42	22·97	53·58	322·23	22·61	—	—

Table IX B. READING

Grouping: Expenditure on Food per Man (Weekly).
Means and Standard Deviations.

Group I: 3/- to 6/-.
„ II: 6/- to 8/-.
„ III: 8/- to 11/-.
„ IV: 11/- to 15/-.

Group.	No. of Families.	Man Value.			Diet per Man per Day.				Calories per Id.	Income (Weekly).	
		Family.	Diet.		Protein (g.).	Fat (g.).	Carbohydrate (g.).	Calories.		Per Man.	No. of Families.
I. 3/- to 6/-	19	5·06	4·99	—	62·6	79·0	355·8	2,439	281·5	9/8¼	18
(Mean = 5/0¾)	—	—	—	%T.C.	10·5	30·0	59·5	—	—	—	—
	—	—	—	S.D.	9·02	13·41	73·44	401·82	30·29	8·73	—
II. 6/- to 8/-	20	4·64	4·57	—	75·7	105·4	426·7	3,026	247·4	12/10¾	16
(Mean = 7/1¼)	—	—	—	%T.C.	10·2	32·2	57·6	—	—	—	—
	—	—	—	S.D.	10·53	17·29	70·33	362·28	26·22	7·44	—
III. 8/- to 11/-	15	3·36	3·35	—	82·7	117·3	421·1	3,143	198·0	16/-	12
(Mean = 9/3¾)	—	—	—	%T.C.	10·7	34·6	54·7	—	—	—	—
	—	—	—	S.D.	10·10	24·86	61·36	277·91	18·26	10·92	—
IV. 11/- to 15/-	3	2·96	2·78	—	110·8	125·4	552·3	3,876	180·2	16/8¼	3
(Mean = 12/8)	—	—	—	%T.C.	11·7	30·0	58·3	—	—	—	—
	—	—	—	S.D.	13·51	9·54	36·73	116·91	15·75	18·14	—

part, we believe, explained by the slightly different grouping of expenditure which we were compelled to adopt.

If, instead of comparing expenditure on food, we turn to total income per man per week (see Tables X A and B), we get a somewhat different result. Here, again, we obtain very clear evidence of the financial differences which exist between the two sets of families studied. About 53 per cent. of the Cardiff population examined have weekly incomes per man under 14*s*., whereas in the Reading families more than 71 per cent. have weekly incomes per man below this level. There are other differences also to be noted. Although the mean per man weekly income of the similar number of families in both towns in Group I is practically identical, the diet in the case of the Reading households is quite definitely inferior in every respect, except that they get a somewhat bigger return in calories per penny spent, due probably to the fact that the Cardiff families spend of their income some 78 per cent., whilst the Reading families spend only about 62 per cent. on food. In Group II, however, the position is almost reversed, although the percentage of the income spent on food is practically identical in both sets of families. The weekly per man income

TABLE X A.

CARDIFF

Grouping: Income per Man (Weekly). 55 Families.
Means and Standard Deviations.

GROUP I: 6/- to 10/-.
„ II: 10/- to 14/-.
„ III: 14/- to 18/-.
„ IV: 18/- to 23/-.
„ V: 23/- upwards.

Group.	*No. of Families.*	*Man Value.*			*Diet per Man per Day.*				*Expenditure on Food per Man (Weekly).*	*Calories per 1d.*	*Per cent. Expenditure on Food.*
		Family.	*Diet.*		*Protein (g.).*	*Fat (g.).*	*Carbohydrate (g.).*	*Calories.*			
I. 6/- to 10/-	15	5·83	5·86	—	74·2	87·5	447·2	2,939	6/8¼	263·0	78
(Mean = 8/6)	—	—	—	%T.C.	10·3	27·6	62·1	—	—	—	—
	—	—	—	S.D.	14·76	17·76	90·37	502·90	18·15	40·02	—
II. 10/- to 14/-	14	4·57	4·47	—	70·4	101·7	408·2	2,897	7/0¾	239·8	62
(Mean = 11/4¾)	—	—	—	%T.C.	9·9	32·5	57·6	—	—	—	—
	—	—	—	S.D.	13·96	22·07	82·10	557·56	14·37	28·38	—
III. 14/- to 18/-	11	4·70	4·69	—	86·8	132·1	484·3	3,556	9/8	206·8	61
(Mean = 15/9¼)	—	—	—	%T.C.	10·0	34·4	55·6	—	—	—	—
	—	—	—	S.D.	15·97	30·36	60·06	543·25	23·25	39·68	—
IV. 18/- to 23/-	10	2·93	2·87	—	85·0	131·4	455·5	3,423	10/1¾	201·0	48
(Mean = £1/0/9¾)	—	—	—	%T.C.	10·2	35·5	54·3	—	—	—	—
	—	—	—	S.D.	16·55	19·16	51·24	351·34	20·80	28·08	—
IV. 23/- upwards	5	3·19	3·34	—	87·0	151·3	409·4	3,429	12/-	168·7	39
(Mean = £1/10/8¾)	—	—	—	%T.C.	10·4	40·9	48·7	—	—	—	—
	—	—	—	S.D.	15·35	34·35	45·22	521·24	25·61	17·80	—

TABLE X B.

READING

Grouping: Income per Man (Weekly). 49 Families (Reliable).

Means and Standard Deviations.

GROUP I: 5/- to 10/-.
" II: 10/- to 14/-.
" III: 14/- to 18/-.
" IV: 18/- to £1.
" V: over £1.

Group.	*No. of Families.*	*Man Value.*			*Diet per Man per Day.*				*Expenditure on Food per Man (Weekly).*	*Calories per 1d.*	*Per cent. Expenditure on Food.*
		Family.	*Diet.*		*Protein (g.).*	*Fat (g.).*	*Carbohydrate (g.).*	*Calories.*			
I. 5/- to 10/-	15	5·37	5·27	—	64·7	80·5	375·7	2,542	5/2½	285·2	62
(Mean = 8/5)	—	—	—	%T.C.	10·4	29·3	60·3	—	—	—	—
	—	—	—	S.D.	14·17	16·43	104·70	608·36	13·52	22·74	—
II. 10/- to 14/-	20	4·15	4·10	—	79·6	100·4	432·7	3,023	7/6¼	241·0	63
(Mean = 11/10)	—	—	—	%T.C.	10·7	30·8	58·5	—	—	—	—
	—	—	—	S.D.	16·64	19·60	78·12	435·60	22·20	37·72	—
III. 14/- to 18/-	6	3·09	3·09	—	75·3	105·8	382·8	2,851	8/5½	198·9	52
(Mean = 16/2)	—	—	—	%T.C.	10·8	34·4	54·8	—	—	—	—
	—	—	—	S.D.	8·67	20·55	37·26	181·52	12·23	19·70	—
IV. 18/- to £1	5	2·83	2·71	—	78·5	131·1	423·3	3,258	8/11½	215·3	47
(Mean = 19/1)	—	—	—	%T.C.	9·8	37·2	53·0	—	—	—	—
	—	—	—	S.D.	14·06	21·87	34·78	202·87	12·71	28·18	—
V. over £1	3	3·49	3·36	—	80·7	123·9	449·7	3,310	9/4¼	227·1	41
(Mean = £1/2/8¼)	—	—	—	%T.C.	9·9	34·7	55·4	—	—	—	—
	—	—	—	S.D.	12·32	14·27	79·85	369·94	45·18	50·61	—

and the weekly per man expenditure on food is 6*d.* lower in the Cardiff families. The mean income in the case of the Reading families (admittedly the numbers are smaller) is somewhat larger in Group III, but as the expenditure on food is both absolutely and relatively smaller, the diets of the Reading families are very definitely inferior to those of Cardiff. In Groups IV and V the Reading families lag behind the Cardiff families, although the differences are not so marked. The percentage of income spent on food by the two Cardiff Groups is III, 48 per cent., IV, 39 per cent. as compared with 47 and 41 per cent. respectively in Reading.

The difference in the percentage of income spent on food noted between the two sets of families is also evident when the data from what may be considered two exactly comparable groups in the two places are analysed. Tables XI A and B give the diets of 7 unemployed families in Cardiff and of 12 in Reading. The Cardiff families who, on the per man weekly basis, are in the receipt of the lower average income, spend 77 per cent. of it on food against the 58 per cent. spent on food by the Reading families. The number of calories obtained per penny spent are almost identical, but the source from which they are derived is different. In Cardiff, protein, fat, and carbohydrate

TABLE XI A.

CARDIFF

7 Unemployed Families.
Means and Standard Deviations.

Man Value.			*Diet per Man per Day.*				*Expend. on Food per Man (Weekly).*	*Calories per 1d.*	*Income per Man (Weekly).*	*Per cent. Expend. on Food.*
Family.	*Diet.*		*Protein (g.).*	*Fat (g.).*	*Carbo-hydrate (g.).*	*Calories.*				
4·32	4·24	—	75·2	95·1	457·8	3,056	7/-	263·4	9/6¾	77
—	—	%T.C.	10·0	28·8	61·2	—	—	—	—	—
—	—	S.D.	12·44	25·71	88·82	563·82	21·52	49·26	—	—

TABLE XI B.

READING

12 Unemployed Families.
Means and Standard Deviations.

Man Value.			*Diet per Man per Day.*				*Expend. on Food per Man (Weekly).*	*Calories per 1d.*	*Income* per Man (Weekly).*	*Per cent. Expend. on Food.*
Family.	*Diet.*		*Protein (g.).*	*Fat (g.).*	*Carbo-hydrate (g.).*	*Calories.*				
3·84	3·78	—	71·0	98·5	379·5	2,751	6/3¾	263·7	10/10¼	58
—	—	%T.C.	10·5	33·2	56·3	—	—	—	—	—
—	—	S.D.	14·77	23·36	87·50	558·56	23·19	41·40	—	—

* Income is mean of 11 families only.

yield respectively 10·0, 28·8, and 61·2 per cent. of the total calories, whereas in Reading the respective yield is 10·5, 33·2, and 56·3 per cent. If one had the choice of the two diets, that of Reading, although of somewhat lower calorie value, is preferable.

One of the difficulties which is constantly encountered in such dietary studies as these is that for some reason or other one or more of the families during the week in which it is under investigation is not in receipt of the average wage, i.e. the wage on which the budget is by use and wont balanced. Sometimes it is admitted that the income is above and sometimes below the average. This difficulty is well exemplified in four studies obtained during the investigation of the Cardiff families. These fluctuations in wage render it hard for the housewife to adjust her expenditure. There is a natural tendency to maintain the same standard whether there be an increase or a decrease in the income; hence it follows that there may be very wide discrepancies in the percentage of income spent on food. This is clearly shown in the data given in Table XII. It will be noted that of these four families, three, during the study week, were in receipt of an income larger than the average, and the fourth of an income about half that of the average. Family I has purchased an excellent diet at a cost of 150 per cent. of its income, a diet as good as that, as Table X A shows, purchased by Cardiff families in receipt of incomes 50 per cent. better

Table XII.

CARDIFF

4 Families—Variable Income.
Income during Study Week not typical of Normal or Average Weekly Income.

	Man Value.		*Diet per Man per Day.*				*Expenditure on Food per Man (Weekly).*	*Calories per 1d.*	*Income per Man (Weekly).*	*Per cent. Expenditure on Food.*
	Family.	*Diet.*	*Protein (g.).*	*Fat (g.).*	*Carbohydrate (g.).*	*Calories.*				
I.										
Study Week	5·73	5·23	90·8	109·0	524·7	3,526	7/9¾	263·3	5/2¾	150
Normal or Average Week	—	—	—	—	—	—	—	—	10/9¼	72
II.										
Study Week	8·63	8·56	65·3	93·1	355·6	2,585	6/7¾	226·6	9/8	69
Normal or Average Week	—	—	—	—	—	—	—	—	7/11¼	84
III.										
Study Week	3·33	3·33	66·4	92·5	279·2	2,269	6/7¼	199·6	£1/1/0¼	31
Normal or Average Week	—	—	—	—	—	—	—	—	12/0¼	55
IV.										
Study Week	5·93	5·85	105·0	137·1	538·3	3,903	10/10	210·1	19/9¾	55
Normal or Average Week	—	—	—	—	—	—	—	—	14/9	73

than the normal average income for this family. If it be assumed that this house mother was purchasing as she would have done with her normal average income, she would be spending about 72 per cent. of it on food. When the diets of families II and III are examined, it will be noted that they, particularly that of family III, are relatively poor and more in keeping with that which might be purchased with their normal income. Even then, family III is well below the average for its income level. Family II has spent 69 per cent. of its actual income on food; calculated on its average income this would have risen to 84 per cent. In the case of Family III, who only spent in the study week some 31 per cent. of their income on food, it would, if the expenditure be accepted as the average, have spent about 55 per cent. of the normal income on food. Family IV stands in a class by itself, as this housewife, judging from the diet consumed, has evidently been stimulated to make full use of her windfall. Instead of an expenditure of only 31 per cent. as in family III, this family has an expenditure on food of 55 per cent. of the income.

In Table XIII, which tabulates the diets of 9 Cardiff dock labourers with an average income per man of 11*s.* 7*d.*, there is clear evidence from the size of the standard deviation[1] that within an occupationally homogeneous group the diet used varies greatly both in constituents

[1] The use of the standard deviation as a measure is to permit of an estimation of the dispersion of the series of observations, i.e. of the extent of the deviation of the individual observations from the average or mean value.

TABLE XIII.

CARDIFF

Dock Labourers. 9 Families.

Means and Standard Deviations.

Man Value.			*Diet per Man per Day.*				*Expend. on Food per Man (Weekly).*	*Calories per Id.*	*Income per Man (Weekly).*
Family.	*Diet.*		*Protein (g.).*	*Fat (g.).*	*Carbo-hydrate (g.).*	*Calories.*			
4·79	4·70		75·9	102·7	408·8	2,930	7/10½	224·4	11/7
—	—	%T.C.	10·6	32·5	56·9	—	—	—	—
—	—	S.D.	22·65	28·82	73·36	615·59	28·92	31·75	—

and total energy yield. The mean value of all the families conforms on the whole well with the mean value for all the Cardiff studies, although the per man income and expenditure on food is definitely below this average value.

IV. POOR DIETS.

We make no apology for giving the data in full with reference to the 8 Cardiff and the 12 Reading families who, according to the current man value calculation, obtain 2,500 cals. and under per man. A great deal is spoken and written about diet. The general assumption is that the problem is one of widespread interest. Academically this is undoubtedly true; practically it is not. The only question asked by the majority of people is whether there is enough to eat. In the case of those whose incomes barely suffice to purchase the necessary materials to maintain life, the proper expenditure of the available funds is of real importance. Hence such dietaries as those about to be considered are deserving of serious study. Before turning to their consideration, however, certain points must be emphasized. The question may quite properly be asked—How, if food be the material which makes good the wear and tear and supplies the energy for the performance of work, can this necessary repair substance and energy be obtained from intakes of 2,500 cals. and less, assuming that the recipients are healthy? In our St. Andrews study we dealt with this problem and we produced, we believe, fairly reliable evidence which would suggest that the wage-earner is never reduced to this low level. Those who suffer are the wife, and possibly the children.

Another point which arises is the causal factor in these abnormally low diets. Smallness of income is only partially responsible. Far more important on the whole than the size of the income is improvidence on the part of one or both parents or simply general incapacity. Frequently the wife, who may be an intelligent, capable woman, is hampered by the small allowance made to her by the man. To us, used for the most part to Scottish dietary studies, two facts stand out in these English studies very clearly, viz. the relatively low percentage of the income, at this level, spent on food and the relatively high

charge of housing. Very few of the Scottish families with comparable incomes would be found paying 14*s.* to 15*s.* a week for housing accommodation. The mode in which the income is spent differs markedly from that ruling in the north.

In Table XIV is given the mean values and the separate data for

Table XIV.

CARDIFF

8 Families under 2,500 Calories.

Man Value.		*Diet per Man per Day.*				*Expend. on Food per Man (Weekly).*	*Calories per Id.*	*Income per Man (Weekly).*	*Per cent. Expend. on Food.*
Family.	*Diet.*	*Protein (g.).*	*Fat (g.).*	*Carbo-hydrate (g.).*	*Calories.*				
I. 5·16	5·16	68·3	102·1	310·9	2,493	7/1½	204·2	12/11¾	54·9
II. 6·13	6·13	63·5	74·6	376·5	2,486	5/5½	265·6	8/2	67·0
III. 5·83	5·84	62·8	75·6	366·0	2,450	6/7¾	215·3	15/5¼	43·0
IV. 6·66	6·66	60·2	56·3	412·5	2,450	4/6¾	312·9	8/7½	52·9
V. 5·96	5·44	57·0	88·4	340·4	2,441	6/2	231·2	11/2	55·2
VI. 2·53	2·13	57·4	69·9	354·0	2,327	5/11½	227·5	10/3¼	58·1
VII. 3·33	3·33	66·4	92·5	279·2	2,269	6/7½	199·6	12/0¼	55·2
VIII. 7·23	7·23	48·6	53·8	352·2	2,125	4/4¼	284·5	7/10½	55·3

Mean Values of all 8 Studies.

Man Value.			*Diet per Man per Day.*				*Expend. on Food per Man (Weekly).*	*Calories per Id.*	*Income per Man (Weekly).*	*Per cent. Expend. on Food.*
Family.	*Diet.*		*Protein. (g.)*	*Fat. (g.)*	*Carbo-hydrate. (g.)*	*Calories.*				
5·35	5·24	—	60·5	76·6	348·9	2,380	5/10½	242·6	10/9¾	54
—	—	%T.C.	10·4	29·8	59·8	—	—	—	—	—
—	—	S.D.	5·84	15·90	38·5	123·71	11·34	38·17	—	—

the 8 Cardiff families receiving less than 2,500 cals. per man, who, on the average, spend 54 per cent. of the income on food. The mean calorie intake is definitely low, due to a fairly uniform fall in the consumption of all three proximate principles, as a comparison of the percentage yield of calories from the various sources shows.

	Protein.	*Fat.*	*Carbohydrate.*
General Average	10·1	33·2	56·7
Present Series	10·4	29·8	59·8

The total calorie value is nearly 800 calories below the general mean for Cardiff.

Consideration of the separate values shows that there is no correspondence between the percentage of the income spent on food and the return in calories. The only two percentage expenditures which differ markedly from the average are II, with 67 per cent., and III, with a total income nearly double that of II, who spent 43 per cent., yet the

Table XV.

READING

12 Families under 2,500 Calories.

Man Value.		*Diet per Man per Day.*				*Expend. on Food per Man (Weekly).*	*Calories per Id.*	*Income per Man (Weekly).*	*Per cent. Expend. on Food.*
Family.	*Diet.*	*Protein (g.).*	*Fat (g.).*	*Carbo-hydrate (g.).*	*Calories.*				
I. 4·13	4·13	59·9	73·0	388·5	2,500	5/2¼	279·1	9/8¼	54·0
II. 4·93	4·86	58·7	82·7	362·9	2,485	5/6¼	260·6	10/1¾	54·8
III. 3·83	3·75	58·2	94·5	329·2	2,458	5/7¾	253·8	7/2¼	78·6
IV. 5·63	5·67	67·6	74·2	364·5	2,453	5/3½	270·3	9/6	55·7
V. 7·46	7·46	67·9	71·0	359·0	2,403	5/2¾	268·3	9/9½	53·4
VI. 5·63	5·63	63·6	89·5	285·5	2,254	4/11	267·6	8/2	60·1
VII. 3·93	3·93	57·4	75·9	321·7	2,249	4/11¼	266·1	10/0½	49·1
VIII. 3·93	3·93	56·3	90·0	274·4	2,184	5/5¾	232·9	12/1	45·3
IX. 5·43	5·43	61·8	67·4	307·2	2,134	5/5¼	228·8	—	—
X. 6·03	6·03	49·5	52·3	260·5	1,752	3/6	291·7	5/9¾	60·4
XI. 4·33	4·33	46·9	62·8	235·3	1,730	3/1½	322·2	7/4¾	42·4
XII. 5·23	4·86	43·1	62·8	233·3	1,708	4/-	249·1	8/2¾	48·6

Mean Value of all 12 Studies.

Man Value.			*Diet per Man per Day.*				*Expend. on Food per Man (Weekly).*	*Calories per Id.*	*Income per Man (Weekly).*	*Per cent. Expend. on Food.*
Family.	*Diet.*		*Protein (g.).*	*Fat (g.).*	*Carbo-hydrate (g.).*	*Calories.*				
5·04	5·00	—	57·6	74·7	310·2	2,193	4/10¼	265·9	8/11	54
—	—	%T.C.	10·7	31·5	57·8	—	—	—	—	—
—	—	S.D.	7·37	12·11	50·5	290·89	9·79	24·25	—	—

difference in yield is only 36 calories. Both families were admirable, with steady fathers and intelligent mothers. Family II was hampered by irregular work and III by ill-health.

Families IV and VIII, both large families, are interesting for comparison in that their expenditures per man on food are practically

identical, yet family IV receives some 19 per cent. more protein, over 4 per cent. more fat, about 15 per cent. more carbohydrate, and over 13 per cent. more energy, with naturally a corresponding increase in the return per penny spent. The difference here lies in the character of the two housewives. The mother in family IV was intelligent and hard-working, whereas that in family VIII, in addition to being of a lower type, was further handicapped by ill-health. Families III and VII also spend almost identical amounts per man on food, yet family III obtains a somewhat higher calorie yield with a slightly lower content of protein and quite a definite reduction in fat. The lower energy-yielding diet is in many respects the better of the two. The mother in III, as already noted, although delicate, was a good housewife, while in family VII the mother was a poor manager.

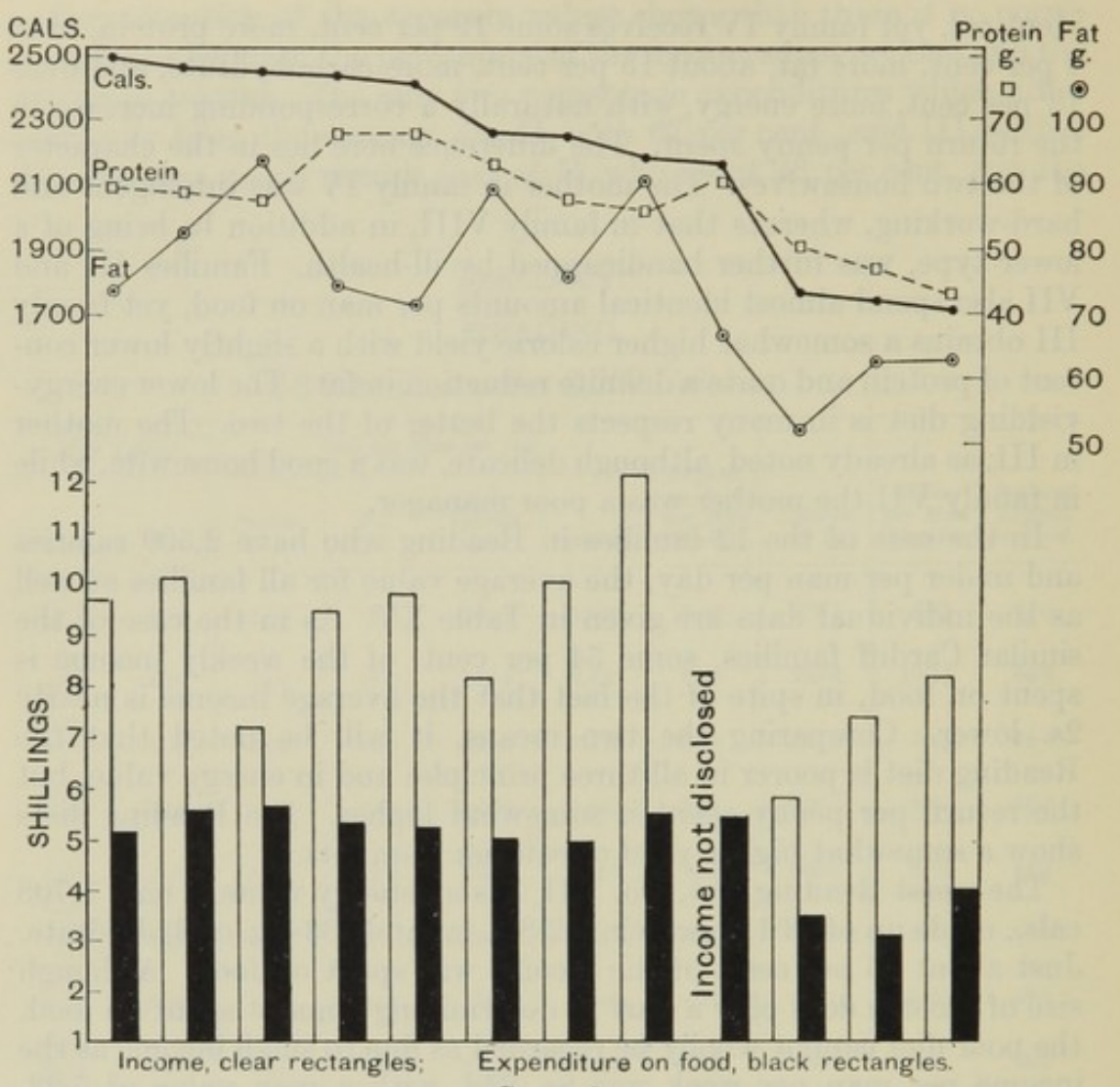
In the case of the 12 families in Reading who have 2,500 calories and under per man per day, the average value for all families as well as the individual data are given in Table XV. As in the case of the similar Cardiff families, some 54 per cent. of the weekly income is spent on food, in spite of the fact that the average income is nearly 2*s*. lower. Comparing the two means, it will be noted that the Reading diet is poorer in all three principles and in energy value, but the return per penny spent is somewhat higher. The Reading diets show a somewhat higher yield of calories from fats.

The worst Reading diet, No. XII, has an energy value of only 1,708 cals., made up of 43·1 g. protein, 62·8 g. fat and 233·3 g. carbohydrate. Just about 45 per cent. of the income was spent on food. Although size of income does play a part in determining amount spent on food, the poor diet cannot wholly be regarded as due to small income as the income per man per week was 8*s*. 2¾*d*. with a man value of 5·23. Another family, No. III, with a per man weekly income of 7*s*. 2¼*d*. (man value 3·83) obtained 2,458 calories with 58·2 g. protein, 94·5 g. fat and 329·2 g. carbohydrate, but they spent just over 78 per cent. of their income on food. It is difficult to adjudicate in such cases. Neither of the husbands was very satisfactory and the two housewives differed markedly. In the first household she was happy-go-lucky, careless, and cheerful, in the second a struggling, intelligent woman. Graph 1 makes it very clear that there is but a small correlation in these particular cases between income per man and the per man calorie value of the diet.

V. Relation of Diet to Parental Capacity.

As in the previous study, we investigated, so far as we were able, the relation between the parental efficiency and the dietary standard. It is a pleasure to record that in both Cardiff and Reading, as the data given in Table XVI show, the standard of parental efficiency was fairly high. The methods adopted in the determination of this capacity were the same as those used, we believe quite successfully, in previous studies.[1] So far as Cardiff is concerned, with both parents

[1] *Sp. Rep. Ser. Med. Res. Coun.* London. Nos. 101, 151.



GRAPH 1.

TABLE XVI.

Parental Efficiency.

Cardiff and Reading Parents combined.

Grouping.	*No.*	*Per Man per Day.*			*Total. Calories.*
		Protein (g.).	*Fat (g.).*	*Carbo-hydrate (g.).*	
I (*a*). Both parents good; (*b*) mother good, father dead or absent	80	79	111	428	3,093
II (*a*). 1 parent good, other fair or bad; (*b*) mother fair, father dead or absent	23	74	102	416	2,947
III (*a*). Both parents fair or bad; (*b*) 1 fair or bad; (*c*) mother bad, father dead or absent	9	68	86	398	2,701

alive, in nearly 65 per cent. of the families both parents could be classed as good, and where the mothers alone are considered, it was found that fully 73 per cent. of them were passed as good, some 20 per cent. as fair, and only 7 per cent. as frankly bad.

In the case of Reading, the results in the matter of parental

efficiency are still better; indeed, they may be regarded as excellent. Where both parents are concerned, some 78 per cent. of the families can be classed as good, and when the mothers alone are considered, they reach the very high figure of 93 per cent.

Owing to the preponderance of good parents and the limited number of families at our disposal it was impossible with any pretence to accuracy to tabulate the various 'less good' parents in special groups. In order to give a fairer conspectus of the whole we have tabulated our data, from Cardiff and Reading combined, in three groups (Table XVI). The result shows most clearly that the parental goodness does play an undoubted role in the nature of the household diet, both as regards quantity and quality.

Whether the result be due to mere accident, to the fewness of the families available, or to some more fundamental difference between the inhabitants of Cardiff and Reading, it is interesting to note that the data at our disposal show that in Cardiff the 'good' mothers expend about 54 per cent. of the total income on food, the 'fair' mothers just over 65 per cent., and the 'bad' mothers nearly 70 per cent.: whereas in Reading the 'good' mothers spend about 58 per cent., the 'fair' mothers only about 48 per cent., and the 'bad' mothers about 45 per cent. of their income on food

VI. Physical Condition of Children in relation to Diet.

Naturally the question arises as to the adequacy, from a general health point of view, of these diets. Under the conditions of our investigation it was impossible to deduce anything definite or reliable, either from direct observation of the subjects or otherwise, as regards their state of health. Speaking generally, from objective examination only, the various members of the different families did not suggest serious mal-nutrition. If such mal-nutrition existed, at any rate if it existed in a marked and chronic form, it might be assumed that this state of affairs would render itself evident in the condition of the children. Admittedly in the small number of families examined the number of children available is correspondingly small. Still, if allowance be made for the smallness of numbers, we feel it may justly be claimed that our data give no evidence of ill-result. If reference be made to Tables XVII and XVIII and Graph 2, it is very evident that the Cardiff children, both in height and weight, compare very favourably with the average of the great body of English school-children incorporated in the data of A. Greenwood[1] and with the more limited body of figures supplied by the Cardiff School Medical Officer.

In Table XIX we have tried, by combining certain of the age groups, to obtain a more definite picture. The resulting figures do suggest that our sample of children is slightly under weight as compared with the general Cardiff school-children, although in the matter of growth there is little to pick and choose between them. Our sample compares well with the greater mass of the Greenwood data. It is impossible, however, with the data at our command, to state whether or not these

[1] *The Health and Physique of School Children.* A. Greenwood. London, 1913.

slight variations are significant, i.e. due to the nature of the diet, or to chance in sampling.

Table XVII.

Cardiff Children. Mean Weights and Heights.

Males. Total 114.

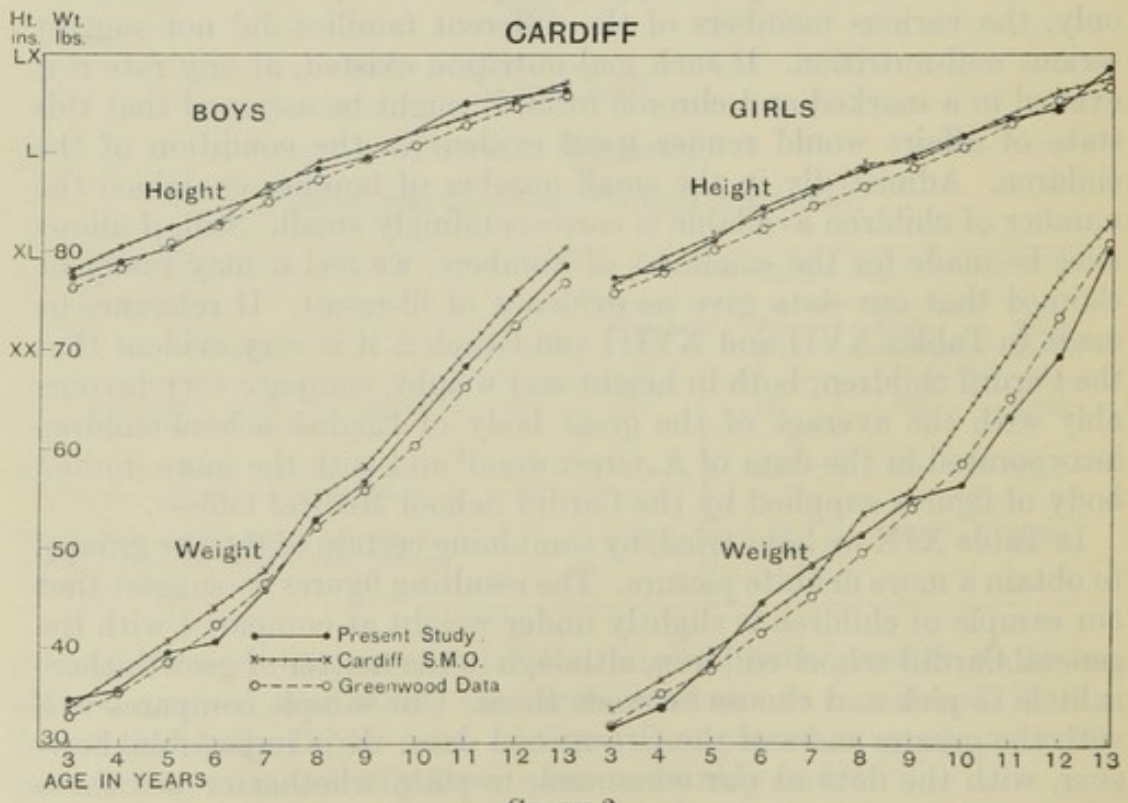
Ages.	$-\frac{5}{12}$	$\frac{6}{12}-\frac{11}{12}$	1	2	3	4	5	6	7	8	9
Weight (lb.)	11·70	17·31	20·39	29·34	34·73	35·87	39·63	40·62	45·75	52·59	56·81
Height (in.)	22·55	27·25	29·17	34·22	37·39	38·85	40·42	42·92	46·61	48·50	49·56
No. of Boys	5	4	9	8	11	13	6	15	7	8	4

Ages.	10	11	12	13	14	15	16	17	18	19 *and over.*
Weight (lb.)	—	68·44	62·63	78·50	81·00	89·33	107·50	121·00	—	129·50
Height (in.)	—	55·00	55·13	56·08	57·00	61·67	64·00	70·00	—	66·00
No. of Boys	—	4	2	6	1	3	4	1	—	3

Females. Total 131.

Ages.	$-\frac{5}{12}$	$\frac{6}{12}-\frac{11}{12}$	1	2	3	4	5	6	7	8	9
Weight (lb.)	10·64	13·88	21·43	27·50	31·64	33·98	37·88	44·24	48·29	51·28	54·92
Height (in.)	21·82	25·00	28·50	33·59	37·36	38·61	41·56	44·66	46·75	48·50	49·83
No. of Girls	11	4	11	8	7	11	8	20	6	9	3

Ages.	10	11	12	13	14	15	16	17	18	19 *and over.*
Weight (lb.)	56·75	63·50	69·58	79·92	84·00	112·00	110·50	—	115·00	134·00
Height (in.)	51·82	53·44	54·29	58·75	59·00	63·00	63·00	—	62·67	64·00
No. of Girls	7	9	6	3	1	1	2	—	3	1



Graph 2.

Table XVIII.

Comparison—School Medical Officer's Report. Cardiff 1928 and Greenwood Data.

Boys. Age (Years).	Average Weight in lb.						Boys. Age (Years).	Average Height in in.					
	Present Study.	No.	Cardiff.	No.	Greenwood Data.	No.		Present Study.	No.	Cardiff.	No.	Greenwood Data.	No.
3.	34·73	11	34·00	141	32·91	12,266	3.	37·39	11	37·60	141	36·46	12,069
4.	35·87	13	37·30	639	35·77	33,200	4.	38·85	13	40·10	639	38·63	33,240
5.	39·63	6	40·70	921	38·68	97,268	5.	40·42	6	42·10	921	40·68	98,560
6.	40·62	15	44·10	548	42·24	29,219	6.	42·92	15	44·30	548	42·83	28,916
7.	45·75	7	47·70	258	46·40	46,439	7.	46·61	7	46·20	258	45·04	47,391
8.	52·59	8	55·10	207	52·90	17,856	8.	48·50	8	48·60	207	47·39	17,896
9.	56·81	4	58·40	954	55·90	11,430	9.	49·56	4	50·00	954	49·18	11,582
10.	—	—	—	—	60·35	27,654	10.	—	—	—	—	50·84	27,775
11.	68·44	4	—	—	66·41	10,489	11.	55·00	4	—	—	52·98	10,732
12.	62·63	2	75·90	1,025	72·66	45,978	12.	55·13	2	55·30	1,025	54·88	47,784
13.	78·50	6	80·30	356	77·40	77,595	13.	56·08	6	56·50	356	56·07	76,407
Girls.							*Girls.*						
3.	31·64	7	33·20	100	31·68	11,118	3.	37·36	7	37·10	100	35·95	11,111
4.	33·98	11	36·50	563	34·98	30,983	4.	38·61	11	39·80	563	38·39	30,959
5.	37·88	8	39·30	805	37·74	93,769	5.	41·56	8	41·80	805	40·44	95,103
6.	44·24	20	42·70	576	41·24	29,394	6.	44·66	20	44·10	576	42·53	29,426
7.	48·29	6	46·20	271	44·93	39,943	7.	46·75	6	46·40	271	44·76	44,504
8.	51·28	9	53·20	144	49·55	17,187	8.	48·50	9	48·60	144	46·94	17,621
9.	54·92	3	55·90	357	54·09	11,543	9.	49·83	3	49·50	357	48·71	11,379
10.	56·75	7	—	—	58·69	26,361	10.	51·82	7	—	—	50·60	26,716
11.	63·50	9	—	—	65·52	10,605	11.	53·44	9	—	—	53·01	10,709
12.	69·58	6	77·90	1,145	73·86	44,128	12.	54·29	6	56·30	1,145	55·48	44,784
13.	79·92	3	82·80	251	80·37	74,195	13.	58·75	3	57·20	251	56·81	72,710

Table XIX.

Sex.	Age Group.*	Mean Weights. Present Study.	Mean Weights. Cardiff Schools.	Mean Weights. Greenwood Data.	Mean Heights. Present Study.	Mean Heights. Cardiff Schools.	Mean Heights. Greenwood Data.
Males	3–4	35·35 (24)†	36·70 (780)†	35·00 (45,466)†	38·18	39·65	38·05 (45,309)
	5–7	41·69 (28)	42·82 (1,727)	41·35 (172,926)	43·31	43·41	42·22 (174,867)
	8–9	54·00 (12)	57·81 (1,161)	53·52 (29,286)	48·85	49·75	48·09 (29,478)
	12–13	74·53 (8)	77·03 (1,381)	75·64 (123,573)	55·84	55·61	55·61 (124,191)
Females	3–4	33·07 (18)	36·00 (663)	34·11 (42,101)	38·12	39·39	37·75 (42,070)
	5–7	43·46 (34)	41·62 (1,652)	40·13 (163,106)	44·30	43·36	41·94 (169,033)
	8–9	52·19 (12)	55·12 (501)	51·37 (28,730)	48·83	49·24	47·63 (29,000)
	12–13	73·03 (9)	78·78 (1,396)	77·94 (118,323)	55·78	56·46	56·30 (117,494)

* No observations in 10–11 group.
† Numbers of children examined : similar for weight and height except in Greenwood figures.

Table XX a. *Reading. Boys—Birth to 13 Years. Number = 104. Mean Weight and Height.*

Age (Yrs.).	Weight. Present Study.	Weight. No.	Weight. School-children in England and Wales.*	Weight. British Association.	Height. Present Study.	Height. No.	Height. School-children in England and Wales.*	Height. British Association.†
Birth	lb.‡				in.			
to $\frac{5}{12}$	16·25	4	—	—	25·38	4	—	—
$\frac{6}{12}$–$\frac{11}{12}$	17·45	5	—	—	25·25	5	—	—
1	25·67	9	—	—	30·50§	9	—	—
2	29·88	12	—	—	34·60	12	—	—
3	31·92	12	32·91	34·0	36·19	12	36·46	36·82
4	37·48	10	35·77	37·3	40·15	10	38·63	38·46
5	42·78	8	38·68	39·9	41·84	8	40·68	41·03
6	44·58	3	42·24	44·4	43·58	3	42·83	44·00
7	47·75	9	46·40	49·7	45·14	9	45·04	45·97
8	50·86	7	52·00	54·9	46·68	7	47·39	47·05
9	58·25	5	55·90	60·4	49·05	5	49·18	49·70
10	65·78	8	60·35	67·5	52·25	8	50·84	51·84
11	68·75	4	66·41	72·6	52·81	4	52·98	53·50
12	86·75	4	72·66	76·7	57·25	4	54·88	54·90
13	84·25	4	77·40	82·6	58·81	4	56·07	56·91

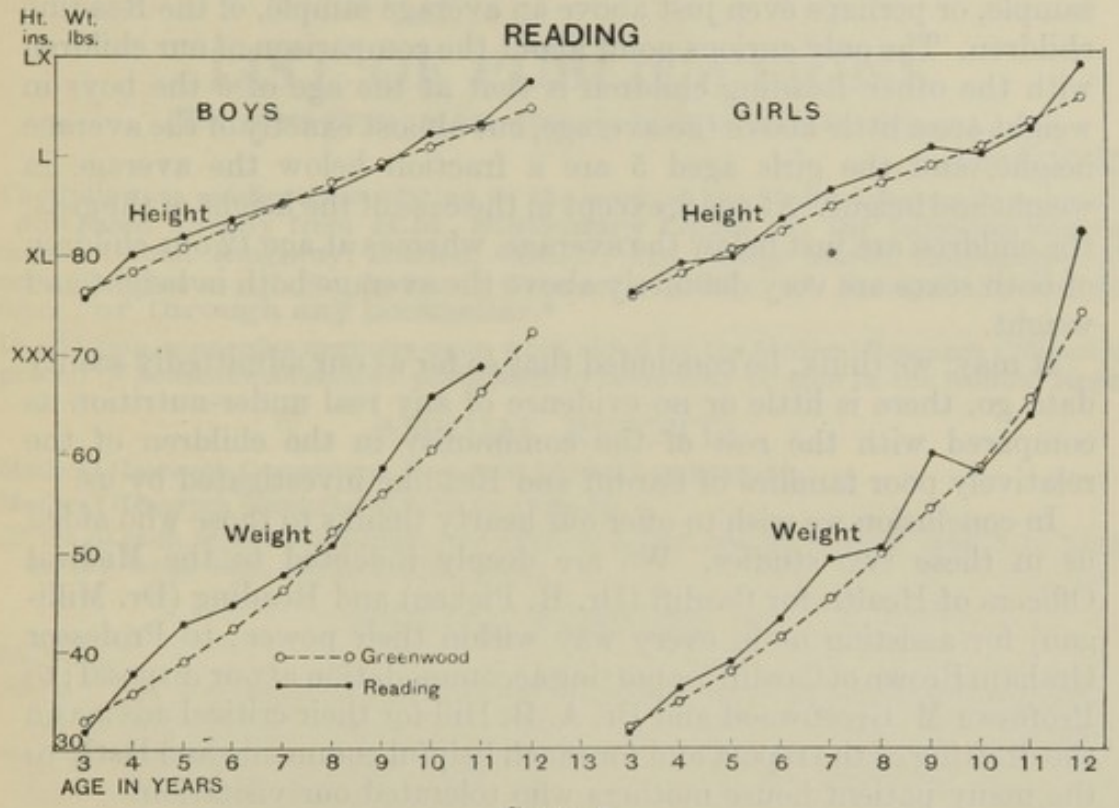
* Elementary school-children in England and Wales (Greenwood). London 1913.
† British Association Report 1883.
‡ Birth to 1 yr.: babies weighed in clothes. 1–13 yrs.: ordinary indoor clothing without shoes.
§ Measurements without shoes.

Table XX b. *Reading. Girls—Birth to 15 Years. Number = 112. Mean Weight and Height.*

Age (Yrs.).	Weight. Present Study.	Weight. No.	Weight. School-children in England and Wales.	Weight. British Association.	Height. Present Study.	Height. No.	Height. School-children in England and Wales.	Height. British Association.
Birth	lb.				in.			
to $\frac{5}{12}$	14·88	4	—	—	25·75	4	—	—
$\frac{6}{12}$–$\frac{11}{12}$	19·80	5	—	—	28·25	5	—	—
1	23·29	13	—	—	29·88	13	—	—
2	28·18	10	—	—	33·93	10	—	—
3	31·45	10	31·68*	31·6	36·55	10	35·95†	36·23
4	36·25	7	34·98	36·1	39·43	7	38·39	38·26
5	38·72	9	37·74	39·2	39·61	9	40·44	40·55
6	42·78	9	41·24	41·7	43·22	9	42·53	42·88
7	49·39	11	44·93	47·5	46·34	11	44·76	44·45
8	49·92	6	49·55	52·1	48·04	6	46·94	46·60
9	60·00	8	54·09	55·5	50·69	8	48·71	48·73
10	58·50	3	58·69	62·0	50·08	3	50·60	51·05
11	63·81	9	65·52	68·1	52·47	9	53·01	53·10
12	82·31	4	73·86	76·4	58·44	4	55·48	55·66
13	85·63	2	80·37	87·2	57·75	2	56·81	57·77
14	112·00	1	88·11	96·7	59·00	1	58·93	59·80
15	119·00	1	96·24	106·3	64·00	1	60·26	60 93

* Grand total, 395,720. † Grand total, 401,634.

When we consider the Reading results as exemplified in Tables XX A and B and Graph 3, the same deductions may be drawn, although here we have only the Greenwood data and the older British Associa-



GRAPH 3.

tion data for comparison. No local comprehensive school data were available. A certain amount of very limited data were obtained by means of which we were enabled to compare the children of the Reading families investigated with certain groups of school-children in the Reading area. These data have been incorporated in Table XXI. It

TABLE XXI.

Comparison—Reading Children. Ages 5, 8, and 12 Years.

Age (Yrs.).	*Present Study.*	*No.*	*Age (Yrs.).*	*Reading Elementary Schools, 1929.*	*No.*	*Age (Yrs.).*	*Schools at Kates Grove, St. Giles' and St. Mary's.*	*No.*	*Age (Yrs.).*	*Schools at Alfred Sutton, Wilson, and George Palmer.*	*No.*
BOYS. Weight.											
5	42·8	8	$5\frac{3}{12}$	40·5	389	5	39·5	36	5	40·8	69
8	50·9	7	$8\frac{3}{12}$	54·8	773	8	52·0	91	8	56·3	200
12	86·8	4	$12\frac{3}{12}$	76·5	445	12	73·3	51	12	77·5	143
BOYS. Height.											
5	41·8	8	$5\frac{3}{12}$	41·3	389	5	41·0	36	5	41·3	69
8	46·7	7	$8\frac{3}{12}$	47·8	773	8	47·0	91	8	48·0	200
12	57·3	4	$12\frac{3}{12}$	55·0	445	12	54·0	51	12	55·3	143
GIRLS. Weight.											
5	38·7	9	$5\frac{3}{12}$	39·3	416	5	37·5	30	5	40·0	74
8	49·9	6	$8\frac{3}{12}$	52·5	719	8	50·5	91	8	53·0	167
12	82·3	4	$12\frac{3}{12}$	77·8	515	12	73·5	42	12	77·8	178
GIRLS. Height.											
5	39·6	9	$5\frac{3}{12}$	41·0	416	5	40·3	30	5	41·0	74
8	48·0	6	$8\frac{3}{12}$	47·5	719	8	47·0	91	8	47·3	167
12	58·4	4	$12\frac{3}{12}$	55·8	515	12	55·0	42	12	55·8	178

will be noted that there are only three age groups which limit the numbers of children measured by us very definitely. It will be observed that our sample again may be regarded as a fair average sample, or perhaps even just above an average sample, of the Reading children. The only curious point about the comparison of our children with the other Reading children is that at the age of 5 the boys in weight are a little above the average, but almost exactly of the average height, and the girls aged 5 are a fraction below the average in weight and height. At age 8, except in the case of the height of the girls, the children are just below the average, whereas at age 12 our children of both sexes are very definitely above the average both in height and weight.

It may, we think, be concluded that so far as our admittedly scanty data go, there is little or no evidence of any real under-nutrition as compared with the rest of the community in the children of the relatively poor families of Cardiff and Reading investigated by us.

In conclusion we wish to offer our hearty thanks to those who aided us in these two studies. We are deeply indebted to the Medical Officers of Health for Cardiff (Dr. R. Picken) and Reading (Dr. Milligan) for assisting us in every way within their power; to Professor Graham Brown of Cardiff for putting accommodation at our disposal; to Professor M. Greenwood and Dr. A. B. Hill for their critical advice on the drafting of the report and for much helpful comment; and lastly to the many patient house mothers who tolerated our visitation.

VII. Summary.

1. The average calorie intake determined by the study of the family diet is approximately 3,000 calories.

2. As in the St. Andrews study we have found that the percentage of calories derived from fat is higher than that generally accepted as the average.

3. The percentage derivation of calories is approximately 10 per cent. from protein, 32 per cent. from fat, and 58 per cent. from carbohydrate.

4. The increase in the intake of fat with rising income is more marked than the rise in the consumption of protein.

5. Judging from the state of the physique of the, admittedly limited, number of children at our disposal there is no evidence of serious malnutrition in the families studied.

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No. 140. Diet and the Teeth : An Experimental Study. Part I. Dental Structure in Dogs. By May Mellanby. [1929.] 17*s.* 6*d.* (18*s.*).

No. 153. Diet and the Teeth: An Experimental Study. Part II. A. Diet and Dental Disease. B. Diet and Dental Structure in Mammals other than the Dog. By May Mellanby. [1930.] 2*s.* 6*d.* (2*s.* 8½*d.*).

No. 159. The Influence of Diet on Caries in Children's Teeth (Interim Report). By the Dental Committee. [1931.] 6*d.* (7*d.*).

Diphtheria :

No. 115. The Prevention of Diphtheria. By J. Graham Forbes. [1927.] 2*s.* (2*s.* 1½*d.*).

(Book). Diphtheria: its Bacteriology, Pathology, and Immunology. By the Bacteriological Committee. [1923.] 12*s.* 6*d.* (13*s.* 3*d.*).

See also Epidemiology (No. 75), Statistics (No. 137).

Dysentery :

Reports upon Investigations in the United Kingdom of Dysentery Cases received from the Eastern Mediterranean :—

No. 4. I. Amoebic Dysentery and the Protozoological Investigation of Cases and Carriers. By Clifford Dobell. [1917.] *Out of print.*

No. 5. II. Report upon 878 Cases of Bacillary Enteritis. By L. Rajchman and G. T. Western. [1917.] *Out of print.*

No. 6. III. Report upon recovered Cases of Intestinal Disease in the Royal Naval Hospital, Haslar, 1915–16. By Paul Fildes and others. IV. Report upon combined Clinical and Bacteriological Studies of Dysentery Cases from the Mediterranean. By S. R. Douglas and L. Colebrook. [1917.] 4*s.* 6*d.* (4*s.* 7½*d.*).

No. 7. V. Report upon 2,360 Enteritis 'Convalescents' received at Liverpool from various Expeditionary Forces. By E. Glynn and others. [1918.] 2*s.* (2*s.* 2*d.*).

No. 15. A Study of 1,300 Convalescent Cases of Dysentery from Home Hospitals : with special reference to the Incidence and Treatment of Amoebic Dysentery Carriers. By Clifford Dobell, H. S. Gettings, Margaret W. Jepps, and J. B. Stephens. [1918.] 1*s.* 3*d.* (1*s.* 4*d.*).

No. 29. A Contribution to the Study of Chronicity in Dysentery Carriers. By W. Fletcher and Doris L. Mackinnon. [1919.] 9*d.* (10*d.*).

No. 30. An Investigation of the Flexner-Y Group of Dysentery Bacilli. By H. S. Gettings. [1919.] 1*s.* (1*s.* 1*d.*).

No. 40. Studies of Bacillary Dysentery occurring in the British Forces in Macedonia. By L. S. Dudgeon and others. [1919.] 3*s.* (3*s.* 1½*d.*).

No. 42. A Study of the Serological Races of the Flexner Group of Dysentery Bacilli. By F. W. Andrewes and A. C. Inman. [1919.] 2*s.* (2*s.* 1½*d.*).

See also Food Poisoning.

Encephalitis :

No. 108. The Sheffield Outbreak of Epidemic Encephalitis in 1924. [1926.] 1*s.* 9*d.* (1*s.* 10½*d.*).

Enteric Infections :

No. 9. A Report upon the Use of Atropine as a Diagnostic Agent in Typhoid Infections. By H. F. Marris. [1917.] 1*s.* (1*s.* 1*d.*).

No. 48. A Report on the probable Proportion of Enteric Infections among Undiagnosed Febrile Cases invalided from the Western Front since October 1916. By W. W. C. Topley, S. G. Platts, and C. G. Imrie. [1920.] 3*s.* (3*s.* 1½*d.*).

See also Bacteriology ; Food Poisoning.

Epidemiology :

No. 75. The Schick Test, Diphtheria and Scarlet Fever. By S. F. Dudley. [1923.] 1*s*. (1*s*. 1½*d*.).

No. 111. The Spread of Droplet Infection in Semi-isolated Communities. By S. F. Dudley. [1926.] 1*s*. 6*d*. (1*s*. 7½*d*.).

No. 120. An Inquiry into the Relationship between Housing Conditions and the Incidence and Fatality of Measles. By J. L. Halliday. [1928.] 1*s*. (1*s*. 1*d*.).

See also SMALL-POX ; STATISTICS ; TUBERCULOSIS ; etc.

Flying, Medical Problems of :

Reports of the Air Medical Investigation Committee :—

No. 28. The Sense of Balance and Stability in the Air. By Henry Head. [1919.] 9*d*. (10*d*.). (Included in No. 53.)

No. 37. The Effects of Diminished Tension of Oxygen, with especial reference to the Activity of the Adrenal Glands. By C. H. Kellaway. The Ear in relation to certain Disabilities in Flying. By S. Scott. [1919.] 1*s*. (1*s*. 1*d*.).

No. 53. The Medical Problems of Flying (including reports on oxygen want, selection of candidates for flying, sense of balance, and flying strain). [1920.] 6*s*. (6*s*. 4*d*.).

No. 84. The Application of the Air Force Physical Efficiency Tests to Men and Women. By L. D. Cripps. [1924.] 1*s*. 6*d*. (1*s*. 7½*d*.).

Food Poisoning:

No. 24. A Report on the Investigation of an Epidemic caused by Bacillus aertrycke. By H. Marrian Perry and H. L. Tidy. [1919.] 9*d*. (10*d*.).

No. 91. An Investigation of the Salmonella Group, with Special Reference to Food Poisoning. By W. G. Savage and P. Bruce White. [1925.] 3*s*. 6*d*. (3*s*. 8*d*.).

No. 92. Food Poisoning : a Study of 100 Recent Outbreaks. By W. G. Savage and P. Bruce White. [1925.] 2*s*. 6*d*. (2*s*. 8*d*.).

No. 103. Further Studies of the Salmonella Group. By P. Bruce White. [1926.] 5*s*. (5*s*. 2½*d*.).

Goitre:

No. 154. Iodine Supply and the Incidence of Endemic Goitre. By J. B. Orr. [1931.] 4*d*. (5*d*.) *See also* NUTRITION (No. 123).

Haemoglobin: *see* BLOOD.

Heart:

No. 8. Report upon Soldiers returned as Cases of 'Disordered Action of the Heart' (D.A.H.), or Valvular Disease of the Heart. By Sir Thomas Lewis. [1917.] 1*s*. (1*s*. 1*d*.).

No. 147. The Electrocardiogram. By W. H. Craib. [1930.] 1*s*. 3*d*. (1*s*. 4½*d*.).

Industrial Health:

The Annual Reports of the Industrial Health (formerly Fatigue) Research Board, and special reports on particular subjects, are published for the Council in separate series. The subjects dealt with include accident causation, rest pauses, spells of work, movement study, vocational selection, and problems of particular industries. A list can be supplied on application to the Secretary of the Board, 38 Old Queen Street, Westminster, S.W. 1.

Influenza:

No. 36. Studies of Influenza in Hospitals of the British Armies in France, 1918. [1919.] 3*s*. 6*d*. (3*s*. 8*d*.).

No. 63. Studies in the Aetiology of Epidemic Influenza. By J. McIntosh. [1922.] 2*s*. 6*d*. (2*s*. 7*d*.).

Jaundice:

No. 113. Spirochaetal Jaundice. By G. Buchanan. [1927.] 4*s*. (4*s*. 2*d*.).

Light Treatment:

No. 131. Irradiation and Health. Two Experimental Studies. By Dora Colebrook. [1929.] 1*s.* 6*d.* (1*s.* 7*d.*).

Malaria: *see* QUININE.

Maternal Mortality: *see* CHILD LIFE and STREPTOCOCCAL INFECTIONS.

Measles: *see* EPIDEMIOLOGY (No. 120).

Miners' Dietaries: *see* NUTRITION.

Miners' Diseases, etc.:

No. 89. Report on Miners' 'Beat Knee', 'Beat Hand', and 'Beat Elbow'. By E. L. Collis and T. L. Llewellyn. [1924.] 1*s.* 6*d.* (1*s.* 7*d.*).

See also JAUNDICE (No. 113).

Miners' Nystagmus: *see* VISION.

Nephritis:

No. 43. Albuminuria and War Nephritis among British Troops in France. By H. MacLean. [1919.] 2*s.* 6*d.* (2*s.* 8*d.*).

No. 142. A Classification of Bright's Disease. By Dorothy S. Russell. [1929.] 8*s.* 6*d.* (8*s.* 10*d.*).

Nerve Injuries:

Reports of the Committee upon Injuries to the Nervous System:—

No. 54. The Diagnosis and Treatment of Peripheral Nerve Injuries. [1920.] 2*s.* (2*s.* 1½*d.*).

No. 88. Injuries of the Spinal Cord and Cauda Equina. [1924.] 1*s.* 6*d.* (1*s.* 7½*d.*).

Nutrition:

No. 13. An Enquiry into the Composition of Dietaries, with special reference to the Dietaries of Munition Workers. By Viscount Dunluce and Major Greenwood.

Out of print.

No. 38. Report on the Present State of Knowledge of Accessory Food Factors (Vitamins). By a Committee appointed jointly by the Lister Institute and Medical Research Council. Second Edition. [1924.] 4*s.* 6*d.* (4*s.* 8½*d.*).

No. 87. Report on the Nutrition of Miners and their Families. By the Committee upon Quantitative Problems in Human Nutrition. [1924.] 1*s.* 3*d.* (1*s.* 4*d.*).

No. 105. Diets for Boys during the School Age. By H. C. Corry Mann. [1926.] 2*s.* 6*d.* (2*s.* 7½*d.*).

No. 123. Iodine in Nutrition: a Review of Existing Information. By J. B. Orr and I. Leitch. [1929.] 2*s.* 6*d.* (2*s.* 8*d.*). *See also* GOITRE (No. 154).

No. 135. The Carbohydrate Content of Foods. By R. A. McCance and R. D. Lawrence. [1929.] 2*s.* (2*s.* 1½*d.*).

No. 146. The Antiscurvy Vitamin in Apples. By Mary F. Bracewell, E. Hoyle, and S. S. Zilva. [1930.] 9*d.* (10*d.*).

No. 151. A Study in Nutrition. An Inquiry into the Diet of 154 Families of St. Andrews. By E. P. Cathcart and A. M. T. Murray, assisted by M. Shanks. [1931.] 1*s.* (1*s.* 1½*d.*).

No. 155. Studies of Nutrition: The Physique and Health of Two African Tribes. By J. B. Orr and J. L. Gilks. [1931.] 2*s.* (2*s.* 2*d.*).

No. 158. The Quantitative Estimation of Vitamin D by Radiography. By R. B. Bourdillon, H. M. Bruce, C. Fischmann, and T. A. Webster. [1931]. 1*s.* (1*s.* 1½*d.*).

See also CHILD LIFE; RICKETS; DENTAL DISEASE.

Pituitary Extract: *see* STANDARDS.

Pneumonia:

No. 79. Bacteriological and Clinical Observations on Pneumonia and Empyemata, with special reference to the Pneumococcus and to Serum Treatment. By E. E. Glynn and Lettice Digby. [1923.] 5*s.* (5*s.* 3*d.*).

Pneumothorax, Artificial: *see* TUBERCULOSIS.

Print, Legibility of: *see* VISION.

Protozoan Infections :

No. 59. A Report on the Occurrence of Intestinal Protozoa in the inhabitants of Britain. By Clifford Dobell. [1921.] 2*s.* (2*s.* 1½*d.*).

Quinine :

No. 96. Clinical Comparisons of Quinine and Quinidine. By the Committee upon Cinchona Derivatives and Malaria. [1925.] 1*s.* (1*s.* 1*d.*).

Radium :

No. 62. Medical Uses of Radium : Studies of the Effects of Gamma Rays from a large Quantity of Radium. By various authors. [1922.] 5*s.* (5*s.* 3*d.*).

No. 90. Medical Uses of Radium : Summary of Reports from Research Centres for 1923. [1924.] 1*s.* (1*s.* 1*d.*).

No. 102. Ditto for 1924. [1926.] 1*s.* 6*d.* (1*s.* 7*d.*).

No. 112. Ditto for 1925. [1926.] 1*s.* 3*d.* (1*s.* 4*d.*).

No. 116. Ditto for 1926. [1927.] 1*s.* (1*s.* 1½*d.*).

No. 126. Ditto for 1927. [1928.] 1*s.* (1*s.* 1½*d.*).

No. 144. Ditto for 1928. [1929.] 1*s.* (1*s.* 1*d.*).

No. 150. Ditto for 1929. [1930.] 9*d.* (10*d.*).

No. 160. Ditto for 1930. [1931.] 1*s.* (1*s.* 1*d.*).

Rheumatism : *see* CHILD LIFE (No. 114).

Rickets :

No. 20. A Study of Social and Economic Factors in the Causation of Rickets, with an Introductory Historical Survey. By L. Findlay and Margaret Ferguson. [1918.] *Out of print.*

No. 61. Experimental Rickets. By E. Mellanby. [1921.] 4*s.* (4*s.* 2*d.*).

No. 68. Rickets : the Relative Importance of Environment and Diet as Factors in Causation. By H. Corry Mann. [1922.] 2*s.* 6*d.* (2*s.* 7½*d.*).

No. 71. The Aetiology and Pathology of Rickets from an experimental point of view. By V. Korenchevsky. [1922.] 4*s.* (4*s.* 3*d.*).

No. 77. Studies of Rickets in Vienna, 1919–22. [1923.] 7*s.* 6*d.* (7*s.* 10½*d.*).

No. 93. Experimental Rickets : The Effect of Cereals and their Interaction with other factors of Diet and Environment in producing Rickets. By E. Mellanby. [1925.] 3*s.* 6*d.* (3*s.* 8*d.*).

Salvarsan : *see* VENEREAL DISEASES ; STREPTOCOCCAL INFECTIONS ; STANDARDS, BIOLOGICAL (No. 128).

Scarlet Fever: *see* STATISTICS (No. 137).

Scurvy : *see* NUTRITION (No. 146).

Shock, Surgical :

Reports of the Committee on Surgical Shock and Allied Conditions:—

No. 25. Wound-Shock and Haemorrhage. [1919.] 4*s.* (4*s.* 5½*d.*).

No. 26. Traumatic Toxaemia as a Factor in Shock. [1919.] 1*s.* (1*s.* 1*d.*).

No. 27. Blood Volume Changes in Wound-Shock and Primary Haemorrhage. By N. M. Keith. [1919.] 9*d.* (10*d.*).

Small-pox :

No. 98. Studies of the Viruses of Vaccinia and Variola. By M. H. Gordon. [1925.] 3*s.* 6*d.* (3*s.* 8½*d.*).

No. 106. Small-pox and Climate in India : Forecasting of Epidemics. By Sir Leonard Rogers. [1926.] 2*s.* (2*s.* 1½*d.*).

No. 143. Diagnostic Value of the 'Vaccinia Variola' Flocculation Test. By W. L. Burgess, James Craigie, and W. J. Tulloch. [1929.] 1*s.* 3*d.* (1*s.* 4*d.*).

No. 156. Further Investigations on the Variola-Vaccinia Flocculation Reaction. By James Craigie and W. J. Tulloch. [1931.] 3*s.* (3*s.* 2½*d.*).

Spinal Deformities: *see* ANATOMY (No. 161).

Standards, Biological :

No. 69. I. Pituitary Extracts. By J. H. Burn and H. H. Dale. [1922.] 1*s.* 6*d.* (1*s.* 7*d.*)

No. 128. II. Toxicity Tests for Novarsenobenzene (Neosalvarsan). By F. M. Durham, J. H. Gaddum, and J. E. Marchal. [1929.] 1*s.* 9*d.* (1*s.* 10*d.*).

See also VENEREAL DISEASES (No. 44) and NUTRITION (No. 158).

Statistics (MISCELLANEOUS).

No. 16. A Report on the Causes of Wastage of Labour in Munition Factories. By Major Greenwood. [1918.] 1*s.* 6*d.* (1*s.* 7*d.*).

No. 60. The Use of Death-rates as a Measure of Hygienic Conditions. By John Brownlee. [1922.] 3*s.* (3*s.* 1½*d.*).

No. 95. Internal Migration and its Effects upon the Death-rates: with Special Reference to the County of Essex. By A. B. Hill. [1925.] 3*s.* 6*d.* (3*s.* 8*d.*).

No. 137. Scarlet Fever, Diphtheria, and Enteric Fever, 1895–1914: A Clinical-Statistical Study. By E. W. Goodall, M. Greenwood, and W. T. Russell. [1929.] 2*s.* (2*s.* 1½*d.*).

Streptococcal Infections:

No. 119. A Study of some Organic Arsenical Compounds with a view to their Use in certain Streptococcal Infections. By L. Colebrook. [1928.] 1*s.* 3*d.* (1*s.* 4*d.*).

Surgery.

No. 125. A Study of Intracranial Surgery. By H. Cairns. [1929.] 3*s.* (3*s.* 2½*d.*).

No. 138. The Preparation of Catgut for Surgical Use. By W. Bulloch, L. H. Lampitt and J. H. Bushill. [1929.] 4*s.* (4*s.* 3*d.*).

See also BURNS; SHOCK, SURGICAL.

T.N.T. Poisoning:

No. 11. The Causation and Prevention of Tri-nitro-toluene (T.N.T.) Poisoning. By Benjamin Moore. [1917.] 1*s.* (1*s.* 1½*d.*).

No. 58. T.N.T. Poisoning and the Fate of T.N.T. in the Animal Body. By W. J. O'Donovan and others. [1921.] 3*s.* (3*s.* 1½*d.*).

Tuberculosis:

No. 1. First Report of the Special Investigation Committee upon the Incidence of Phthisis in relation to Occupations.—The Boot and Shoe Trade. [1915.] 3*d.* (3½*d.*).

No. 18. An Investigation into the Epidemiology of Phthisis Pulmonalis in Great Britain and Ireland. Parts I and II. By John Brownlee. [1918.] 1*s.* 3*d.* (1*s.* 4½*d.*).

No. 22. An Inquiry into the Prevalence and Aetiology of Tuberculosis among Industrial Workers, with special reference to Female Munition Workers. By Major Greenwood and A. E. Tebb. [1919.] 1*s.* 6*d.* (1*s.* 7*d.*).

No. 33. Pulmonary Tuberculosis: Mortality after Sanatorium Treatment. By Noel D. Bardswell and J. H. R. Thompson. [1919.] 2*s.* (2*s.* 2*d.*).

No. 46. An Investigation into the Epidemiology of Phthisis in Great Britain and Ireland. Part III. By John Brownlee. [1920.] 2*s.* 6*d.* (2*s.* 7½*d.*).

No. 67. Report on Artificial Pneumothorax. By L. S. T. Burrell and A. S. MacNalty. [1922.] 2*s.* 6*d.* (2*s.* 8*d.*).

No. 76. Tuberculosis in Insured Persons accepted for Treatment by the City of Bradford Health Committee. By H. Vallow. [1923.] 6*d.* (7*d.*).

No. 83. Tuberculosis of the Larynx. By Sir St. Clair Thomson. [1924.] 2*s.* 6*d.* (2*s.* 8*d.*).

No. 85. An Inquiry into the After-Histories of Patients treated at the Brompton Hospital Sanatorium at Frimley, during the years 1905–14. By Sir P. H.-S. Hartley, R. C. Wingfield, and J. H. R. Thompson. [1924.] 1*s.* 6*d.* (1*s.* 7*d.*).

No. 94. Tuberculin Tests in Cattle, with special reference to the Intradermal Test. By the Tuberculin Committee. [1925.] 3*s.* (3*s.* 3*d.*).

No. 122. The Intradermal Tuberculin Test in Cattle: Collected Results of Experience. By J. B. Buxton and A. S. MacNalty. [1928.] 1*s.* 6*d.* (1*s.* 7½*d.*).

No. 149. Tuberculosis in Man and Lower Animals. By H. H. Scott. [1930.] 4*s.* (4*s.* 4*d.*).

No. 152. Studies of Protection against Tuberculosis: Results with B. C. G. Vaccine in Monkeys. By A. Stanley Griffith. [1931.] 9*d.* (10½*d.*).

No. 164. The Value of Tuberculin Tests in Man with special reference to the Intracutaneous Test. By P. D'Arcy Hart. [1932.]

Venereal Diseases:

No. 14. The Wassermann Test. By the Committee upon Pathological Methods. *New Edition.* [1921.] 1*s.* (1*s.* 1*d.*).

No. 19. The Laboratory Diagnosis of Gonococcal Infections. Methods for the Detection of *Spironema pallidum*. By the Bacteriological Committee. *New Edition*. [1923.] 1*s*. 6*d*. (1*s*. 7½*d*.).

No. 21. The Diagnostic Value of the Wassermann Test. By the Committee upon Pathological Methods. [1918.] *Out of print.*

No. 23. An Analysis of the Results of Wassermann Reactions in 1,435 Cases of Syphilis or Suspected Syphilis. By Paul Fildes and R. J. G. Parnell. [1919.] 2*s*. (2*s*. 1*d*.).

No. 41. (I.) An Investigation into the Ultimate Results of the Treatment of Syphilis with Arsenical Compounds. By Paul Fildes and R. J. G. Parnell. (II.) A Clinical Study of the Toxic Reactions which follow the Intravenous Administration of '914'. By R. J. G. Parnell and Paul Fildes. [1919.] 2*s*. (2*s*. 1*d*.).

No. 44. Reports of the Special Committee upon the Manufacture, Biological Testing, and Clinical Administration of Salvarsan and of its Substitutes. I. [1919.] 1*s*. (1*s*. 1*d*.).

No. 45. Unsuspected Involvement of the Central Nervous System in Syphilis. By Paul Fildes, R. J. G. Parnell, and H. B. Maitland. [1920.] 1*s*. (1*s*. 1*d*.).

No. 47. The Accuracy of Wassermann Tests, applied before and after death, estimated by Necropsies. I. The Wassermann Test applied before death. By H. M. Turnbull. [1920.] 2*s*. 6*d*. (2*s*. 7½*d*.).

No. 55. (I.) Results of the Examination of Tissues from Eight Cases of Death following Injections of Salvarsan. By H. M. Turnbull. (II.) The Influence of Salvarsan Treatment on the Development and Persistence of Immunity, as indicated by Measurements of Agglutinins. By E. W. Ainley Walker. [1920.] 3*s*. (3*s*. 1½*d*.).

No. 66. Toxic Effects following the Employment of Arsenobenzol Preparations. By the Salvarsan Committee. [1922.] 2*s*. (2*s*. 1½*d*.).

No. 78. The Serum Diagnosis of Syphilis: The Wassermann and Sigma Reactions compared. [1923.] 5*s*. 6*d*. (5*s*. 9*d*.).

No. 107. The Effect of Treatment on the Wassermann Reactions of Syphilitic Patients. By E. E. Glynn, R. E. Roberts, and P. M. Bigland. [1926.] 3*s*. 6*d*. (3*s*. 8*d*.).

No. 129. The Wassermann Test. Technical Details of No. 1 Method M.R.C. (Modified). By E. J. Wyler. [1929.] 9*d*. (10*d*.).

No. 132. The Treatment of Syphilis: A Survey of Records from St. Thomas's Hospital. By L. W. Harrison. [1929.] 2*s*. (2*s*. 1½*d*.).

Ventilation, etc.:

No. 32. The Science of Ventilation and Open-air Treatment. Part I. By Leonard Hill. [1919.] 10*s*. (10*s*. 5½*d*.).

No. 52. The Science of Ventilation and Open-air Treatment. Part II. By Leonard Hill. [1920.] 6*s*. (6*s*. 4½*d*.).

No. 73. The Kata-thermometer in Studies of Body Heat and Efficiency. By Leonard Hill and others. [1923.] 5*s*. (5*s*. 2½*d*.).

No. 100. Methods of Investigating Ventilation and its Effects. By H. M. Vernon and others. [1926.] 2*s*. (2*s*. 1½*d*.).

Vision:

No. 65. First Report of the Miners' Nystagmus Committee. [1922.] *Out of print.*

No. 80. Second Report of the Miners' Nystagmus Committee. [1923.] 9*d*. (10*d*.).

No. 110. The Legibility of Print. By R. L. Pyke. [1926.] 4*s*. (4*s*. 2*d*.).

Reports of the Committee on the Physiology of Vision:

No. 104. I. Illumination and Visual Capacities. By R. J. Lythgoe. [1926.] 2*s*. 6*d*. (2*s*. 7½*d*.).

No. 127. II. Dark Adaptation (a Review of the Literature). By Dorothy Adams. [1929.] 5*s*. (5*s*. 2½*d*.).

No. 130. III. Two Studies in the Psychology of Reading. By M. D. Vernon and R. W. Pickford. [1929.] 2*s*. (2*s*. 1½*d*.).

No. 133. IV. Experiments on Binocular Vision. By N. M. S. Langlands. [1929.] 2*s*. 6*d*. (2*s*. 8*d*.).

No. 134. V. The Adaptation of the Eye: its Relation to the Critical Frequency of Flicker. By R. J. Lythgoe and K. Tansley. [1929.] 2*s*. 6*d*. (2*s*. 7½*d*.).

No. 136. VI. Some Experiments on Peripheral Vision. By Myer Salaman. [1929.] 2*s.* 6*d.* (2*s.* 7*d.*).

No. 139. VII. A Redetermination of the Trichromatic Mixture Data. By W. D. Wright. [1929.] 1*s.* 3*d.* (1*s.* 4*d.*).

No. 148. VIII. The Movements of the Eyes in Reading. By M. D. Vernon. [1930.] 9*d.* (10*d.*).

No. 163. IX. Psychological Factors in Peripheral Vision. By C. C. Grindley. [1932.] 1*s.* (1*s.* 1*d.*)

Vitamins : *see* NUTRITION.

Wassermann Test : *see* VENEREAL DISEASES.

Wound Infections :

No. 12. The Classification and Study of the Anaerobic Bacteria of War Wounds. By J. McIntosh. [1917.] *Out of print.*

No. 39. Report on the Anaerobic Infections of Wounds and the Bacteriological and Serological Problems arising therefrom. By the Committee upon Anaerobic Bacteria and Infections. [1919.] 6*s.* (6*s.* 3½*d.*).

No. 57. Studies in Wound Infections. By S. R. Douglas, A. Fleming, and L. Colebrook. [1920.] 4*s.* 6*d.* (4*s.* 8½*d.*).

The following books were published under the direction of the Medical Research Committee and are obtainable from the publishers named :

Milk and its Hygienic Relations. By Janet E. Lane-Claypon. 9*s.* net. [Longmans, Green & Co.]

The Amoebae living in Man. By Clifford Dobell. 7*s.* 6*d.* net. [Bale, Sons & Danielsson, Ltd.]

The Intestinal Protozoa of Man. By Clifford Dobell and F. W. O'Connor. 15*s.* net. [Bale, Sons & Danielsson, Ltd.]

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AUSTRALIA : Messrs. Angus & Robertson, Ltd., 89 Castlereagh Street, Sydney, New South Wales. Messrs. Albert & Son, Ltd., 180 Murray Street, Perth, Western Australia. Messrs. Fuller Oldham & Morris Pty., Ltd., 36 Elizabeth Street, Hobart, Tasmania.

SOUTH AFRICA : The Central News Agency, Ltd., Cape Town, P.O. Box 9 ; Johannesburg, P.O. Box 1033 ; Durban, P.O. Box 938 ; Port Elizabeth, P.O. Box 356 ; Pretoria.

INDIA : Messrs. Thacker, Spink & Co., Calcutta and Simla. Messrs. Thacker & Co., Ltd. Bombay. Messrs. Higginbothams, Ltd., Madras and Bangalore.

GOLD COAST: The Wesleyan Methodist Book Depot, P.O. Box 100, Cape Coast. Branches at Accra, Kumasi, and Sekondi.

U.S.A. : The British Library of Information, 270 Madison Avenue, New York.

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I. Studies in the Localization of Sound

A. The Localization of Sounds in the Median Plane.
By J. H. Shaxby and F. H. Gage

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PREFACE

Four years ago the attention of the Medical Research Council became particularly directed towards the need for co-ordinating and extending research in the physiology of hearing. They had in view not only the advancement of primary knowledge of the subject, and the investigation of various problems of medical and scientific interest within its field, but also some practical questions of a special kind in which help was sought by the Departments concerned with national defence. It was, in fact, through problems in the Defence Services, which were at that time being investigated by an Inter-Service Committee on Acoustics under the direction of the Royal Engineer Board, that their attention was first drawn to immediate requirements.

In 1928, therefore, the Council appointed a Committee on the Physiology of Hearing, to advise them on the initiation and supervision of research work and on co-operation with other Government Departments, especially the Service Departments, in the study of problems involving questions of hearing. The Committee thus constituted included representatives of physiological, psychological, otological, and physical science.

Since their appointment the Committee have been active in advising the Defence Services, and they have also considered various other questions such as the detection of defects in hearing. They have also kept before them the desirability of encouraging the study of the subject at the Universities and other centres.

The present report is issued by the Council on the advice of the Committee, and is the first report which has been submitted by the Committee for publication. The report gives the results of two separate studies, both dealing with scientific aspects of sound localization. This question has some important practical applications. The correct estimation of the position of a source of sound in space, for example, is a matter of prime importance in the work of the Defence Services, as for instance in the detection of aircraft and submarines, and is also involved in judgements which have to be made in various industrial occupations.

A review of existing literature on the subject of sound localization is being prepared, and will be published in a subsequent report.

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STUDIES IN THE LOCALIZATION OF SOUND

A. THE LOCALIZATION OF SOUNDS IN THE MEDIAN PLANE: AN EXPERIMENTAL INVESTIGATION OF THE PHYSICAL PROCESSES CONCERNED

BY

J. H. Shaxby, D.Sc., and F. H. Gage, M.Sc.

(from the Physiology Institute, Cardiff)

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1. Introduction

The estimation of the position of a source of sound can be made in either of two ways. The observer may regard his own orientation as fixed and describe the sound as being to his right or left, at an azimuth of so many degrees; or he may himself turn until the sound appears to be directly ahead of him. The first method is less exact than the second, and presents special disturbing phenomena which add to the difficulties of its practical use. The second method, that of localizing the source of sound in the median plane ('son de milieu') is the one used with Sound Locators. For these reasons the work to

be described here is confined to this aspect of the subject, the factors which determine the judgement that a source lies in the median plane, directly in front of the observer. The estimation of the height of the source, i.e. of its angular altitude, is not considered, since with locators this problem is reduced again to the median plane determination by the use of two horns one above the other.

As is well known, the basis of the judgement referred to has been variously stated to be (1) difference of intensity or loudness at the two ears: the sound is considered to be in the median plane when the loudness is the same for both ears, (2 *a*) difference of phase, the median position being that in which there is no phase difference, and (2 *b*) difference of time of arrival, simultaneity at the two ears being the criterion of the median position. In the case of continuous sounds this, of course, means that the crests or other significant features of the two series of waves arrive simultaneously at the two ears.

It has also been suggested that both (1) and (2 *a*) or (1) and (2 *b*) act together in localization. Some authors, notably the late Lord Rayleigh, ascribed the localizing of comparatively low tones primarily to the phase factor, that of high ones to the loudness factor. Perrin finds a real physiological discontinuity in this respect at about fa_4 or sol_4, and remarks that this is about the pitch at which the wave-length is comparable with the distance between the ears, so that ambiguity would occur for phase perceptions on the one hand, and the head would begin to be an effective screen in producing loudness differences at the two ears on the other hand.

As between the theories (2 *a*) and (2 *b*), the use of phase differences or time intervals, opinion seems to be turning to the latter view. In the first place it is difficult to conceive of any physiological mechanism which could react directly to anything so abstract as phase difference, i.e. to the fractional value of a period or wave-length of the incident sound waves. Most of the work by which the phase theory has been supported as against the loudness theory is equally valid for the time-difference view.

It is clear, however, that experiment is capable of deciding between the two conceptions; on the phase theory, a given deviation in localization should be associated with the same fraction of a period for notes of different frequencies, i.e. with time differences proportional to those periods or inversely proportional to the frequency. On the time-interval theory, the given deviation should always require the same time difference, whatever the pitch of the note localized. Experiments described below appear to show definitely that this is the case.

That such time differences of arrival of the sound at the two ears and not differences of intensity are the important factor of localization in the median plane is also shown; differences of loudness can affect localization, but the differences ordinarily met with in the case of natural sources of sound are negligible in this respect as compared with the factor of time difference. It is shown that loudness differences can be experimentally balanced against time differences, so that, for

example, a sound reaching the left ear before the right but at a notably weaker intensity may be judged to have its source in the median plane. As has just been remarked, the difference of loudness required to effect this balancing of moderate time difference is far greater than those occurring under natural conditions.

Also, as has been noted repeatedly by other observers, a moderate deafness in one ear or the partial closing of a tube through which sound reaches one ear does not affect localization.

It is further to be observed that in work with locators the mean angle of error in setting for a given observer is practically independent of the distance of the source of sound, so long as this is not so great as to make hearing difficult, i.e. so long as the intensity is well above the threshold. This follows at once if the time difference at the horns of the locator is the deciding factor. But if the determining quantity were the absolute difference of intensity at the horns the accuracy of locating would vary inversely as the cube of the distance; if it were the relative difference of intensity it would vary inversely as the distance.

The accuracy of working under the best possible conditions with modern locators appears to approach that corresponding to the least time interval which the hearing mechanism is physiologically capable of appreciating, as measured by experiments in which a note is presented to the two ears at a measured difference of time, and with equal intensities. This also is what one would expect if localization is by time difference; it would be difficult to explain on the loudness hypothesis.

It will be shown later (Section 6) that the extent to which the intensity factor can influence localization of this kind must be negligible in comparison with the effect of time difference.

A further argument in favour of the time-interval theory is the well-ascertained fact that, in working with locators, a complex sound is more easily and accurately located than a comparatively pure tone. If phase differences were in question, since these differences would have different values for the various components of the complex sound, they would tend to introduce confusion rather than to make localization easier. On the time-difference view the component tones are a number of clues all acting in agreement in aiding localization. It may be noted in passing that in some of the experiments we have performed this difference in accuracy as between pure and complex notes did not appear. These cases are exactly those in which the time differences involved were extremely small, a few microseconds; it may well be supposed that this approaches the physiological limit of localization imposed by the structure of the ear and its nervous and central connexions. In that event, it is obvious that the use of a complex tone cannot bring about any further improvement as compared with a pure tone.

We conclude that for ordinary sounds, at any rate for frequencies lower than about 1,200 vibrations per second, the physical phenomenon on which localization in the median plane depends is the

interval of time elapsing between the arrival of the sound waves (i.e. of some significant feature, maximum pressure-variation, velocity, or the like) at the two ears.

For notes higher than the limit stated the factor of loudness may play a part, in view of the statement by more than one experimenter that phase differences artificially produced fail to give localization for such high notes. We have not ourselves observed this failure, in the methods we have employed.

2. Preliminary Experiments with Double-Tube Method

A certain amount of preliminary work was done, using the well-known method of conducting the sound to the two ears by separate acoustical paths, by means of two tubes whose lengths could be varied. In our experiments the apparatus consists of a T-piece sliding quietly over the ends of two tubes, each of which at its further end is continued by a length of wide rubber tubing leading to one side of an ordinary double stethoscope. The sound is introduced by a telephone receiver fixed to the free end of the stem of the T. The sound then divides between two paths, one running to each ear. The difference of time of arrival at the two ears is at once given in terms of the difference of path length corresponding to the setting of the T-piece, and the velocity of sound in the tubes.

It is assumed that no perceptible difference of loudness is introduced by this small path difference: as far as could be judged by listening to each side of the stethoscope separately this assumption is justified. Owing to the symmetry of the two limbs there is no reason to suspect disturbing factors depending on reflections or resonances in the branches: observations made with notes of very varied types and pitches were quite consistent among themselves, which one would hardly expect if there was resonance. We are aware that there are theoretical objections to this type of apparatus, and for that reason we used it only for certain preliminary investigations, for which it proved quite satisfactory, and for obtaining data with a considerable number of observers for the tests which are given in the following section.

Upon the observations made with this simple apparatus we base certain conclusions which are in our judgement of some importance.

(1) The double-tube method is satisfactory and simple both for selecting and training listeners as a preliminary to work with locators. It familiarizes them with the phenomenon to be observed, viz. the passing of a sound from one side to the other of the head, introduces them easily to the technique of bracketing, and detects good and bad listeners with a minimum of trouble.

(2) In analysing a listener's results, it is desirable to keep clearly in mind two distinct elements:

(*a*) his BIAS, i.e. the average error of his central determination from the 'theoretical' value (geometrical centre of the tube apparatus or sighted zero of the locator);

(*b*) his UNCERTAINTY, i.e. the standard deviation of a given number of observations.

The Bias is analogous to a consistent one-sided error in firing at a target, the Uncertainty is analogous to the scatter of a series of successive shots.

We have not found that there is a Bias of any considerable magnitude in the large majority of subjects. The Uncertainty differs largely from observer to observer, and even to some extent for the same observer at different times.

These facts are exhibited in Table 1, which is based on data of observations made by forty medical students. These worked in pairs, acting alternately as observers and adjustors of the apparatus. They were first instructed in using the method of bracketing in finding the 'central' position (i.e. sliding the T-piece to and fro past the required point with gradually decreasing amplitude of motion) and subsequently carried out the experiments unaided.

TABLE 1

(*Each observer made ten observations*)

Bias.			*Bias.*		
Left.	*Right.*	*Uncertainty.*	*Left.*	*Right.*	*Uncertainty.*
mm.	mm.	mm.	mm.	mm.	mm.
..	16·3	6·7	..	11·3	19·1
..	6·7	7·8	1·4	..	19·5
3·3	..	8·1	..	29·7	19·6
..	14·3	8·3	..	30·5	21·6
26·6	..	8·7	3·1	..	22·6
1·6	..	9·1	2·1	..	22·7
15·3	..	9·4	16·8	..	24·1
18·9	..	11·2	12·0	..	24·5
..	28·0	11·4	1·0	..	25·9
5·3	..	12·8	16·3	..	27·9
10·7	..	12·8	..	3·6	31·4
5·3	..	13·8	..	30·2	35·8
..	7·8	14·2	25·6	..	36·0
..	17·7	14·4	1·2	..	39·0
..	13·3	14·5	9·1	..	45·8
5·9	..	15·5	1·8	..	46·8
12·3	..	16·1	..	8·6	47·5
..	2·9	16·4	1·3	..	56·8
..	14·7	17·1	50·3	..	77·5
..	0·2	18·2	..	5·4	86·8

The table is arranged in the order of increasing values of the Uncertainty. It is to be noticed that about 13 mm. corresponds to a rotation of 1° of a locator whose horns are 150 cm. apart. It is seen that only one of the observers makes a mean error equivalent to as much as 3°, but it does not follow from this that they are good listeners. A good listener is one who can be reasonably expected to show a small error in any one trial, i.e. one whose Uncertainty is small as well as his Bias. With the exception of the last but one of the above observers, the mean error never exceeds the equivalent of

2°; for 23 of the 40 observers it is less than 1°. But the last few subjects would obviously be very poor listeners: the last of the list, for instance, despite the good centrality of the mean of all his trials, will have more than 5° error in setting the locator in half his separate attempts.

3. The Pure-Tone Generator

In order to obtain a note of any desired frequency a heterodyne generator was constructed.

1. A valve oscillator is contained in the copper box indicated by the dotted lines A in Fig. 1. This oscillator is of fixed frequency of approximately 75,000 per second.

2. A similar valve oscillator of variable frequency is contained in the copper box B. This oscillator covers a frequency range of from 75,000 down to approximately 64,000 per second. Four variable air condensers are added to the fixed 0·005 mfd. condenser F to make this possible. One of the 0·00025 mfd. condensers is used as a rough zero adjustment, the vernier condenser V is used to bring the frequencies of the two oscillators finally into unison. The other 0·00025 condenser takes the heterodyne note to a frequency of roughly 2,000 per second, while the 0·001 condenser raises the frequency further to 11,000 per second. The oscillating and reaction coils X_1, X_2, X_3, and X_4 are wound of Litz wire on wooden formers of diameter 5 cm., the number of turns on the respective coils being shown in the diagram. Each of the high-frequency chokes consists of three coils of 600 turns of 40 s.w.g. wire in series and placed close together. The diameter of the coils is 8 cm.

The copper boxes A and B are directly connected, and B is separately joined to the third copper compartment C by means of copper tubing. The latter compartment contains the anode-bend rectifying valve. Insulated wire connexions are made through the copper tubing previously mentioned. Oscillator 2 is coupled to the grid of the rectifying valve in compartment C by means of the 15 turn coil Y placed near the oscillating coils in B. Oscillator 1 is coupled in a more complicated way. A single turn of wire is placed near the oscillating coils of that oscillator, and this forms part of a tuned circuit, the remainder of which is in compartment C. This consists of the 65 turn coil placed near the 10 turn coil and the two condensers C_5 and C_6 of the magnitude shown. C_6 is a variable air condenser and the resonant circuit is tuned by adjusting this to the fundamental frequency of oscillator 1. This can be indicated by the loudness of the telephones in the output circuit, the position of maximum loudness corresponding to resonance.

This resonant circuit filters out the harmonics of oscillator 1. In this way there are no harmonics to heterodyne with the harmonics of oscillator 2. The lowest heterodyne note caused by the harmonics of oscillator 2 and the fundamental of oscillator 1 is of the order of 50,000 cycles per second and is therefore supersonic.

The whole of this apparatus is decoupled as is usual in radio

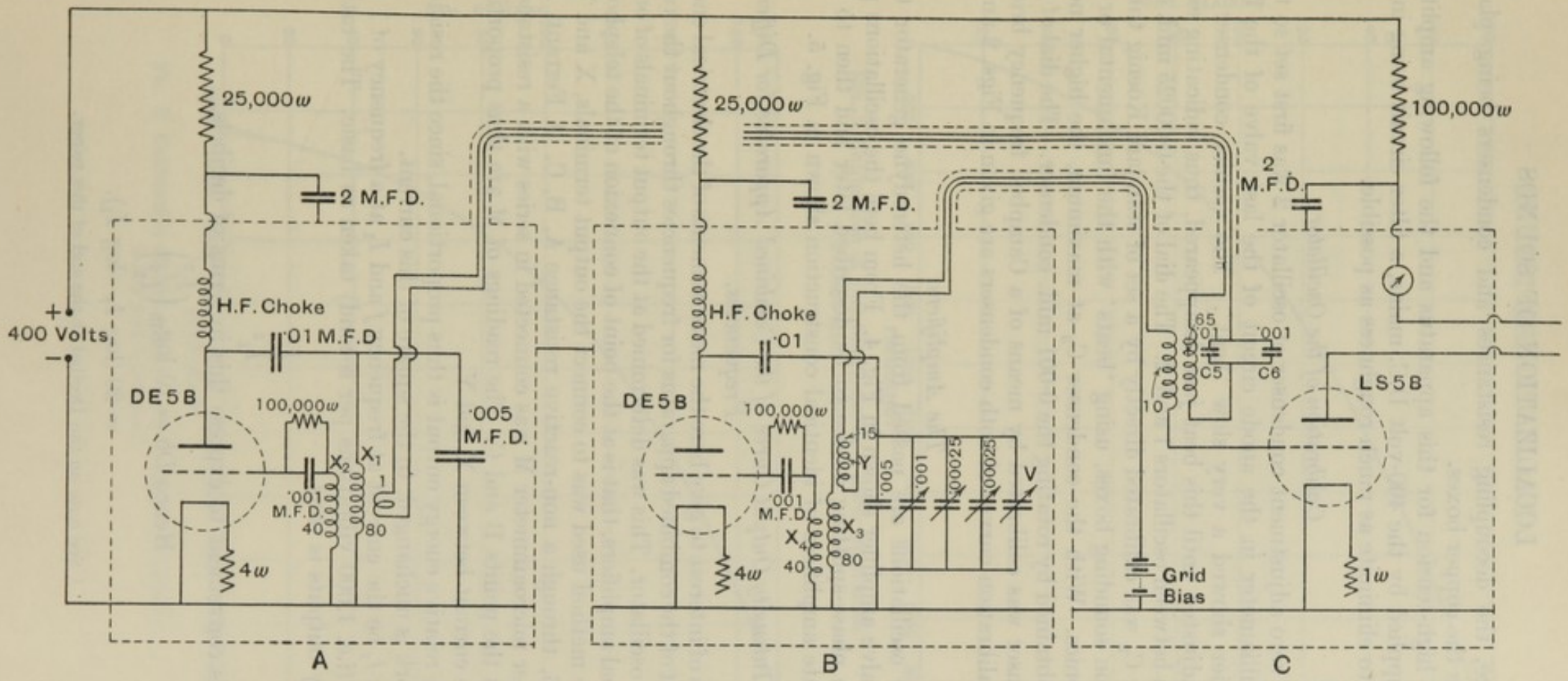


FIG. 1. Pure tone generator.

practice, the decoupling resistances and condensers being placed outside the copper boxes.

The high-tension for this apparatus and the following amplifiers was supplied by the 400-volt D.C. mains, a filter circuit being interposed to eliminate as much roughness as possible.

Calibration of the Oscillator

The zero adjustment condenser of oscillator 2 was first set so that the milliameter in the anode circuit of the last valve of the final amplifier showed a very slow 'beat'. The vernier condenser was then adjusted until this beat just disappeared, thus indicating exact unison between oscillators 1 and 2. The dial of the 0·00025 mfd. condenser C_3 was calibrated directly by a set of standard Koenig tuning forks on sounding boxes, using 'beats' with the fundamental or the harmonics. With the condenser C_3 at maximum, the higher notes were obtained by rotating the 0·001 mfd. condenser. The dial of this condenser was calibrated by means of a Campbell frequency bridge. The calibration curves of both condensers are given in Figs. 2 and 3.

The Amplifiers.

The oscillations are passed from the heterodyne generator to a two-valve amplifier shown in Fig. 4. From here the oscillations pass to the phase-splitting device to be described later and then to two separate amplifiers of identical construction shown in Fig. 5.

The Intensity Output Curve of the Combined Apparatus for Different Frequencies.

It is of interest to give here the measurements of the electrical energy output of the combined apparatus for frequencies throughout the range of the oscillator. This was determined at the output terminals of one of the final amplifiers, that is at the point of connexion of the telephones.

The method used was to connect the output terminals, X and Y in Fig. 6, through a non-reactive resistance A, B, C. A Ferranti A.C. rectifier microammeter M was connected in series with a resistance R across the points B and C. The readings of M are thus proportional to the current between X and Y.

The relative energy output is thus proportional, since the resistance network is unchanged, to the square of this current.

Let I_f be the current at frequency f and I_1 at a frequency of 1 kilocycle (i.e. 1,000 vibrations per second) taken as basic. The ratio of energy outputs is thus

$$\frac{I_f^2}{I_1^2}$$

It is convenient to express this in terms of decibels.[1]

$$\text{Hence } Db = 10 \log_{10} \left(\frac{I_f^2}{I_1^2} \right)$$

$$= 20 (\log I_f - \log I_1).$$

[1] See note on the Decibel at the end of this paper.

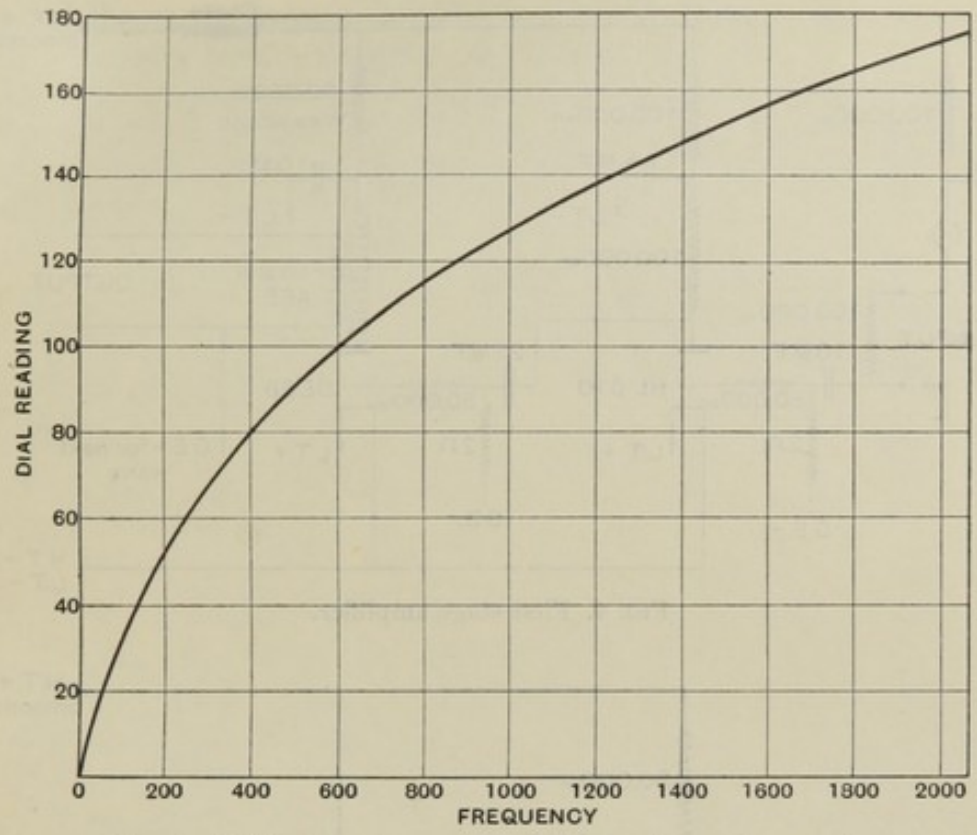


FIG. 2. Calibration of pure tone generator: lower frequencies 0·00025 mfd. condenser.

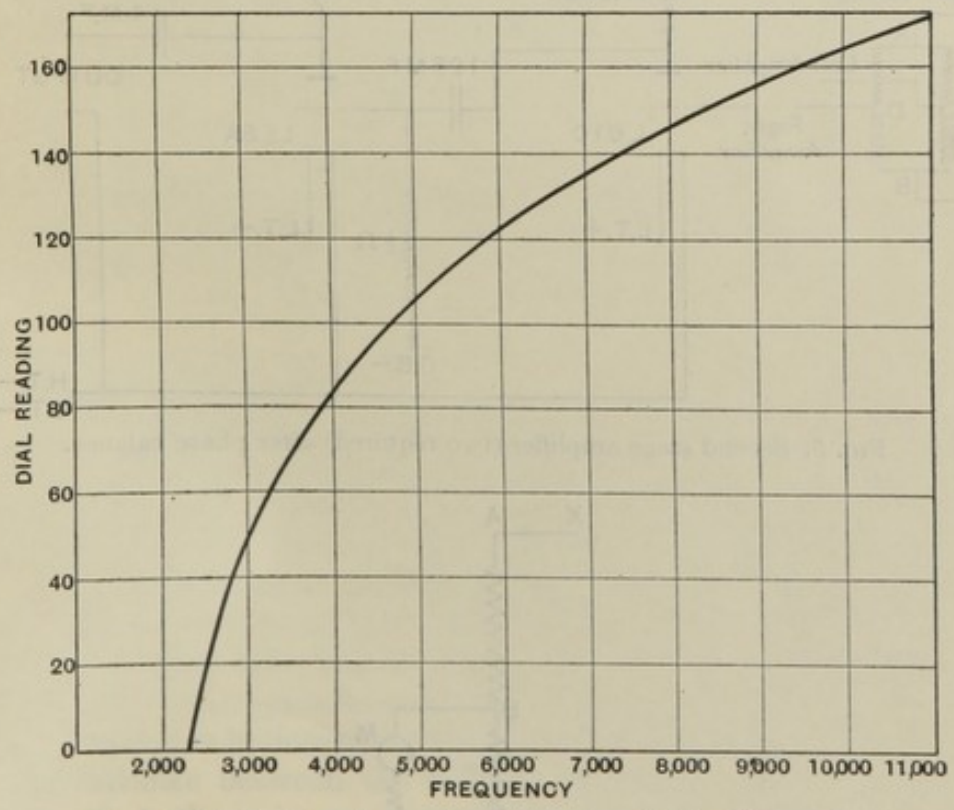


FIG. 3. Calibration of pure tone generator: higher frequencies 0·001 mfd. condenser.

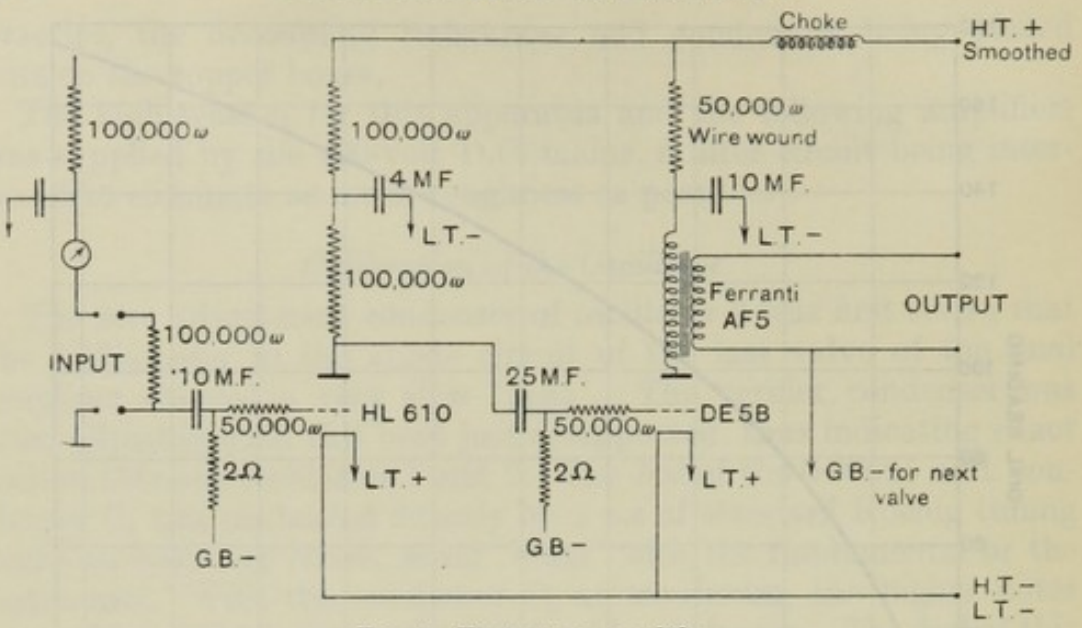


FIG. 4. First stage amplifier.

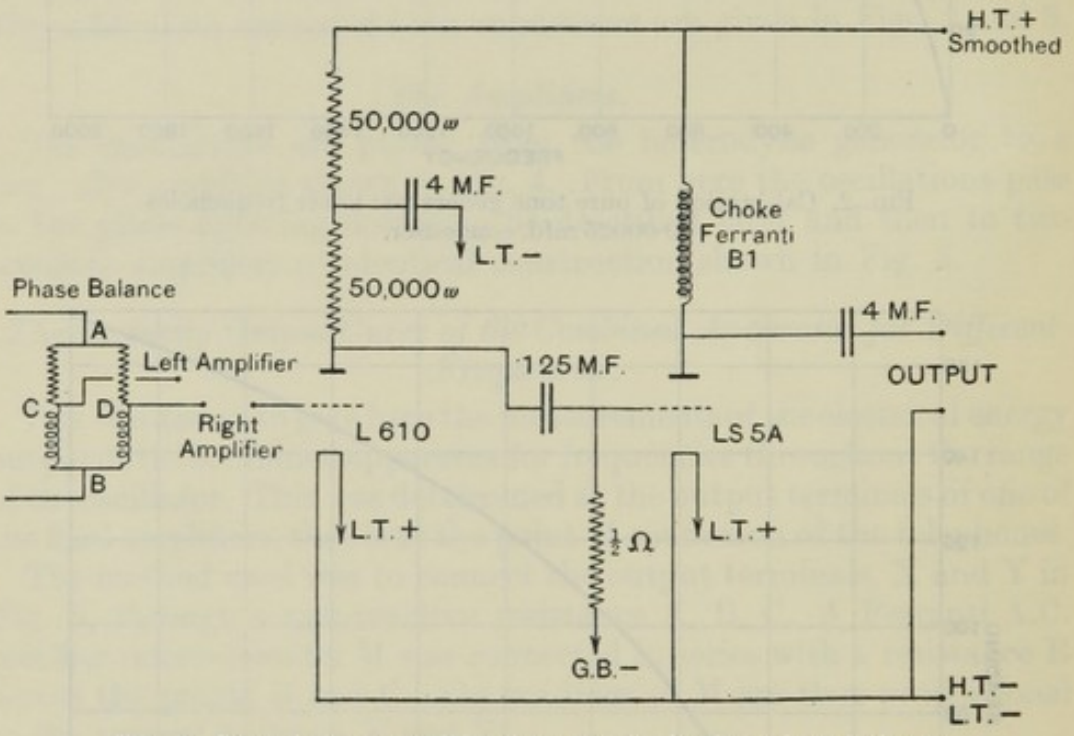


FIG. 5. Second stage amplifier (two required) after phase balance.

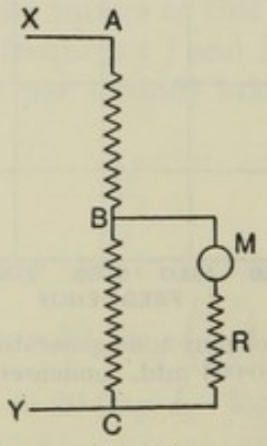


FIG. 6

Fig. 7 shows the energy output expressed in decibels in this way, the zero intensity level being that at a frequency 1,000.

It will be seen that from a frequency of about 40 to one of about 5,500 there is never more than approximately 1·5 *db.* variation in output, while between 200 and 2,800 there is no variation greater than the experimental error.

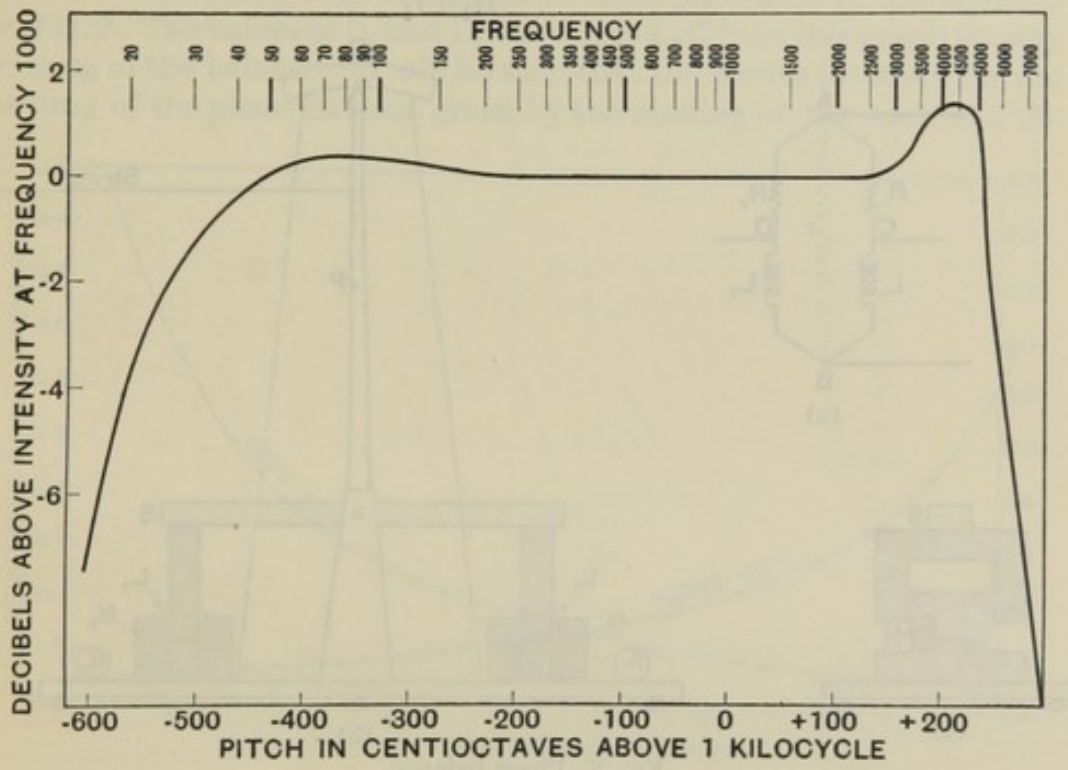


Fig. 7

It is to be understood that this constancy does not imply constancy of acoustical output, since this depends on frequency response of the telephones employed.

4. The 'Phase Balance'

This apparatus consists of the components diagrammatically shown in Fig. 8 *a*. R_1 and R_2 are two wire-wound resistances of 20,000 ohms each. L_1 and L_2 are two iron core inductances wound on small transformer stampings. Constructional details of the 'phase balance' are shown in Figs. 8 *b* and 8 *c*. The coils N are wound on the T section stampings, while the U section stampings are fixed to the balanced arm. When one side of this arm is depressed the self-inductance of the coil on that side will be increased; at the same time the self-inductance of the opposite coil will be decreased. The position of the balance arm S is indicated by the pointer P working over the scale Q.

The distance between the coils L_1 and L_2 is sufficiently large to ensure that there is no appreciable mutual inductance between them.

The points A and B in Fig. 8 *a* are connected to the output terminals of the amplifier following the oscillator. Points C and D are

joined to the grids of the first valves in the following amplifiers as shown in Fig. 5.

If we consider the current through the fictitious resistance r in Fig. 8 *a*, then the current in ACB will lag behind the current in r by a phase-angle given by

$$\theta_1 = \tan^{-1} \frac{L_1\omega}{R_1+r_1}$$

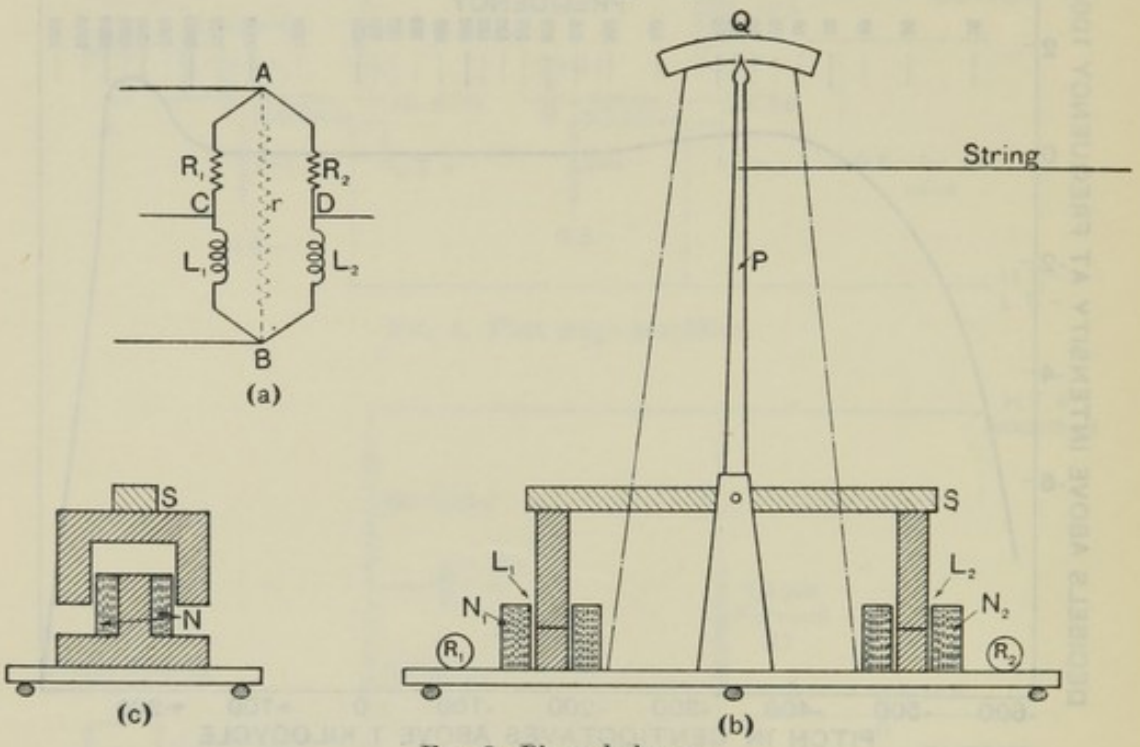


Fig. 8. Phase balance.

where ω is the angular frequency $= 2\pi \times$ frequency f. r_1 is the resistance of the coil L_1, and L_1 its inductance.

Similarly the current in L_2 will lag behind that in r by a phase-angle given by

$$\theta_2 = \tan^{-1} \frac{L_2\omega}{R_2+r_2}$$

Now θ will be small owing to the magnitude of R_1 and R_2 so we can put

$$\theta_1 = \frac{L_1\omega}{R_1+r_1} \text{ and } \theta_2 = \frac{L_2\omega}{R_2+r_2}$$

The corresponding time lags will be $\frac{\theta_1}{\omega}$ and $\frac{\theta_2}{\omega}$ seconds.

Thus if t_1 be the time lag in the branch ACB, and t_2 that in ADB

Then $$t_1 = \frac{L_1\omega}{(R_1+r_1)\omega} = \frac{L_1}{(R_1+r_1)} \text{ seconds.}$$

Similarly $t_2 = \frac{L_2}{(R_2+r_2)}$ seconds.

Therefore $$t_1-t_2 = \frac{L_1}{R_1+r_1} - \frac{L_2}{R_2+r_2} \text{ seconds.}$$

Now in the apparatus as constructed

$$R_1+r_1 = R_2+r_2 = R \text{ say,}$$

so that

$$t_1-t_2 = \frac{L_1-L_2}{R}$$

The values of L_1 and L_2 were measured for different positions of the phase balance, using Hay's A.C. bridge. A graph of these results is shown in Fig. 9. The values of L_1 and L_2 can be read off from this graph for any setting of the balance. It will be seen that the curves intersect for the setting of the phase balance given by the reading on the scale of 5·92.

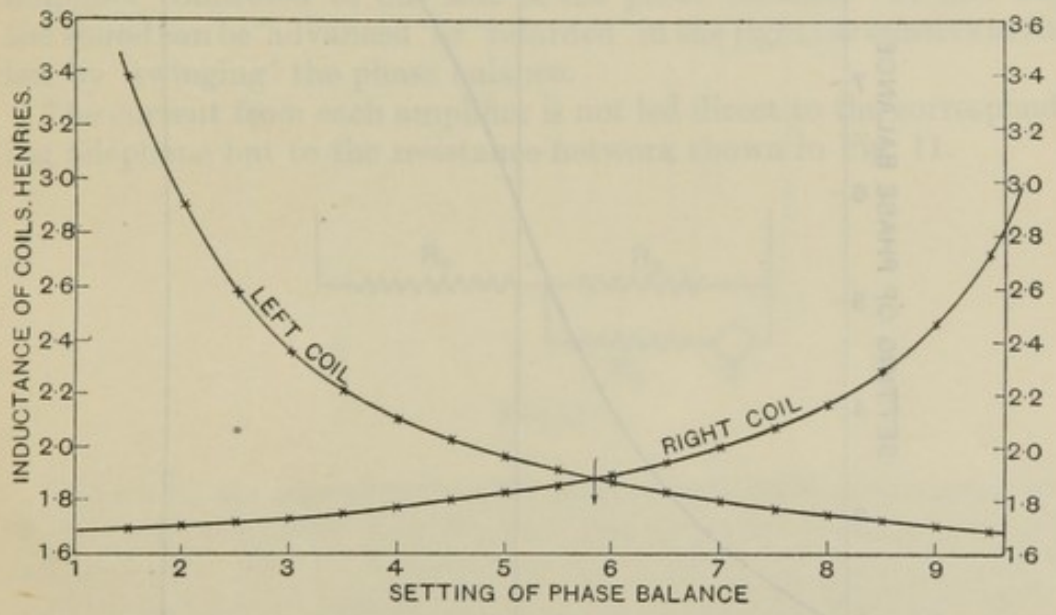


FIG. 9

Table 2 shows the calculated values of t_1-t_2 for different positions of the balance.

TABLE 2

$R = 20{,}100$ *ohms.*

Setting of phase balance.	L_1-L_2 *henries.*	$\frac{L_1-L_2}{R}$ *expressed as* t *microseconds.*
1·5	1·654	82·3
2·0	1·188	59·1
2·5	0·865	43·0
3·0	0·621	30·9
3·5	0·466	23·2
4·0	0·333	16·6
4·5	0·229	11·4
5·0	0·138	6·9
5·5	0·051	2·5
5·92	0·000	0·0
6·0	−0·025	− 1·2
6·5	−0·119	− 5·9
7·0	−0·207	−10·3
7·5	−0·304	−15·1
8·0	−0·425	−21·1
8·5	−0·567	−28·2
9·0	−0·763	−38·0
9·5	−0·014	−50·4

A further graph shown in Fig. 10 was drawn from these results. This graph shows the relation between the setting of the phase balance and the time difference between the two sides in micro-seconds.

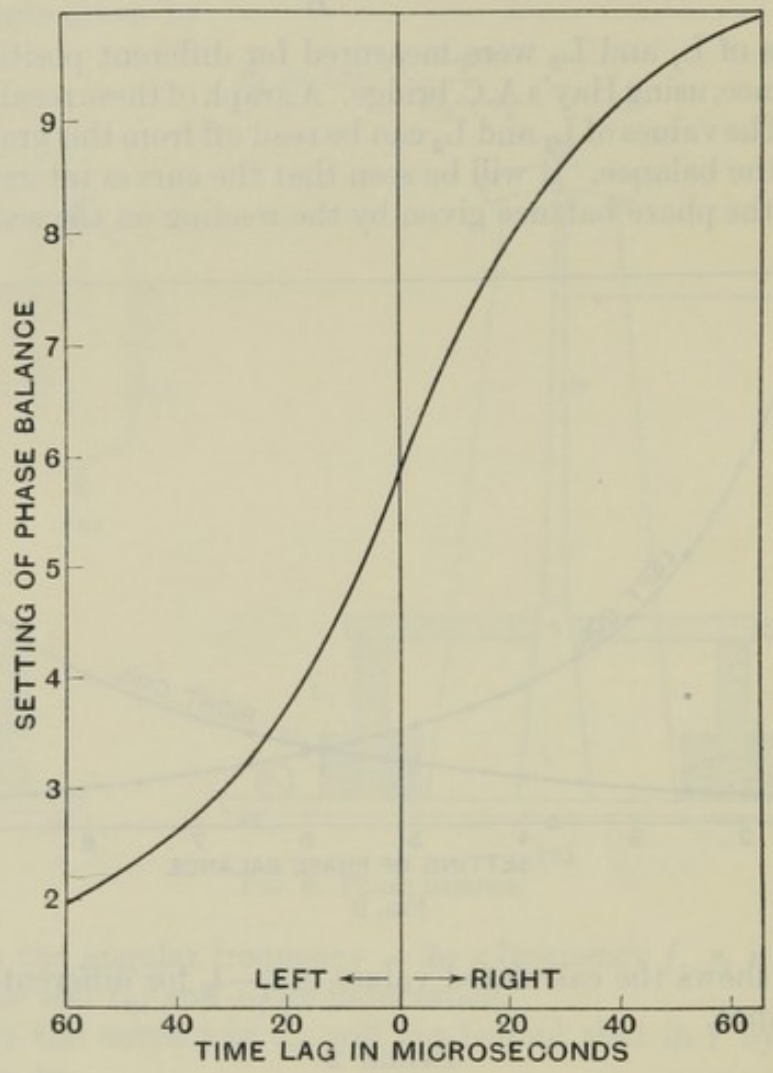


Fig. 10

From this graph the results shown in Table 3 were deduced. This shows the settings of the balance for a set of pre-determined values of time differences.

Table 3

Time differences in microseconds.	*Settings of balance.*	
0	..	5·92 (centre)
5	5·18	6·42
10	4·60	6·95
15	4·15	7·46
20	3·72	7·90
25	3·40	8·25
30	3·07	8·60
35	2·80	8·85
40	2·60	9·10
45	2·42	9·30
50	2·26	9·48
60	1·98	9·83

Column 2 represents positions where the left side of the balance lags behind the right, while column 3 gives the similar positions where the right side lags behind the left.

The calculated time differences between the two circuits differ somewhat from those found experimentally, for several reasons, chiefly because the self-capacities of the coils and the loading effect of the following valves have been neglected in the above theoretical considerations. A correcting factor was determined experimentally by a separate investigation described in paragraph 4.

Each ear-piece of the telephone is supplied with current from the amplifier connected to one side of the phase balance. In this way the sound can be 'advanced' or 'retarded' in the right ear relative to the left by 'swinging' the phase balance.

The current from each amplifier is not led direct to the corresponding telephone but to the resistance network shown in Fig. 11.

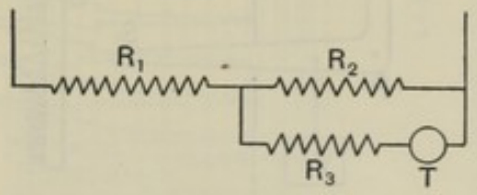


FIG. 11

R_1 and R_2 are placed across the output terminals of the amplifier, R_1 is large compared with R_2 and also with the impedance of the output valve. R_3 is a large resistance in series with the telephone, both being placed across R_2. In this way the amplitude of the oscillations in the telephone is made proportional to R_2.

The Sound-Proof Room.

The phase balance, together with its amplifiers and telephones, is set up in a 'sound-proof' room. This consists of a wooden structure about 10 ft. by 8 ft. by 8 ft. high, the inner side of the walls being padded with felt. Inside this is hung by ropes a second chamber 4 ft. 6 in. by 4 ft. 6 in. by 6 ft. high made of a wooden framework covered with a double casing of canvas stuffed with coarse wool. The door of this chamber is made in the same way.

The Self-Recording Apparatus.

In order to make self-recording experiments, a string attached to the pointer of the phase balance (see Fig. 8 *b*) and passing through a glass tube penetrating the walls of the sound-proof room, is connected to the apparatus shown in Fig. 12.

The string pulls on the crank lever V which is pivoted at P and counterbalanced by the weight W. At the other end of V is a stylus S which writes on the smoked paper of the kymograph drum, D. On this paper is also recorded a seconds scale made by the time-marker C which is electrically connected to a pendulum, not shown in the

diagram. Alternatively, a time scale in fifths of seconds could be inscribed by a time-marker controlled by a synchronous motor, run from the 50-cycle A.C. supply.

A series of horizontal lines is simultaneously ruled on the smoked paper by a 'comb' B made of thin copper sheet. This 'comb' is constructed by calibrating the position of the lever V so that the intervals shown in Table 3 will be registered on the smoked paper record. The central position is indicated with a thicker line than the

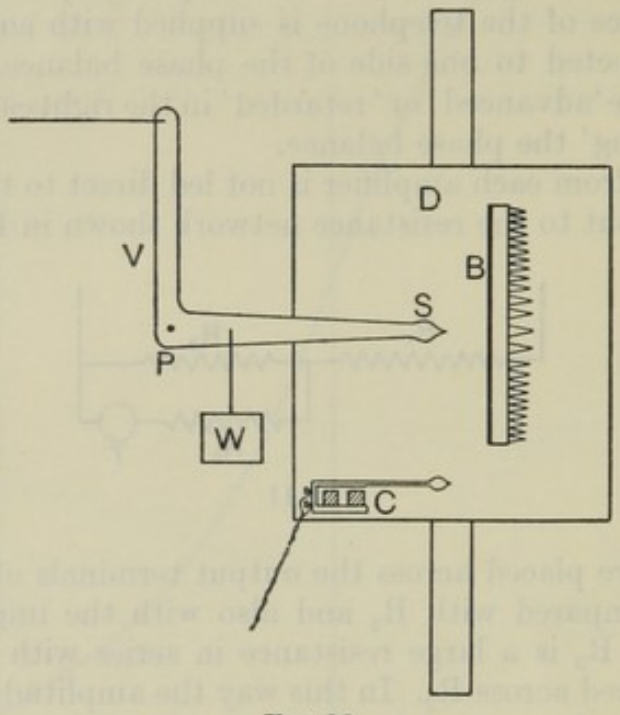


FIG. 12

rest, by making the central tooth of the comb rather more blunt than the other teeth. A typical record is shown in Fig. 13, in which the time scale is in fifths of seconds.

Experimental Check of the Time Differences introduced by the Phase Balance.

It was thought advisable to check the time differences set up by the phase balance experimentally and also to determine whether the amplitude of the sound in the telephones varied with the setting of the phase balance. This latter point is very important, since one object of the experiments was to separate the time factor and loudness factor in localization.

Two vibration galvanometers were therefore constructed as described in Section 5. These are made so that the mirror of one rotates about a horizontal axis and the mirror of the other about a vertical axis. Unfortunately it was found that sufficient sensitivity could be obtained only when the galvanometers were working near resonance.

In general, it can be shown that if a spot of light is reflected from these mirrors successively when they are vibrating with the same

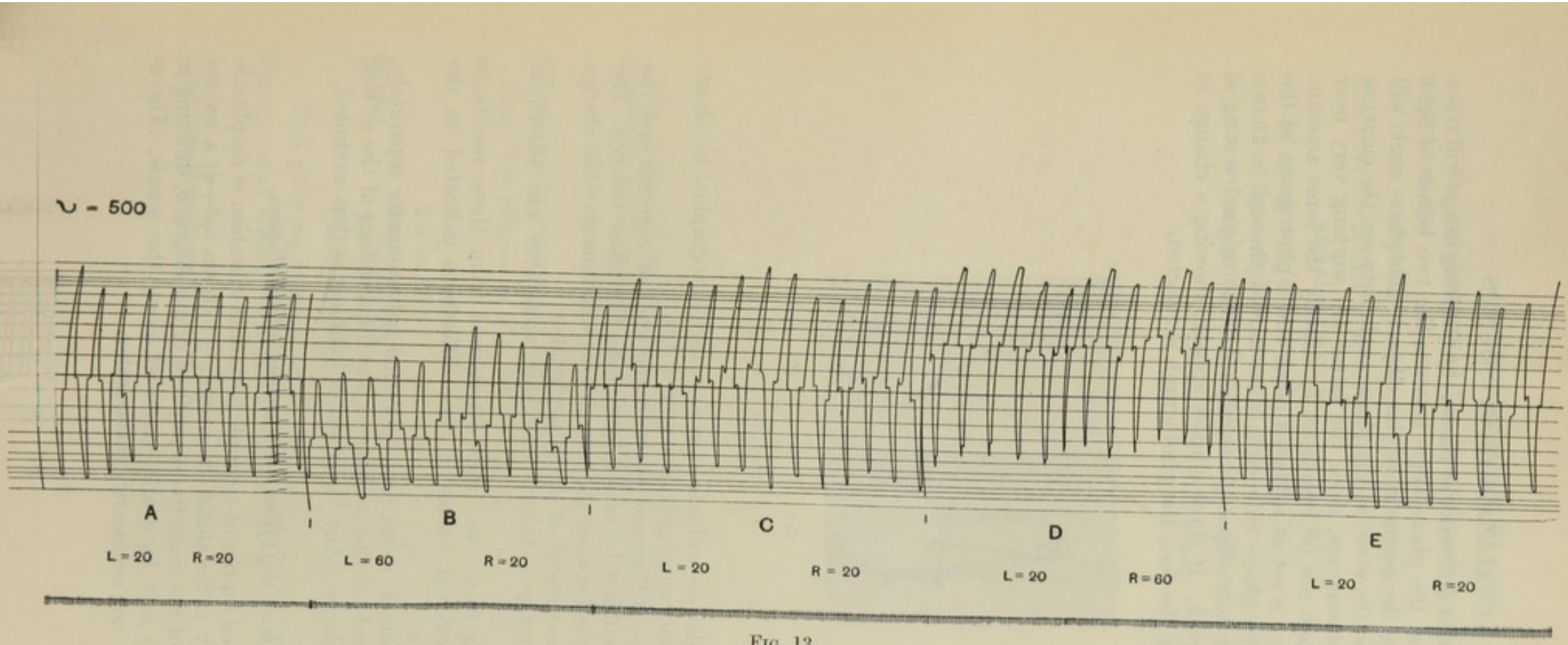
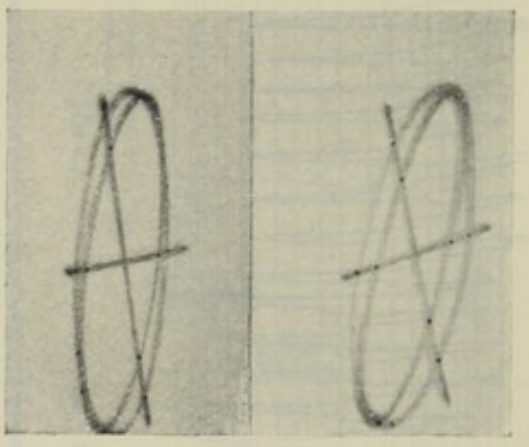


FIG. 13

frequency but with a difference of phase, the resulting image will trace out an ellipse, and the phase angle between the two beams of light can be found from the geometry of this ellipse. This phase angle will not necessarily be the same as the phase angle between the currents driving the galvanometers, since the latter are working very near resonance, although not off resonance by exactly the same amount. The phase balance is set at a known point and a photograph of the ellipse taken. The phase balance is then moved through a known distance and the new ellipse photographed. The original setting is repeated as a check. If three ellipses appear, showing a change in conditions during the test, the photograph is rejected.



(*a*) (*b*)

FIG. 14

Two rectangular co-ordinate axes are also photographed by short-circuiting each vibration galvanometer in turn.

Now the phase differences between the applied currents and the beams of light will not be varied by shifting the phase balance. The difference of phase between the two ellipses will thus give the change caused by the movement of the phase balance.

Actual photographs taken with this apparatus are shown in Fig. 14.

A multiplying factor was thus determined from these results to correct the calculated values of time differences indicated on the kymograph drum.

It was also found, using each vibration galvanometer separately, that the amplitude of the oscillations was independent of the setting of the phase balance. Any variation of loudness is thus excluded.

Calculation of phase shift from measurement of the ellipses.

Let $x = a \sin \omega t$ represent a simple harmonic vibration of amplitude a and frequency $\omega/2\pi$ along the x-axis; $y = b \sin (\omega t + \phi)$ a second such vibration of the same frequency and of amplitude b, differing in phase from the first by ϕ and executed along the y-axis. The x- and y- axes are not necessarily rectangular.

Then the combination of the two vibrations traces the elliptical path given by:

$$a^2y^2+b^2x^2-2ab\cos\phi\, xy = a^2b^2\sin^2\phi$$

This cuts the x-axis at $x_1 = \pm a\sin\phi$,

and the y-axis at $y_2 = \pm b\sin\phi$,

so that $\sin\phi = x_1/a = \pm y_2/b$. (see Fig. 15).

If the phase angle is changed from ϕ to ϕ' by swinging the phase balance we can similarly find $\sin\phi'$ from the corresponding ellipse.

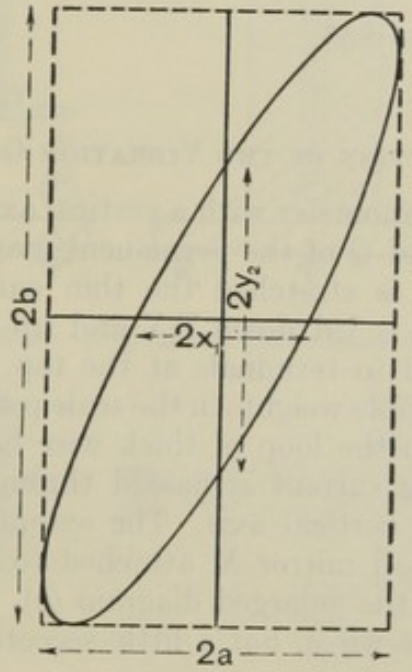


FIG. 15

The difference $\phi-\phi'$ thus determined is the phase shift due to the movement of the balance; the time difference is

$(\phi-\phi')/2\pi n$, where n is the frequency of the vibrations.

Calculation of phase-angle shift obtained.

From the ellipses in Fig. 14*a* the results obtained for one ellipse were:

	$2\,x_1 = 0{\cdot}93$	$2\,y_2 = 2{\cdot}75$
	$2\,a = 1{\cdot}10$	$2\,b = 3{\cdot}24$
so that	$\dfrac{2\,x_1}{2\,a} = 0{\cdot}846$	$\dfrac{2\,y_2}{2\,b} = {\cdot}849$
	Phase angle $\phi = 57°43'$	Phase angle $\phi = 58°4'$

This gives an average value $\phi = 57°54'$.

For the other ellipse the value obtained was

$$\phi = 41°\ 0'$$

Therefore the phase shift if given by

$$57°54'-41°\ 0'$$
$$= 16°54'$$

In the same way the phase shift found from Figure 14*b* was 17°16′.

The average value obtained from a number of results was 17·08°.

The frequency = 461, so that the time difference corresponding to the phase angle is

$$\frac{17{\cdot}08}{360 \times 461} = 102{\cdot}9 \text{ microseconds.}$$

The calculated value is 120·3 microseconds.

Therefore all time differences recorded on the drum must be multiplied by $\frac{102{\cdot}9}{120{\cdot}3} = {\cdot}855$.

5. Construction of the Vibration Galvanometers

The vibration galvanometer with a vertical axis is shown in Fig. 16. The pole pieces R and Q of the permanent magnet G create a magnetic field in which is stretched the thin eureka wire (44 s.w.g.) T_1VT_2. The current is led down T_1V and up VT_2, the ends of the wire being connected to terminals at the top. The wire is held in tension by the adjustable weights in the scale pan P hung at the end of the lever L to which the loop of thick wire holding V is attached. When an alternating current is passed through the loop T_1VT_2 it will vibrate about a vertical axis. The extent of this oscillation is indicated by the small mirror M attached to the small stretching-piece T as shown in the enlarged diagram (c). This is kept in place by the tension in the wires, but a little seccotine is introduced into the corners C and C_2.

The horizontal axis galvanometer is of similar design except that the weights are hung on the bent lever W (Fig. 17).

It is necessary that the two galvanometers be placed near together so that the whole length of the light-beam from the first will be caught on the mirror of the second.

The apparatus is assembled as shown in Fig. 18. Light from a 'pointolite' lamp passes through the convex lens X and then enters the light-tight box ABCD through the window W. It is reflected from the mirror of the vertical axis galvanometer G_v and then from the mirror of the horizontal galvanometer G_h. The beam of light is next received by the plane mirror P which is fitted with a ball and socket adjustment so that the reflected beam can be directed in any desired path. The light is finally focussed on the photographic paper held in the shallow box K fitted with a simple shutter. The latter is worked by a piece of cotton passing through a small hole in the light-proof box.

As pointed out above, the galvanometers were used near resonance. It was found that the best procedure was to tune both galvanometers as accurately as possible to the same frequency by adjustment of the weights in the pan and then work slightly off their resonance points.

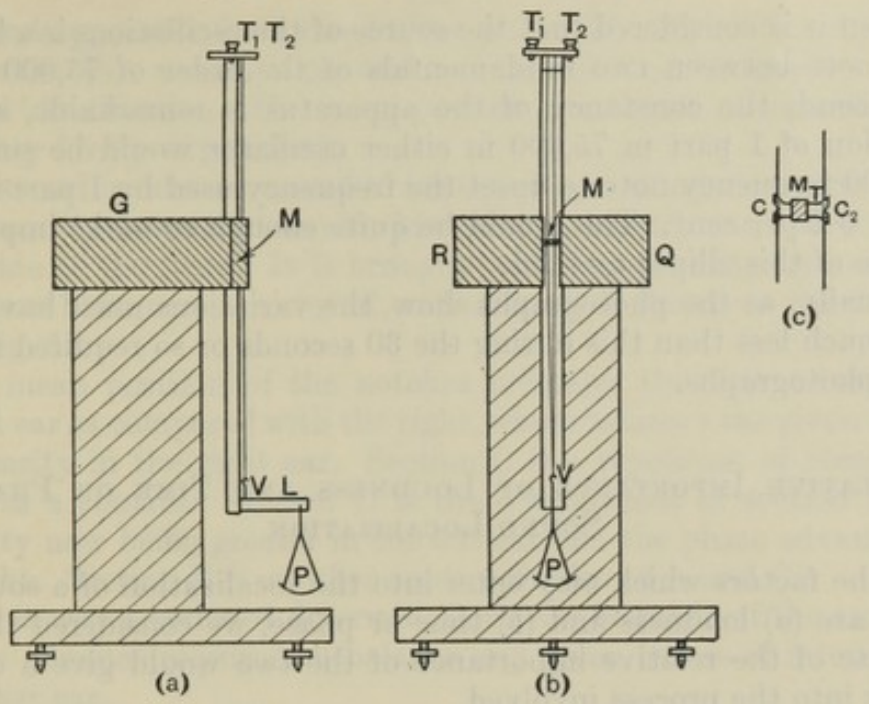


FIG. 16. Vertical axis galvanometer.

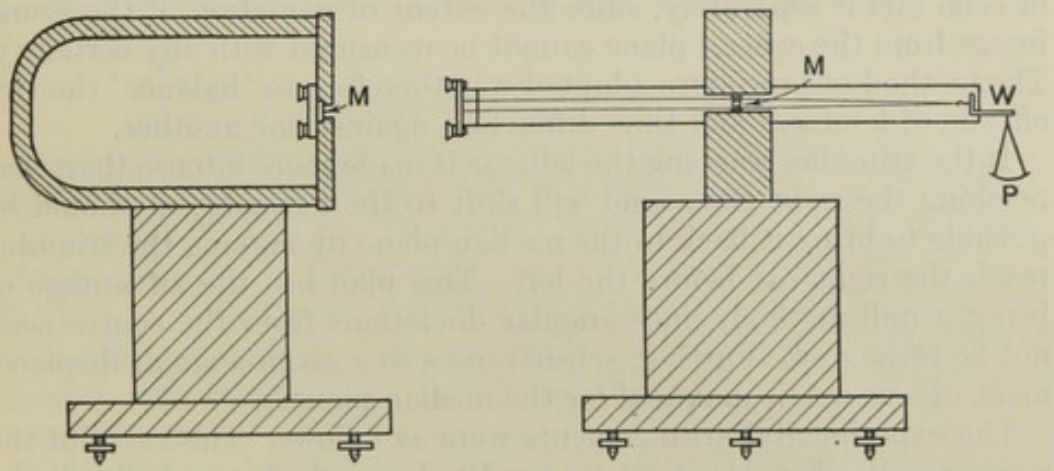


FIG. 17. Horizontal axis galvanometer.

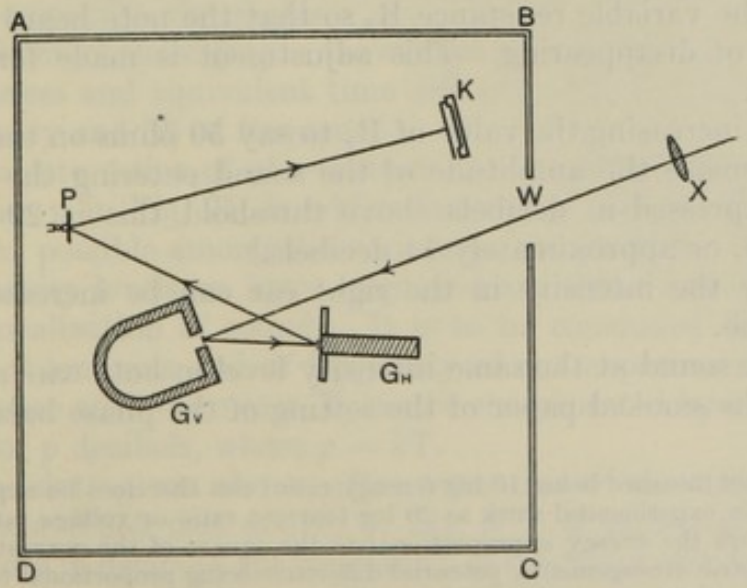


FIG. 18

When it is considered that the source of the oscillations is a heterodyne note between two fundamentals of the order of 75,000 cycles per second, the constancy of the apparatus is remarkable, since a variation of 1 part in 75,000 in either oscillator would be sufficient in a 500 frequency note to upset the frequency used by 1 part in 500, i.e. by 0·2 per cent. This would be quite enough to make impossible the use of this ellipse method.

Actually, as the photographs show, the variations must have been very much less than this during the 30 seconds or so required for the three photographs.

6. Relative Importance of Loudness and Time or Phase in Sound Localization

As the factors which may enter into the localization of a source of sound are (*a*) loudness and (*b*) time or phase, we considered that an estimate of the relative importance of the two would give a clearer insight into the process involved.

It was found to be extremely difficult to get a quantitative estimate of each factor separately, since the extent of deviation of the sound image from the central plane cannot be measured with any certainty. The method of procedure adopted was therefore to 'balance' the two effects, of loudness and time difference, against one another.

If the stimulus reaching the left ear is made more intense than that reaching the right the sound will shift to the left side; it should be possible to bring it back to the median plane by making the stimulus reach the right ear before the left. This plan has the advantage of being a null method, since angular deviations from the centre need not be estimated. Further, sensitiveness to a given angular displacement of a source is maximal for the median plane.

The experimental arrangements were as follows. The value of the resistance R_2 (Fig. 11) is set to say 10 ohms, which we shall call the threshold resistance. The observer in the sound-proof room, then increases the variable resistance R_3 so that the note heard is just on the point of disappearing. This adjustment is made for each ear separately.

By now increasing the value of R_2 to say 50 ohms on the left side, we can increase the amplitude of the sound entering the left ear 5 times. Expressed in decibels above threshold this is $20 \log_{10} 5 = 20 \times 0{\cdot}6990$, or approximately 14 decibels.[1]

Similarly the intensity in the right ear can be increased in any desired ratio.

With the sound at the same intensity level in both ears a record is taken on the smoked paper of the setting of the phase balance when

[1] The decibel measure being 10 log (energy ratio) can therefore be expressed most conveniently in experimental work as 20 log (current ratio or voltage ratio), since in a given network the energy is proportional to the square of the current or voltage. In potentiometric arrangements, potential difference being proportional to resistance, the decibels are thus measured by 20 log (resistance ratio).

the sound appears to be in the median plane (Fig. 13, Section A). The notches in this record represent a series of such estimates of this 'central' localization. The mean position is adopted as a standard of centrality for the experiment.

The sound is next increased in intensity by a known amount in the right ear, which in itself would throw the apparent sound over to the right side of the head. It is brought back to centrality by swinging the phase balance. A series of such records is shown in the portion B of Fig. 13.

The mean position of the notches indicates the time advance in the left ear as compared with the right, which balances the given excess of intensity in the right ear. Section C is a repetition of Section A, taken as a control. Section D is the complement of section B, the intensity now being greater in the left ear and the phase advanced in the right. Section E is another control similar to A and C.

In this way a series of determinations can be made of the amounts of time-advance required to balance any given excess of intensity at the other ear.

To allow for any lack of symmetry in the observer, the average of the mean displacements in sections B and D is taken.

Experimental Results.

Series of measurements were made for different frequencies, intensity levels and intensity differences. The data thus obtained are given in extenso in Table 4.

These results are exhibited graphically in Figs. 19, 20 and 21. Fig. 19 is a plot of all points for frequency 500, different intensity levels being distinguished; the abscissae are decibels denoting the intensity difference at the two ears, the ordinates the compensating time difference.

In Fig. 20 are plotted in the same way the points for the various frequencies employed, for a constant intensity level of 20 decibels above the respective thresholds.

Examination of these graphs shows that the relation between intensity differences and equivalent time differences is independent (1) of basic intensity and (2) of frequency.

The complete series of observations for all pitches and intensity levels is shown in Fig. 21, in which the straight line is drawn to lie as evenly as possible among the various points plotted.

The intensity levels we have employed are those met with in the practical localization of sounds. It is to be concluded that, within the limits of loudness and of frequency of our experiments, a given time difference at the two ears, T, can be compensated by an intensity difference of p decibels, where $p = k\mathrm{T}$.

From the data given above, after correction of the time intervals by the factor 0·855 (see above, p. 24), if T is expressed in microseconds and p in decibels, the constant k of the linear relation between them is found to be 0·60.

TABLE 4

Serial number of experiments.	*Decibels of weaker sound above threshold.*	*Decibels difference.*	*Time difference (microseconds).*	*Number of observations.*
	Frequency 500.			
35	20·0	3·5	9·8±0·4	54
32	..	6·0	13·0±0·4	55
34	..	8·0	10·5±0·6	45
33	..	9·5	29·7±0·4	53
36	..	11·0	18·9±0·8	43
52	..	12·0	26·0±0·4	79
53	..	14·0	25·0±0·3	105
54	..	15·6	31·8±0·4	84
55	..	18·1	32·2±0·4	110
20	26·0	1·9	1·9±0·3	59
21	..	3·5	5·9±0·25	63
42	..	4·9	10·0±0·3	41
22	..	4·9	10·1±0·3	49
19	..	6·0	9·9±0·3	68
43	..	7·0	11·6±0·3	55
23	..	8·0	14·2±0·3	54
25	..	8·0	15·6±0·4	53
44	..	9·5	16·6±0·4	46
24	..	11·5	27·5±0·5	59
60	..	12·0	23·9±0·3	48
61	..	14·0	26·6±0·4	59
62	..	15·6	25·9±0·5	42
39	29·5	3·5	9·4±0·5	56
37	..	6·0	11·8±0·3	51
40	..	8·0	19·8±0·5	43
38	..	9·5	13·8±0·5	44
41	..	11·0	24·3±0·5	41
	Frequency 800.			
69	20·0	6·0	12·9±0·5	76
71	..	8·0	11·5±0·6	51
70	..	9·5	20·2±0·6	73
72	..	11·0	20·2±0·8	42
73	..	12·0	23·8±0·5	53
74	..	14·0	27·3±0·4	68
75	..	15·6	26·6±0·4	50
76	..	18·1	32·4±0·4	53
	Frequency 1,200.			
47	20·0	3·5	18·2±0·5	41
65	..	3·5	3·4±0·3	76
26	..	6·0	15·4±0·4	53
45	..	6·0	14·5±0·8	35
66	..	6·0	3·2±0·2	58
50	..	6·0	10·4±0·5	37
48	..	8·0	13·4±0·5	39
68	..	8·0	4·7±0·5	43
46	..	9·5	15·4±0·5	41
51	..	9·5	18·0±0·6	28
63	..	9·5	19·9±0·3	46
67	..	9·5	11·2±0·6	56
49	..	11·0	24·3±0·8	35
64	..	11·0	27·5±0·3	56
56	..	12·0	24·2±0·2	95
57	..	14·0	24·0±0·3	95
58	..	15·6	25·8±0·3	75
59	..	18·1	22·8±0·3	94
28	26·0	1·9	3·3±0·3	56
27	..	3·5	9·0±0·3	55
29	..	4·9	9·6±0·5	65
30	..	8·0	15·7±0·3	66
31	..	11·5	22·2±0·1	56

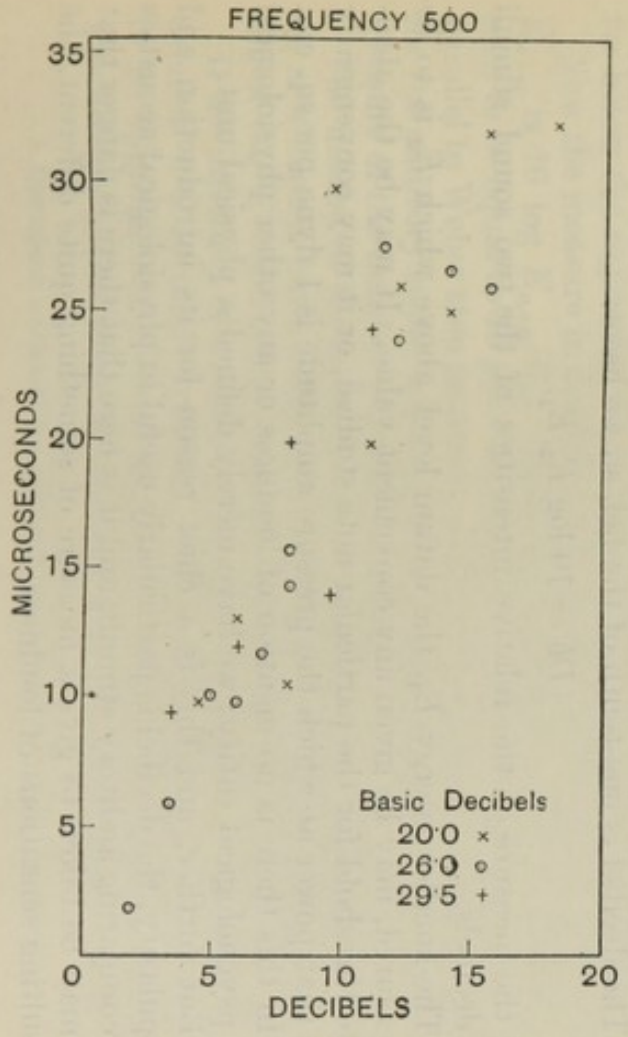


FIG. 19

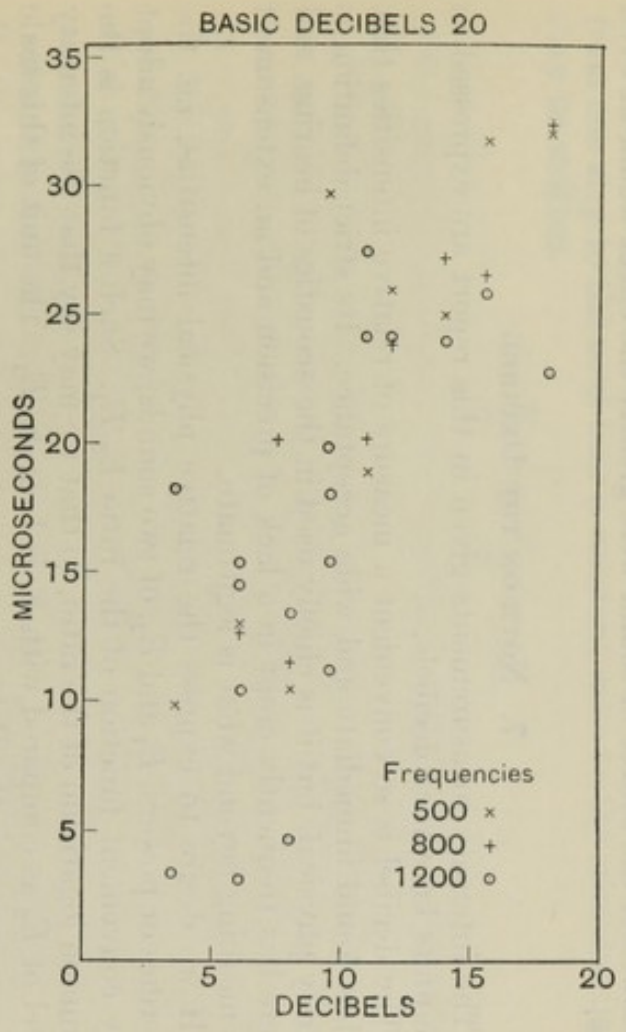


FIG. 20

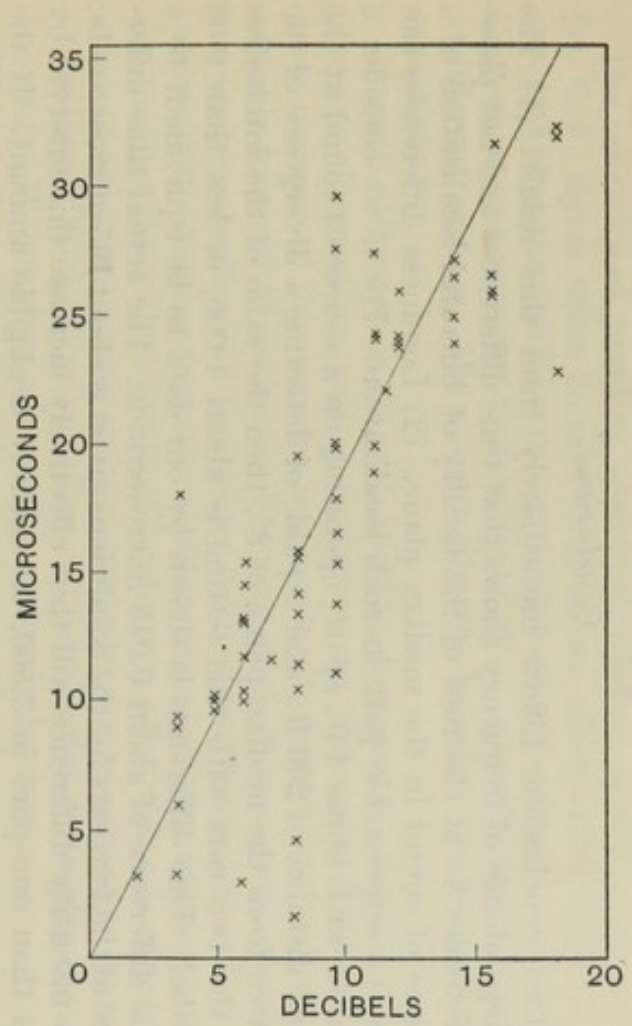


FIG. 21

Conclusions.

Two conclusions follow immediately from this result: (1) The independence of frequency shows that time differences and not phase differences lie at the root of the faculty of binaural localization of a source of sound in the median plane. (2) Loudness differences can play no appreciable part in such localization. For if we consider a locator with horns 4 ft. apart, operating on a source of sound at the small distance of 200 ft., and capable of detecting a divergence of the source from the median plane of 3°, then the ratio of the intensities at the two ears with this setting is about 1·006, or less than 0·03 decibel. This difference is shown by our data to be equivalent to a time difference of about 0.045 microseconds. The actual time difference at the two ears for the conditions given is about 165 microseconds; the intensity difference will thus effectively increase this interval by less than one part in 3,500, an entirely negligible amount. If the distance of the source of sound were greater the effect would be even less.

7. Note on the Decibel

The intensity measurements given in this report are expressed in the units known as decibels.

The decibel is so convenient a measure of relative intensities that it has found immediate and wide acceptance. Its strict definition is purely physical, but it is chiefly used in the acoustics of hearing, and there has frequently crept in a lack of precision and an extension of its meaning beyond what is legitimate.

If we desire to express the relative physical intensities, i.e. the energies or powers, E_1 and E_2, of two sounds, we may obviously adopt any convenient function of the ratio E_2/E_1. Such a function is the common logarithm of the ratio, so that we may say that the intensity level of E_2 as compared with E_1 is $\mathrm{Log}_{10}E_2/E_1$. The unit of this scale of comparison is the Bel.

The decibel is one-tenth of the bel, so we have:

$$Db = 10 \log E_2/E_1,$$

as the measure of the relative intensities of the two sound stimuli in decibels.

The smaller energy E_1, the datum level above which E_2 is to be measured, may be given any convenient value. It may be the absolute threshold for the particular note studied, or it may conveniently be that power at which the pressure amplitude is 1 dyne per sq. cm.

In this there is no mention of loudness or any other physiological or psychological entity: we have merely defined a physical unit.

But further, and this is a chief reason for its introduction and popularity, the decibel is particularly useful in physiological acoustics in comparing auditory stimuli, and it is here that there is danger that it may be taken to give a measure of something quite different, the resulting sensations of loudness.

The necessity of guarding against this fallacy is well expressed by Dr. C. S. Myers when he says:[1] 'It seems, often unwittingly, to be assumed that the decibel serves as a measure of loudness', adding 'All that we can measure are the strengths of . . . physical stimuli—quite a different matter. We cannot directly measure the strengths of sensations.'

As with other senses so with hearing, increase of stimulus causes increase of sensation, and at any point on the scale of intensity a finite increase of the stimulus is necessary to bring about a just perceptible increase of loudness. We may then, if we choose, set these successive sensations of increasing loudness in order, and call them successively (starting from the absolute threshold) loudnesses 1, 2, 3, &c., so that the n'th sound is of loudness n. But this n is simply an index of order in a series, and not in the least a quantitative measure of loudness. We have no experimental justification for saying that the successive Weber steps are equal increases in loudness.

Weber's Law, that the increments of stimulus necessary to pass from one step to the next bear a constant ratio to the stimulus itself, may be written

$$\frac{E_{n+2}}{E_{n+1}} = \frac{E_{n+1}}{E_n}, \text{ \&c.,}$$

if the energies associated with the n'th $(n+1)$th, &c., steps are E_n, E_{n+1}, &c.

From this follows the Weber-Fechner logarithmic equation, which is a formula for the number of Weber steps corresponding to a change from one stimulus to a second. It is not legitimate to interpret it as an equation measuring change of sensation in a quantitative way.

Thus in the form $p = k \log E_{n+p}/E_n$ it is valid (provided both E_n and E_{n+p} lie in the region where Weber's Law holds) as an expression of the number p of steps of sensation which occur when the stimulus is increased from E_n to E_{n+p}.

But even in this narrow sense it is not true if for E_n we substitute E_o, the value of the stimulus at the absolute threshold, since this lies far below the region for which Weber's Law is approximately true.

Now the measure in decibels of the interval of intensity from E_n to E_{n+p} is 10 log E_{n+p}/E_n. From the expression for p above this is seen to be equal to 10 p/k. In other words, decibel measure is exactly parallel to Weber step measure, and since $10/k$ is approximately unity for the auditory sense, p is numerically equal to Db, i.e. decibels are a measure of the number of sensation steps.

The misuse of the term to which attention has been called is thus no new heresy, but a carrying over of loose thinking about the meaning of the Weber-Fechner formula into the special case of hearing.

If the intensity of a sound is changed by equal steps as measured in decibels it is certainly true the loudness also changes by what seem to us approximately equal amounts. This is the psychological fact which lends plausibility to the idea that decibels measure loudness.

[1] Discussion on Audition, Physical Society, June 19, 1931, p. 135.

But to admit this parallelism between decibels and our subjective estimate of loudness changes does not entitle us to say, as has been done, that 'steps of one decibel are equal loudness changes'.

To sum up, decibels measure the relative levels of two physical intensities (energies or powers) and this measure may be legitimately extended (if it is thought worth while) to that of the number of Weber sensation steps which occur in the change from one of these two intensities to the other. But decibels do not measure the change in sensation, i.e. in loudness.

Further, this secondary use is only valid provided both the energies referred to lie in the Weber's law region. If we speak of 'the number of decibels above the absolute threshold' we mean 10 log E/E_o, where E is the energy at the level dealt with and E_o the energy at the threshold, far below the Weber region. This is a purely physical measure and a very convenient one. To interpret it as having a sensory meaning is mere nonsense.

In the work above the term decibel is used *strictly in the physical sense, to denote* 10 *times the logarithm of the ratio of the physical energies* concerned at two specified intensity levels: it must not be taken to imply any quantitative measure of *loudness* differences.

* * * * * * * * *

We desire to express our thanks to a former colleague, Mr. C. Handford, for invaluable help in the design and construction of the pure-tone oscillator.

B. SOME FACTORS IN AUDITORY LOCALIZATION

BY

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1. INTRODUCTION

AT the outset of the investigation two problems were envisaged. These were: (1) Is there any difference in efficiency between the binaural localization of continuous and that of intermittent sound, and (2) what effect has the method of locating a sound mid-way between the two ears upon the judgement of this mid position?

Consideration of these two problems forms the substance of this report. But other problems arose in the course of the investigation. In regard to one of these, namely: (3) How far is the efficiency of binaural localization a function of the frequency of the sound localized, some preliminary data are herein reported.

Other problems chiefly concern the effect upon any position judged to be centrally situated between the two ears, of periods of monaural stimulation, of periods during which there is a constant phase-difference between the sound at the two ears and of periods during which the physical sound intensities at the two ears are unequal. But the results so far obtained in regard to these matters are of too tentative a nature to permit of a concise account at present. They will be dealt with in a later report.

2. APPARATUS AND GENERAL PROCEDURE

Originally it was intended to duplicate each part of the investigation on distinct types of apparatus in order adequately to 'control' instrumental factors. But of the two 'set-ups' projected, one has only recently been completed and brought into successful operation, too late for controlling the data given here. It will, however, be used for later work and will be described in detail when that work is reported upon.

The other 'set-up'—that used for all the experiments described here—consisted essentially of a valve-oscillator for the production of a note of the required frequency, of tubes of variable length for

the production and variation of a phase-difference, and of valve-amplifiers for the control of the intensity of the sound. The set-up is shown diagrammatically in Fig. 1.

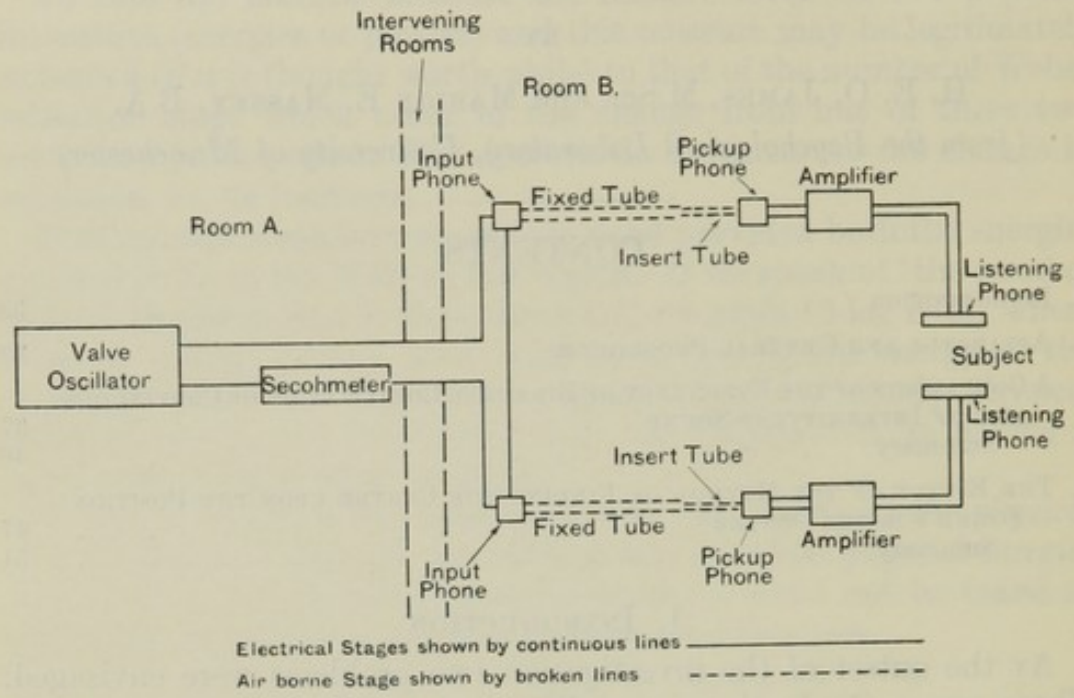

FIG. 1

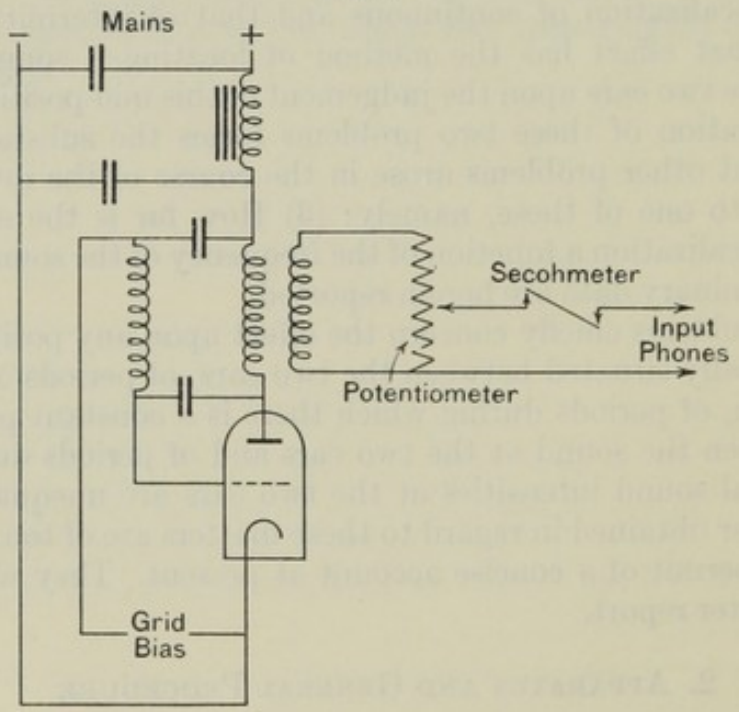

FIG. 2. Oscillator circuit.

The valve-oscillator—the circuit of which is given in Fig. 2—was supplied with anode current from the 200 volt D.C. mains through a smoothing unit. Its inductance was varied in order to obtain the two frequencies used. Its output was controlled by means of a 400 ohm potentiometer shunted across the output coil. In one of the leads from the potentiometer was a secohmeter for the purpose of interrupting the output.

The oscillator and the smoothing unit were housed in metal boxes and the output leads were cased in lead. On account, however, of the noise from the rotating secohmeter and of the possibility of interaction due to the stray magnetic field of the coils it was found advisable to place the oscillator and the secohmeter in a room separate from that in which the rest of the apparatus was placed. The distance between the two sections of the apparatus was approximately 50 feet.

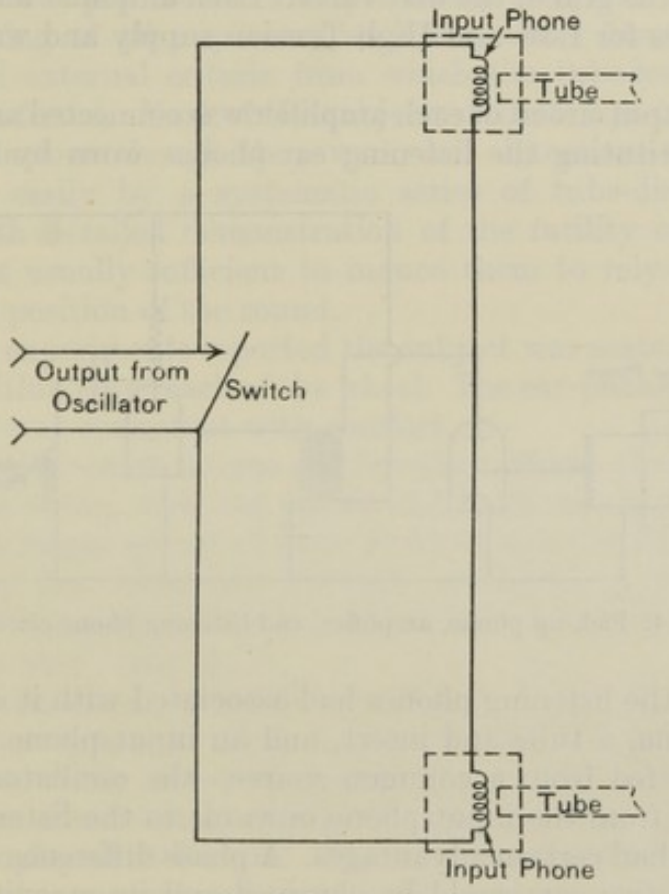

FIG. 3. Input circuit.

The output-leads from the oscillator in Room A (cf. Fig. 1) fed in Room B a pair of series-connected ear-phones (the input phones). Each ear-phone was housed in a funnel-shaped rubber container, padded with cotton-wool, for the purpose of keeping it in position against the input end of the tubes. A switch in series with the ear-phones made it possible to cut off the input from the oscillator (cf. Fig. 3).

The function of the first pair of ear-phones was that of converting the electrical vibrations delivered by the oscillator into air-vibrations for the purpose of transmission through the tubes. The movable insert-tube at the output end of the tube-system made it possible to vary the tube-lengths and to produce a phase-difference of variable magnitude. Fitting to the end of each insert-tube was an ear-phone, housed and held in position in the way described. By means of these ear-phones (the pickup phones), the air-vibrations transmitted through the tubes[1]

[1] The fixed tubes into which the insert tubes fitted as tightly as was consistent with mobility, were 2 metres in length and 2 cm. in diameter. They were firmly fixed in position.

were converted into electrical vibrations. It will be seen that the stage of air transmission was solely for the purpose of producing a variable phase-difference.

Each of the two pickup ear-phones—functioning, it will be noticed, as microphones—was connected into the grid-circuit of an amplifier. (The electrical circuit of one of the two amplifiers is given in Fig. 4). Each amplifier had an input volume-control, consisting of a potentiometer feed to the grid of the first valve. Each amplifier had a separate set of batteries for Low and High Tension supply and was housed in a metal box.[1]

Into the output circuit of each amplifier was connected an ear-phone, the pair constituting the listening ear-phones worn by the subject.

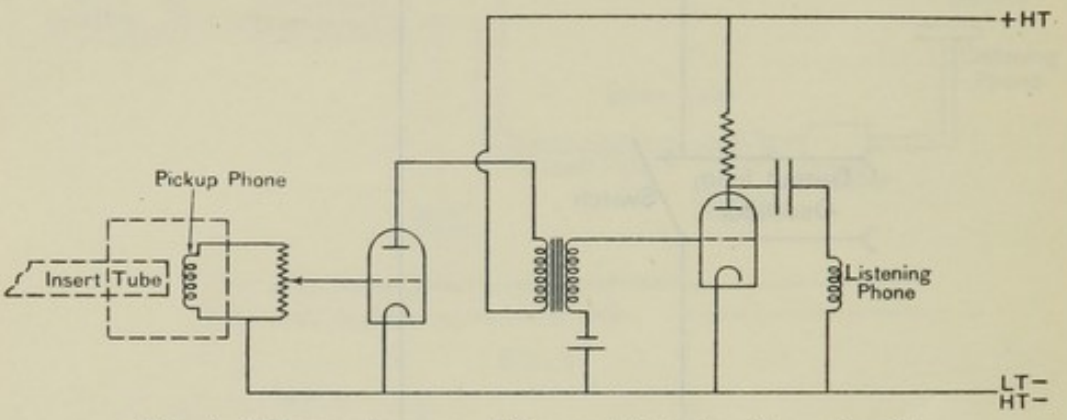

FIG. 4. Pick-up phone, amplifier, and listening phone circuit.

Thus each of the listening phones had associated with it an amplifier, a pickup phone, a tube and insert, and an input phone. These two systems were fed from a common source—the oscillator—but were quite distinct from the input phone onwards to the listening phones.

This set-up had certain advantages. A phase difference between the sounds in the two ears could be obtained and its magnitude varied. Further, the intensity of the sound in the two ears could be controlled independently or together, and it was possible to interrupt independently or together the sound supply to the two ears.

The disadvantage of this set-up is that it contains too many sources of resonance. There are possibilities of resonance in both tubes and ear-phones. Of the three pairs of phones used, at first, two pairs of diaphragm type were used and one pair of reed type. Later —for Subjects Ha. and T.—reed type phones were used throughout. The resonance points of the phones used were not specifically determined, but from measurements made by others on similar types, it is probable that there was more than one resonance point in the neighbourhood of the two frequencies used in the case of the diaphragm type phones, whilst in the case of the reed type there is only one resonance point in this region, and that slightly above the higher frequency.

The intensity of the sound was maintained as low as was consistent with its being easily distinguished from internal and external noises.

[1] All the metallic parts of the apparatus were earthed.

The subject did not vary the tube-length when finding the centre, by direct pushing and pulling. He did this indirectly by rotating a wheel. This worked a clamp holding the insert-tube, up and down a screwed rod. Thus a finer adjustment could be made than was possible by direct push and pull, and further as the insert-tube could be displaced with reference to the clamp, and as this was done for every reading, the possibility of inferring the tube-length from the position of the wheel was precluded. This precaution, together with the complete screening of all apparatus save the wheel from the subject, excluded all external criteria from which the tube-length could be accurately inferred. Most of the subjects did attempt to use external criteria, fallacious though they were, but detection—this could be done quite easily by a systematic series of tube-displacements—together with detailed demonstration of the futility of such a proceeding, was usually sufficient to induce them to rely solely on the experienced position of the sound.

In all the experiments reported the subject was seated comfortably in a chair within easy reach of the wheel. The ear-phones were secured as firmly as was consistent with comfort.

In order to minimise fatigue and boredom effects, the readings were taken in sets of ten, with rest pauses of 5 to 10 minutes between each set and with longer pauses of from 20 to 30 minutes in the middle of the morning and afternoon sittings. In addition the sound was switched off during the intervals between readings, whilst the necessary adjustments were being made.

The subjects were boys and girls of 16 to 18 years of age. They were paid on a bonus system so as to ensure some measure of zeal for improvement. Only one was employed at a time and this one was selected from a number of candidates sent up from the Manchester Juvenile Employment Bureau. The selection was based on an interview, tests of monaural auditory acuity, and on a test of ability to localize sound on the apparatus described.

3. A Comparison of the Efficiency of Binaural Localization of Continuous and of Intermittent Sound

For the purpose of this comparison, efficiency of localization was taken solely in the sense of variability of localization of the centre. It was recognized that a comparison based upon accuracy of localization of the centre would be a desirable complement to the comparison actually made. For instrumental reasons this has as yet proved to be impracticable. It is hoped at a later date to institute this comparison.

The procedure adopted was designed to equalize as far as possible all conditions, other than the intended difference between intermittent and continuous sound, that might be productive of differences in variability. To this end the intermittent and continuous series were given in alternate sets of ten. Moreover, their positions in the day's work were interchanged from day to day. It was thought that these

precautions would tend to equalize the effects of practice, of boredom, of moods and similar mental and physiological conditions as well as those of instrumental irregularity.[1]

The judgements were taken in sets of ten, five of a set with the sound initially well to the right, and five with the sound initially well to the left, left and right being taken alternately till ten readings had been obtained. It was hoped that this plan would tend to minimize the confusion effects noted by Bartlett and Mark.[2]

The subject was instructed that the sound on being switched in was to be moved towards and beyond the centre by an amount just large enough definitely to make the sound cross from one side to the other, and that it was then to be moved to and fro across the centre, but as far as possible with diminishing excursions until an assured estimate of the position of the centre could be made.[3] As will be seen later, these instructions were not always strictly observed. Still, in the case of six subjects the instructions were enforced as far as possible. In the case of the two remaining subjects the standard procedure was described and advised but not enforced. One of the two conformed, whilst the other adopted a different procedure. The effects of this difference in procedure will be described later.

Two sound frequencies were used. The bulk of the results were obtained for a frequency of 784 dv./sec., but results were also obtained for three subjects with a frequency of 946 dv./sec. The rate of interruption for the intermittent sound varied between two and three interruptions per second. The period of 'make' was objectively approximately equal to the period of 'break'.

It should be noted that the interruptions occurred before the first pair of ear-phones, thus cutting off the sound from the tubes. Hence when the circuit was once more 'made', there resulted not only a phase difference between the two ears, but also a difference in the time of arrival of the first disturbances at the two ears, corresponding in magnitude, like the phase difference, to the difference in the lengths of the two tubes.

The statistical treatment adopted was rendered necessary by the fact that generally the means of continuous sets (the same is true of intermittent) differed by amounts greater than could be ascribed to chance variation. How far this variation was due to instrumental factors and how far to subjective factors has not yet been determined, but its effect was to render inadvisable the straightforward plan of throwing all the continuous readings for a given subject (the same is true of intermittent readings) into one distribution and of measuring the variability of continuous localization by the standard deviation round the mean of this distribution. To do this would have been to swamp the variations within the sets by the variations between the sets.

[1] It should be added that the comparison was not begun until the subjects had achieved a reasonable degree of competence.

[2] 'A Note on Local Fatigue in the Auditory System.' *Brit. J. Psychol.* (1922), **13**.

[3] This procedure is later spoken of as the Tacking Method.

The treatment adopted was as follows:

1. For a given subject the standard deviation for each set of ten readings with continuous sound was computed. These S.D.'s were then summed and the sum divided by the number of sets. This gave the mean standard deviation for localization with continuous sound.

The mean standard deviation of localization with intermittent sound was obtained in a similar way.

2. The difference between these two standard deviations was then taken and the standard deviation of the difference was calculated.

3. The difference between the standard deviations for continuous and intermittent sound was expressed as a multiple of the standard deviation of the difference.

The results for a frequency of 784 dv./sec. are shown in Tables 1 and 2. In Table 1 the results are expressed in centimetres, in Table 2, in degrees.

TABLE 1

Subject.	*Sex.*	*No. of Sets.*	*Mean S.D. (Cont.). σC. cm.*	*Mean S.D. (Inter.). σI. cm.*	*Diff. D. cm.*	*S.D. Diff. σD. cm.*	*D./σD.*	*D./σI. × 100*
D.	F.	40	1·164	1·005	+0·159	0·059	+2·74	+15·8
Wi.	M.	20	1·360	1·129	+0·231	0·095	+2·43	+20·5
M.	F.	25	1·525	1·382	+0·143	0·096	+1·49	+10·3
Wa.	F.	20	2·463	2·237	+0·226	0·176	+1·29	+10·1
Ha.	M.	15	1·757	1·573	+0·184	0·143	+1·29	+11·7
P.	F.	12	1·812	1·669	+0·143	0·167	+0·85	+ 8·6
Ho.	F.	17	1·611	1·544	+0·067	0·125	+0·54	+ 4·3
T.	M.	9	1·04	1·06	−0·02	0·117	−0·17	− 1·9

A plus sign indicates that the variability for continuous sound is greater than that for intermittent. A minus sign indicates that the reverse is the case.

M = Male. F = Female.

TABLE 2

Subject.	*Mean S.D. degrees. Continuous.*	*Mean S.D. degrees. Intermittent.*	*Difference. degrees.*
D.	9·93	8·57	+1·36
Wi.	11·60	9·63	+1·97
M.	13·00	11·79	+1·21
Wa.	21·00	19·07	+1·93
Ha.	14·98	13·42	+1·56
P.	15·45	14·23	+1·22
Ho.	13·74	13·16	+0·58
T.	8·87	9·04	−0·17

Plus and minus signs have the same significance as in Table 1.

Frequency = 784 dv./sec. Wave-length = 42·22 cm.

From Table I it may be seen that in seven out of the eight cases localization with intermittent sound is less variable than with continuous sound. In the eighth case the difference is negative but obviously not significant. It is noteworthy that this is the case of the only subject who consistently expressed a preference for continuous

as against intermittent sound, feeling that he localized the former better than the latter. The others, with the exception of Wa., thought that localization was easier or more certain with intermittent than with continuous sound.

In six cases the difference in variability when expressed in terms of the standard deviation of the difference is greater than 0·6745 (the probable error). This implies that in all these six cases the chances are against the differences being due to mere chance variation. In only two cases, however, is the difference greater than three times the probable error. The last column, where the difference in variability is expressed as a percentage of the variability for intermittent sound, confirms this indication. The mean percentage difference for these six cases where the variability difference exceeds 0·6745, is 12·8, and indicates a difference of importance.

Satisfactory as this result is up to a point, it is, however, desirable to consider why it is not more decisive. It is natural to look first for an explanation in the direction of instrumental irregularity. At the time at which these experiments took place, it is probable that instrumental irregularities did occur, though to what extent is as yet unknown, owing to the difficulty of separating instrumental from subjective effects. It is probable that the incidence of whatever instrumental irregularity there was, was, on the whole, fairly evenly distributed over both the intermittent and continuous localization. Even so, its effect would tend to blur whatever variability differences existed between the two kinds of localization. To a certain extent, however, it is to be expected on physical grounds that ear-phone irregularity would tend to be more pronounced with intermittent than with continuous sound. In the circumstances the effect of this would probably be to close the gap in variability between the two kinds of localization.

But there is evidence that psychological as well as physical factors operated to obscure the differences in variability between the two kinds of localization. Firstly, there was the factor of individual differences. In view of T.'s results in Table 1, and still more in Table 9, it seems probable that some subjects may not show an advantage for intermittent as against continuous sound. The individual differences are, however, probably affected by practice differences, and in view of what is said on the effects of practice at a later stage of this discussion, and of the relatively small amount of practice that T. received as compared with, say, D., some caution must be observed in interpreting his results.

Secondly, there was the effect of the tendency on the part of some of the subjects to use accessory criteria in finding the centre. Although this tendency was checked as far as possible the results are not completely free from its influence. And the use of accessory criteria, fallacious or not, for the localization of both intermittent and continuous sound, would tend to blur any existing differences in variability, based upon the differences between the sounds themselves.

A third disturbing influence arose from the procedure of beginning with the sound alternately on the right and on the left, and of putting

into the same distribution both Right and Left Approach Centres, as the centres thus obtained may be termed. It was not expected that there would be any appreciable and consistent differences between Right and Left Approach Centres. The data given in Section 3, however, especially that in Table 14, indicates that this expectation was not generally realized. The fact that the differences between the two kinds of centres were in some cases appreciable, would tend to obscure any differences in variability of the kind sought for. And the fact that the difference between Right and Left Approach Centres was consistently greater in the case of intermittent than of continuous sound, must have tended to diminish the variability differences actually found.

In future comparisons of this kind it would seem advisable, where Right and Left Approaches are taken alternately, that the number of measurements in a set should be increased as far as is consistent with the avoidance of other undesirable effects, so that the variabilities for Right and Left Approach Centres can be calculated separately. The number of ten is too small to permit of this.

Fourthly, it seems probable that the magnitude of the variability differences actually found was affected by the factor of learning. The relevant data are presented in Tables 3, 4, and 5.

For the purpose of these tables the period of time during which the comparison was carried out, was regarded as a period of practice for each subject in the localization of either continuous or intermittent sound. This period was divided into two roughly equal sections and the mean variability obtained for each section. The difference between the variabilities for the two periods shows the amount and direction of the change in variability of localization as a result of practice between the first and second halves of the practice.

The data for localization with continuous sound are shown in Table 3. It will be seen that only three of the eight subjects shows any improvement at all, and that probably only in two cases, those of T. and Wa., is the improvement significant. The total improvement for all the subjects is +0·31 cm.—an indication that improvement did not take place to any appreciable extent as a result of practice in localizing with continuous sound during the period in question.

Table 4, containing the data for intermittent sound, gives a different impression. All the subjects show improvement, although the amount for M. and Ha. is clearly not significant. T., it will be noticed, shows as much improvement with intermittent as with continuous sound. The total improvement for all subjects is +2·24 cm.—seven times that for continuous sound.

Tables 3 and 4 suggest then that during the period of time in which the comparisons shown in Table 1 were being made, improvement took place in the localization of intermittent to a considerably greater extent than in the localization of continuous sound. Certain qualifications of this statement should, however, be noticed. Firstly, the statement refers only to improvement as measured by diminution in variability. No data are available for improvement in accuracy as distinct from improvement in variability.

TABLE 3

Localization with Continuous Sound

Subject.	*Mean S.D. First period. cm.*	*Mean S.D. Second Period. cm.*	*Difference. cm.*
D.	1·13	1·18	−0·05
Wi.	1·32	1·41	−0·09
M.	1·62	1·54	+0·08
Wa.	2·59	2·34	+0·25
Ha.	1·84	1·69	+0·15
P.	1·66	2·02	−0·36
Ho.	1·60	1·62	−0·02
T.	1·25	0·90	+0·35
Sums	13·01	12·70	+0·31

TABLE 4

Localization with Intermittent Sound

Subject.	*Mean S.D. First period. cm.*	*Mean S.D. Second period. cm.*	*Difference. cm.*
D.	1·13	0·93	+0·20
Wi.	1·27	0·87	+0·40
M.	1·39	1·37	+0·02
Wa.	2·50	1·97	+0·53
Ha.	1·62	1·54	+0·08
P.	1·87	1·39	+0·48
Ho.	1·62	1·46	+0·16
T.	1·29	0·92	+0·37
Sums	12·69	10·45	+2·24

Secondly, the statement most emphatically does not imply that no improvement in the variability of the localization of continuous sound took place in the period preceding that in which the measurements given here were taken. What data there are bearing on this earliest period, are for the greater part unsuitable for concise quantitative expression, but their general trend is clear, namely, that this earliest period was the period of greatest improvement for both intermittent and continuous localization. All that the above statement implies is that this improvement had for the greater part come to a standstill for continuous but not for intermittent localization by the time the measurements given in Tables 1–4 began to be taken. Whether the standstill was temporary or permanent in the case of the one, and how long the improvement would have continued in the case of the other, are questions that cannot be decided except by fresh experiments.

Thirdly, it should be borne in mind that originally there was no intention of tracing the course of improvement of the two abilities which were to be compared. Consequently the experiment was not planned with the requirements of a learning experiment in mind. It was for the same reason that no very strenuous or successful attempt was made to obtain an exact record of the subject's earliest attempts to localize. A full and precise record of learning still remains to be obtained.

It should, however, be remarked that in spite of their defects the data do to some extent fulfil expectation. It is to be supposed that the localization of intermittent sound constitutes a somewhat more unfamiliar task than the localization of continuous sound. It is not surprising, then, that the former continues to improve longer than the latter.

The finding that the localization of continuous sound has ceased to improve, whilst that of intermittent sound still continues to improve, suggests two inferences. Firstly, that a continuance of the comparison would have increased the difference in variability between the two kinds of localization. And secondly, that the differences found would have been greater than they are if the comparison had been begun at the end instead of at the beginning of the first period. This second inference is borne out by Table 5. It will be seen from that table that whilst in the first period the total difference for all subjects in variability between the two kinds of localization is +0·32 cm., in the second period it is +2·25 cm.

TABLE 5

Subject.	*First period.*		*Second period.*	
	Mean S.D. (*Cont.*).	— *Mean S.D.* (*Inter.*).	*Mean S.D.* (*Cont.*).	— *Mean S.D.* (*Inter.*).
	cm.		*cm.*	
D.	+0·00		+0·25	
Wi.	+0·05		+0·54	
M.	+0·23		+0·17	
Wa.	+0·09		+0·37	
Ha.	+0·22		+0·15	
P.	−0·21		+0·63	
Ho.	−0·02		+0·16	
T.	−0·04		−0·02	
Sums	+0·32		+2·25	

TABLE 6

Subject.	*S.D.'s larger than median.*			*S.D.'s smaller than median.*		
	Mean S.D. (*Cont.*). *cm.*	*Mean S.D.* (*Inter.*). *cm.*	*Difference. cm.*	*Mean S.D.* (*Cont.*). *cm.*	*Mean S.D.* (*Inter.*). *cm.*	*Difference. cm.*
D.	1·56	1·24	+0·32	0·77	0·77	+0·00
Wi.	1·79	1·51	+0·28	0·93	0·75	+0·18
M.	1·94	1·72	+0·22	1·11	1·06	+0·05
Wa.	3·10	2·65	+0·45	1·83	1·83	+0·00
Ha.	2·50	2·17	+0·33	1·26	1·20	+0·06
P.	2·35	2·00	+0·35	1·28	1·34	−0·06
Ho.	2·00	1·80	+0·20	1·23	1·30	−0·07
T.	1·45	1·41	+0·04	0·78	0·83	−0·05
Sums	16·69	14·50	+2·19	9·19	9·08	+0·11

The examination of the data is carried further in Tables 6, 7, and 8. For the purpose of Table 6 the standard deviations for all the sets of ten were ranked in order of size and then divided into two groups, those larger than and those smaller than the median standard

deviation, the upper and the lower fifty per cents. The mean standard deviation was then obtained for each of these two groups.

This process being carried out separately for the localization of intermittent and continuous sound, it is possible to compare the two kinds of localization on the basis of the upper half and then on that of the lower half of the standard deviations. This comparison is shown in Table 6. It will there be remarked that whilst the total difference for all subjects is +2·19 cm. and all the variability differences positive in the case of the upper half (that containing those S.D's greater than the median), in the case of the lower half the total difference is only +0·11, and three out of the eight differences are negative.

This finding suggests very strongly that the advantage possessed on the average by intermittent over continuous sound in the matter of variability of localization depends very largely upon the upper half of the standard deviations, that is, upon those sets of ten which possess standard deviations greater than the median.

Table 7 brings corroboration on this point. In this table a comparison is instituted between the two kinds of localization in respect of the means of the smallest four, of the median four and of the largest four standard deviations. It will be seen that in respect of the smallest variabilities the comparison shows no essential difference, what little difference there is favouring continuous sound. In the region of the median S.D's the comparison favours intermittent sound (a total difference of +0·95 cm. and seven out of the eight differences positive). At the upper end this advantage has been increased out of all proportion and all the differences are positive. It would, therefore, appear that the difference between intermittent and continuous sound in respect of variability is negligible in the region of the optimal performance, and that it increases in favour of intermittent sound as one proceeds towards the other end, the increase being greatest in the upper half.

TABLE 7

Mean S.D.'s, cm.

	Four smallest S.D.'s.			*Four median S.D.'s.*			*Four largest S.D.'s.*		
Subject.	*C.*	*I.*	*C.—I.*	*C.*	*I.*	*C.—I.*	*C.*	*I.*	*C.—I.*
D.	0·46	0·46	+0·00	1·04	1·01	+0·05	2·42	1·57	+0·85
Wi.	0·75	0·61	+0·14	1·13	1·12	+0·01	2·12	1·88	+0·24
M.	0·90	0·92	−0·02	1·54	1·24	+0·30	2·23	2·12	+0·11
Wa.	1·49	1·65	−0·16	2·39	2·02	+0·37	3·46	3·09	+0·37
Ha.	1·15	0·99	+0·16	1·65	1·58	+0·07	2·51	2·16	+0·35
P.	1·10	1·19	−0·19	1·78	1·69	+0·09	2·56	2·13	+0·43
Ho.	1·03	1·19	−0·16	1·61	1·50	+0·11	2·20	1·99	+0·21
Th.	0·78	0·83	−0·05	1·02	1·05	−0·03	1·45	1·41	+0·04
Sums	7·66	7·84	−0·18	12·16	11·21	+0·95	18·95	16·35	+2·60

C. = Continuous. I. = Intermittent.

It is not proposed to inquire now into the source of these large standard deviations nor even to inquire why they are less frequent with intermittent than with continuous sound. But it is natural to inquire

whether or not those S.D.'s that are greater than the median S.D., are equally well represented in the two practice periods. This question is dealt with in Table 8. Here the percentages of First and Second Period S.D.'s that are greater than the median S.D., are stated separately for intermittent and continuous sound. It appears from the table that whilst for continuous sound there is no appreciable difference between the two periods in respect of the density of larger S.D.'s, the density in the second period for intermittent sound is nearly half that in the first half. This seems to indicate that the improvement already remarked as a result of practice in the case of intermittent sound has consisted to a considerable degree in the reduction of the grosser deviations. In other words, the improvement is shown not only or even mainly as a displacement of the distribution of S.D.'s towards the zero end of the base-line, but as a contraction of the upper end of the curve.

Taking all the various indications together, it would seem that the superiority of intermittent over continuous sound in respect of variability of localization rests upon the greater improvability shown by the former, and it would seem that this difference in improvability is mainly confined to the larger S.D.'s.

TABLE 8

	Percentage of first period S.D.'s in 50 per cent. above median.		*Percentage of second period S.D.'s in 50 per cent. below median.*	
Subject.	*Continuous.*	*Intermittent.*	*Continuous.*	*Intermittent.*
D.	37·5	80·0	58·3	29·2
Wi.	45·5	72·7	55·6	22·2
M.	54·5	63·6	46·2	35·7
Wa.	60·0	70·0	40·0	30·0
Ha.	66·7	42·9	37·5	57·1
P.	57·1	57·1	40·0	40·0
Ho.	37·5	55·6	62·5	42·9
T.	50·0	75·0	50·0	25·0
Means	51·1	64·6	48·8	35·3

Appendix to Section 3.

In the case of three subjects the comparison of intermittent and continuous variabilities was made for a frequency of 946 dv./sec. as well as for that of 784 dv./sec. The data obtained are given in Table 9, expressed both in centimetres and degrees. It will be seen that disregarding Ha., whose variability difference is insignificant, the positive and significant difference of M. is balanced by the negative and significant difference of T. This suggests that variability difference is a function of frequency.

As lower frequency readings were taken on the same days and under the same conditions as the higher frequency readings, a comparison between the two frequencies in respect of variability has been instituted. Table 10 gives the comparison for Intermittent Sound, Table 11 that for Continuous Sound. The comparison is necessarily given in degrees.

The comparison is not very illuminating, partly due to the small number and heterogeneity of the subjects and partly also to the small relative difference between the two frequencies. A comparison based on three such frequencies as 200, 400, and 800 dv./sec. should be much more illuminating and is being undertaken. It seems advisable to defer the discussion of Tables 10 and 11 until this comparison has been carried out.

TABLE 9

Subject.	*No. of sets.*	*Mean S.D. (Cont.). cm.*	*Mean S.D. (Inter.). cm.*	*Diff. cm.*	*S.D. Diff.*	*Diff./S.D. (Diff.).*
M.	14	1·73	1·37	+0·36	0·142	+2·54
Ha.	15	1·70	1·78	−0·08	0·149	−0·55
T.	9	0·63	0·76	−0·13	0·071	−1·83
		degrees	degrees	degrees		
M.	..	17·80	14·09	+3·71	..	..
Ha.	..	17·49	18·31	−0·82	..	..
T.	..	6·48	7·82	−1·34	..	..

Plus and minus signs have the same significance as before.
Frequency = 946 dv./sec.
Wave-length = 34·98 cm.

TABLE 10

Intermittent Sound.

Subject.	*Mean S.D. in degrees.*		*Diff.*	*S.D. Diff.*	*Diff./S.D. Diff.*
	S.D. 784	*S.D.* 946.			
M.	13·73	14·09	−0·36	0·405	− 0·89
Ha.	13·39	18·31	−4·92	0·445	−11·05
T.	9·72	7·82	+1·90	0·297	+ 6·41

TABLE 11

Continuous Sound.

Subject.	*Mean S.D. in degrees.*		*Diff.*	*S.D. Diff.*	*Diff./S.D. Diff.*
	S.D. 784	*S.D.* 946.			
M.	11·68	17·80	−6·12	0·444	−13·79
Ha.	14·84	17·49	−2·55	0·451	− 5·65
T.	9·28	6·48	+2·80	0·295	+ 9·50

Summary of Section 3.

1. A comparison at a frequency of 784 dv./sec. of the variabilities of localization of intermittent and continuous sound respectively showed an advantage for intermittent sound. In six out of eight cases the advantage exceeded the probable error in size. In the remaining two cases the variability differences were insignificant.

2. This superiority of intermittent over continuous sound was, however, less than it should have been, owing to the operation of disturbing factors. These blurred the comparison, partly by increasing the variabilities for both intermittent and continuous sound by equal amounts, and partly by increasing the variability for intermittent to a greater extent than for continuous sound.

3. The disturbing factors demonstrated were: (*a*) instrumental irregularity, (*b*) the use to some extent of accessory criteria, (*c*) the influence of the direction of approach upon the centre found, and (*d*) the relatively early stage of learning at which the comparison was begun. Other disturbing influences were suspected, but not as yet with sufficient certitude for discussion.

4. Analysis of the data showed:

(*a*) That during the period of comparison variability of localization improved much more for intermittent than for continuous sound.

(*b*) That this difference in improvement referred mainly to a greater reduction in size of the larger variabilities for intermittent than for continuous sound.

(*c*) That the difference in variability between intermittent and continuous sound lay chiefly in the tendency for the large variabilities to be greater in the case of continuous than of intermittent sound.

(*d*) That there was no appreciable difference in magnitude existing between the smallest intermittent and the smallest continuous variabilities.

5. A considerably smaller comparison at a frequency of 946 dv./sec. showed no consistent advantage for either continuous or intermittent sound.

6. The advantage in variability of localization of intermittent over continuous sound seems to be conditioned by:

(*a*) individual differences,

(*b*) the stage of learning, and

(*c*) the frequency of the sound.

7. No clear indication of the source of the difference has yet been obtained.

4. The Effect of the Method of Finding the Centre upon the Position Found for the Centre

This problem was first approached in the following way: the subject was instructed to move the sound towards the centre until it appeared to be central and then to halt. There was thus to be no tacking to and fro across the centre, but merely a steady movement towards the centre from the initial position which was either well to the right or well to the left. Thus the problem was that of determining whether or not there was a significant difference between the centres obtained when the sound approached from the right and the centres obtained when the sound approached from the left, and further of determining the direction of any such difference. The general experimental conditions were identical with those already described. Right approaches (those in which the sound was brought in from the right) and left approaches were taken alternately.

The statistical treatment was as follows. The means and standard deviations of sets of ten right approach readings were obtained. From these the mean of all the right approach means and the standard deviation of this mean of means were computed. Similar measures were obtained for left approaches. The amount and sign of the difference between the mean of all the right approaches and the mean of all the left approaches could then be determined, and, the standard deviation of this difference being calculated, its significance tested.

The results, given in Table 12, show that in the case of all five

subjects there is a significant difference between the right and left approach centres, the effect of this difference being that the right approach centre is more to the right than the left approach centre. The magnitude of this difference is appreciable, the weighted average for all subjects being 11·99 degrees.

TABLE 12

Subject.	*Kind of sound.*	*No. of readings.*	*Diff. degrees.*	*Diff. cm.*	*S.D. Diff. cm.*	*Diff./S.D. (Diff.).*
D.	C.	410	+ 7·42	+0·87	0·074	+11·8
Wi.	C.	130	+12·53	+1·47	0·146	+10·0
Ho.	C.	120	+15·52	+1·82	0·210	+ 8·2
P.	C.	50	+ 6·74	+0·79	0·219	+ 3·6
Wa.	C	60	+35·04	+4·11	0·440	+ 9·3
Wa.	I.	60	+16·46	+1·93	0·474	+ 4·1

A plus sign indicates that the right approach centre is more to the right than the left approach centre.
A minus sign indicates the reverse.
C. = Continuous Sound. I. = Intermittent Sound.
The frequency was 784 dv./sec.

TABLE 13

Subject.	*Kind of sound.*	*Difference. Tacking method. degrees.*	*Difference. Non-tacking method. degrees.*
D.	C.	+1·28	+ 7·42
Wi.	C.	+0·43	+12·53
Ho.	C.	+2·64	+15·52
P.	C.	+1·71	+ 6·74
Wa.	C.	−3·41	+35·04
Wa.	I.	−9·98	+16·46

Plus and minus signs, C. and I. have the same significance as in the previous Table (Table 12).

This result is probably an expression, though not perhaps a very simple expression of the fact that the centre is rather an area than a knife-edge. It may be supposed that when a sound is brought in from the right towards the centre, the sound will cease to appear right and will appear central before the middle of the central zone is reached, and that if further the subject is forbidden to tack to and fro, and so to orientate himself as to the limits of this central zone, he will tend on the whole to give as centres positions in the right portion of the central zone.

Evidence that this substantially explains the result is furnished by the above table (Table 13), in which the differences between right and left approach centres occurring when the method described in the present section (the non-tacking method) was used, are shown side by side with the differences obtained with the method described in the previous section (the tacking method). In all cases, it will be seen, the latter differences are appreciably smaller. For four of the subjects they are positive, but for Wa. they are negative, due perhaps to an excessive cross-over which would tend to convert a right approach into a left approach and vice versa.

In the next table (Table 14) are shown the differences between right and left approaches for both continuous and intermittent sound (the tacking method being used) for all the subjects except Ha. It will be noticed that in eleven out of the fourteen cases the differences are positive, two of the minus cases being provided by Wa. and the third being clearly inappreciable. This seems to indicate that the tacking method does not completely eliminate the tendency for right approach centres to be more to the right than left approach centres. It does, however, very considerably diminish the tendency, and possibly with a more skilful use of the method the tendency would be still further diminished.

TABLE 14

Subject.	*No. of readings.*	*Diff. (Inter.). degrees.*	*Diff. (Cont.). degrees.*
D.	200	+ 2·64	+ 1·28
Wi.	100	+ 3·16	+ 0·43
Ho.	90	+ 5·20	+ 2·64
P.	65	+ 3·50	+ 1·71
Wa.	100	− 9·98	− 3·41
M.	125	+ 3·40	− 0·26
T.	50	+11·00	+10·31

Plus and minus signs have the same significance as in Table 12. The method is the tacking method, described in Section 3.

Table 14 is also important in another connexion. The fact that there is a difference of appreciable magnitude between right and left approach centres will tend, where right and left approaches are taken together in calculating the variability of localization, to increase the variability. Further, as the differences for intermittent sound are greater in all cases than those for continuous sound, the variabilities for the former tend to be increased to a greater extent than those for the latter. This finding is important for the discussion of Table 1.

Ha.'s results were omitted from Table 14. This was because his procedure being different from that of the rest, his results were not directly comparable with theirs. Ha's procedure was to convert all left approaches into right approaches. Right approaches were, however, treated as right approaches. What Ha. did when the sound was switched in on the left was to move it quickly over to the right and then to behave as if it were a right approach. On being questioned he stated that he found right approaches easier to centre than left approaches.

In spite of its incompatibility with the instructions, Ha. was allowed to continue with this procedure. This was for the sake of comparing his results with those of T., whose procedure was very closely watched and who did, in fact, adhere very closely to the prescribed procedure.

It was argued that the difference between right and left approaches should be appreciably greater in T.'s case than in Ha.'s. The result shows (Table 15) that not only is this the case for frequencies of both

784 and 946 dv./sec., but whilst the differences are very plainly significant in T.'s case, it is otherwise in the case of Ha.

TABLE 15

Subject.	*Frequency.*	*No. of Readings.*	*Diff. cm.*	*S.D. (Diff. cm.*	*Diff./S.D. (Diff.).*
T.	784	100	+1·23	0·131	+9·4
Ha.	784	130	−0·22	0·223	−1·0
T.	946	120	+0·57	0·081	+7·1
Ha.	946	130	−0·01	0·221	−0·04

Plus and minus signs have the same significance as in Table 12. The data for intermittent and continuous sound are taken together. The magnitude and sign of the differences between right and left approach centres is shown separately below in degrees for the two kinds of sound.

Subject.	*Frequency.*	*Difference (Intermittent). degrees.*	*Difference (Continuous). degrees.*
T.	784	+11·00	+10·31
Ha.	784	− 2·47	− 0·43
T.	946	+ 7·20	+ 5·04
Ha.	946	− 1·44	− 1·75

Taken together the evidence seems to point plainly to the fact that the procedure used in finding the centre is not without effect upon the centre found, and that in particular the direction of approach is important.

It is possible that in the experiments described in Sections 3 and 4 some at least of the subjects varied their procedure to an extent greater than was remarked by the experimenter. Variations of procedure would account for such data as those recorded in Table 16.

TABLE 16

Differences between Right and Left Approach Centres.

July 23.	*July* 24.	*July* 31.	*August* 1.
−0·6	+0·02	+1·3	−0·8
−3·9	−2·1	+0·4	−0·3
+1·0	−2·1	+0·7	−0·1
+2·3	−1·1	+0·9	+0·8
−0·5	−2·1	+0·1	+2·4
−2·0	−1·1	+0·5	+1·5
+1·5	−3·0	+0·1	+1·3
−2·7	−0·3	+0·9	−0·4
−0·2	+0·4	+2·4	−0·2
−1·3	+0·4	−0·1	+0·1
+1·0	−0·5	+0·9	+0·6
+0·3	−0·5	+0·5	+0·3
+0·4	−0·2		
−0·6	−0·5		
+0·2	−0·4		
+0·7	−1·0		

Plus and minus signs have the same significance as in Table 12.
The tacking method was used.

In this table are shown side by side the differences (magnitude and direction) between right and left approaches for successive sets of ten

readings on two pairs of successive days. For instance, the first column gives in order of occurrence the differences for the subject on July 23, the next column those for the next day, July 24. On the first day there is no steady tendency towards either a positive or negative difference, but on the second day there is an unmistakable tendency towards a negative difference. On the first of the second pair of days, July 31, there is an equally unmistakable tendency towards a positive difference, whilst on August 1, positive and negative differences are fairly evenly distributed.

Scattered throughout the records of the experiments there are other indications that can be interpreted as indications of changes of procedure. Whilst the interpretation is probable, it lacks the support of actual observations of changes of procedure corresponding to the changes in the amount and direction of the differences between right and left approach centres. A much closer scrutiny of the subjects' procedure than has so far been attempted, seems to be called for.

Summary of Section 4.

1. The method of finding the centre affects the position found for the centre. In particular the direction from which the sound approaches is important. In general, centres found with the sound approaching from the right are more to the right than centres found with the sound approaching from the left.

2. When the subject is allowed to cross and re-cross the central zone and so to orientate himself as to the position, extent, and limits of this zone (tacking method), the difference between right and left approach centres is much less than when crossing-over is forbidden (non-tacking method).

3. Even where crossing-over is permitted, the difference between right and left approach centres may be appreciable. It may be reversed in sign, probably by an excessive degree of cross-over.

4. The magnitude of the difference is consistently greater with intermittent than with continuous sound.

5. Indications exist of temporary or permanent changes of method on the part of some subjects. Very close observation is necessary in order to enforce a particular procedure.

* * * * * * * *

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PREFACE

In 1919 there was published in this series a 'Report on the Present State of Knowledge Concerning Accessory Food Factors (Vitamines)', prepared by a Committee which the Council had appointed jointly with the Lister Institute. The rapid growth of knowledge in this new field of work soon made that monograph obsolete at many points, and a completely new edition, nearly doubled in content, was prepared by the same Committee and issued in 1924. That this summary of methods and results was found useful by workers in this and other countries has been well indicated by the numbers sold—10,000 copies of successive impressions have been distributed already. Fresh advances of knowledge soon made complete revision necessary again, and this was undertaken by the Committee in 1930. On this occasion, however, much more complete revision was seen to be required, and the report now presented is an entirely fresh resumption of current knowledge and of technical methods in this subject.

The Committee in preparing this report had the great advantage of Dr. Arthur Harden's services as general editor. They were assisted in the preparation of material for particular sections by Dr. W. R. Aykroyd, Dr. C. W. Carter, Professor S. J. Cowell, Mrs. M. A. Boas Fixsen, Miss E. M. Hume, Dr. G. F. Marrian, Dr. R. A. Morton, and Miss M. H. Roscoe. All the members of the Committee named on the preceding page are responsible jointly for the final shaping of every chapter and for the whole work as now produced. To all these the Council are greatly indebted, and they believe that their feelings of gratitude will be shared by the innumerable workers now interested in this field of study.

It can hardly be contemplated that the rapid and accelerating growth of the subject can be conveniently followed further by fresh revisions of such a monograph as this. The labour entailed and the sacrifices of time made by research workers in successive revisions of such a monograph are not likely to be justifiable again. The Council have joined with the Reid Library of the Rowett Institute, Aberdeen, and the Imperial Agricultural Bureaux Council, in giving financial support to a new periodical journal, 'Nutrition Abstracts and Reviews', of which the first number appeared in October last. The Council hope that this will provide for the future a regular means of bringing together and making more readily available the results of research work as they accumulate in the now widely distributed and often disconnected fields of work, medical, agricultural, dietetic, and commercial, in which the subject of nutrition is being so rapidly developed.

In an Appendix is given the report of the International Conference on Vitamin Standards, held under the auspices of the Permanent Standards Commission of the League of Nations in London during June, 1931. The recommendations given here for adoption of

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international standards for those vitamins of which present knowledge makes this practicable, together with definitions of units of activity in terms of the standards for use in each case in quantitative estimation of the vitamin, have now been adopted by the League of Nations. In their first report of 1919, mentioned already, the Committee referred to the scepticism, then shown in some quarters, even of the real existence of the subject of their monograph. They admitted that there was then no knowledge of the actual chemical nature of the vitamins, but they said that 'the study of their functions is progressing on real and objective lines, and has become in certain cases even quantitative'. It is gratifying to notice that within only a dozen years research work has advanced so far as to allow not only quantitative study but a formal international agreement upon precise quantitative standards in the case of no less than four, the so-called A, B_1, C and D, of the vitamins.

MEDICAL RESEARCH COUNCIL,
38, Old Queen Street,
Westminster, S.W. 1.

10th May, 1932.

VITAMINS: A SURVEY OF PRESENT KNOWLEDGE

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CHAPTER I

HISTORICAL INTRODUCTION.

As food constituents vitamins are characterized by the disproportion between the great importance of their nutritional functions and the very small amounts necessary for the adequate fulfilment of those functions. In a normal dietary they are present in quantities far too small to yield any appreciable contributions to the energy supply of the body, or to bulk appreciably as structural elements for the tissues. They are, nevertheless, definite chemical entities, and in the last eight years much light has been thrown upon the actual chemical nature of some among them. It would seem, indeed, that at least one has now been artificially prepared. During the same period, extended researches have shown that they are more numerous than was earlier suspected. Eight years ago only three were clearly recognized; now we must believe in the existence of at least eight. Each one upon good evidence has been shown to exert its own individual functions in nutrition.

Since a supply of them is essential for the animal body, the vitamins are, of course, generally present in the natural foods which are instinctively consumed by men and animals. They are primarily formed during the synthetic activities of the green plant on the land, or those of algae and other smaller organisms in the sea. From these they are transferred to the tissues of animals, terrestrial or marine. There is some evidence, however, to show that one vitamin at least, and perhaps others, may be synthesized in the organs of certain animal species. Such animals can dispense with, or are less dependent upon, an external supply of the vitamin which their own tissues produce. Such cases are rare, however, and we have no knowledge of such synthetic powers in the human body.

It is not surprising that popular experience should have wholly failed in the past to reveal the existence of nutritional agencies which function in such small amounts, and are unconsciously consumed in every adequate dietary. The distribution of vitamins in vegetable and animal foods alike may be partial and irregular, but broadly speaking it is safe to say that the adult individual finds a good supply of vitamins in his food so long as that food is reasonably varied, has received no artificial or accidental separation into parts, and has had no destructive influence applied to it. This generalization does not, however, cover the nutritional demands of infants and children.

In the observation of phenomena the importance of contributory factors is often overlooked until the effects of their removal come to light. In the case of vitamins and their functions, demonstration, so far as it depended upon observation, first came when commercial adventure or other human enterprise had led to the preparation and consumption of foods in which the natural materials had been fractionated for the sake of taste, appearance, or convenience; treated by destructive methods; or, in other cases, only preserved

too long before consumption. Owing on the one hand to the partial and irregular distribution of vitamins in the natural products, or on the other hand to their relative instability, it may happen that they are thus removed or destroyed.

Safety in this connexion is evidently greater when a variety of foods is consumed. In countries where geographical conditions prevent such variety the danger is more imminent. It is a fact, at any rate, that the most striking evidence for the evil effects of the artificial treatment of food first came from rice-eating districts of the East when they had been invaded by milling machinery from the West. The recognition some twenty to thirty years ago of beri-beri and scurvy as deficiency diseases provided good evidence both for the evil effects of fractionating natural foodstuffs such as the cereals, and for the nutritive importance of vitamins.

The evidence from disease would have led sooner to a conception of these food constituents and their functions but for a not unnatural bias in thought. It was difficult to implant the idea of disease as due to deficiency.

Disease is so generally associated with positive agents—the parasite, the toxin, the *materies morbi*—that even expert thought turned naturally to such positive associations and believed with difficulty in causation prefixed by a *minus* sign. Even in the case of deficiencies arising within the body there was a similar tendency. When the importance of internal secretions was first recognized there used to be much hesitation in believing that symptoms might be frankly due to their failure. When each fresh internal secretion was described there was always an effort to show that its function was to 'neutralize' some, always hypothetical, toxic substance. Symptoms on this view were due to the unmasking of a deleterious agent rather than to simple deficiency in a normal and necessary agent. To distinguish between these two possibilities was, of course, a scientific duty; but, at any rate in the earlier literature of internal secretions, a bias against the simpler view interfered with the fair interpretation of experimental results.

So in connexion with the later conception of disease as due to dietetic deficiencies. Even when a distinguished Dutch investigator, through his admirable studies, which will a little later receive reference, had clearly established more than thirty years ago that beri-beri arose during the consumption of decorticated but not of whole rice (making it clear, therefore, that something in the cortex was necessary to normal nutrition), he was led to suggest, not the simple view that some substance in the cortex was of direct use to the body, but rather that it was necessary to neutralize the otherwise deleterious effect of a diet over-rich in starch.

While it is true that the simpler idea of the nature of deficiency diseases is now generally held, it must be stated that there is good evidence, which is referred to later on, to show that there are times when certain dietetic ingredients necessitate a larger intake of specific vitamins for normal development and function. In such cases some investigators regard the vitamin action as antagonizing those of harmful agents.

Fortunately, in the case of deficiency diseases it proved possible

some years ago, by the choice of suitable animals, to supplement clinical and sociological observation by controlled experiments. As usual, experimental work on animals made knowledge more quantitative, and in some cases revealed some well-known pathological conditions as deficiency diseases. Illustrations of this will shortly be given.

If, however, the evidence for the existence and importance of vitamins had arisen entirely from the study of deficiency diseases our views might have remained too limited, too much confined to the standpoint of pathology and therapeutics. But in the very years during which the aetiology of beri-beri was being cleared up, and the suggestion established that a qualitative food deficiency may be responsible for striking pathological symptoms, experiments were in progress on quite independent lines to show that something more than a supply of energy and protein is required to maintain so fundamental a physiological phenomenon as growth, and to demonstrate that normal metabolism as a whole is not possible without the influence of food constituents which, because of their minute amount, could justifiably be spoken of as 'accessory' food factors in nutrition.

From such experiments, then, the conception of the vitamin rose, and at the present time the evidence from all kinds of feeding experiments, whether carried out from the physiological or pathological standpoint, is being consolidated. The earlier stages in the history of such researches will be now reviewed.

As often happens in the development of science, a fundamental idea is foreshadowed in many quarters, but has long to wait before it emerges as a basis of accepted knowledge. As in other cases, so with recognition of vitamins as physiological necessities. Their existence was suggested long ago, but a certain right moment in the history of the science of nutrition had to arrive before it could attain to universal recognition. Some workers had discovered suggestive facts, but failed to realize their full significance; while the work and words of true pioneers lay forgotten because published when average minds were not ready to appraise them at their right value.

No one can deny that the recorded experience of voyagers and explorers in the eighteenth century, and particularly, perhaps, the records of the British Navy which deal with the incidence and cure of scurvy, would have directed thought towards our modern conception of vitamins had the times been ripe. The knowledge concerning nutrition was then, however, entirely vague, and the days of animal experiment in such matters had not yet come. The earliest experiments, indeed, supplied evidence which was of an independent kind, reached from a different angle.

It is now generally agreed that the first clear evidence, based upon experiment, for the existence of dietary factors of the nature of vitamins, came from the school of Bunge at Basel. In 1881 Lunin, one of the workers in that school, fed mice upon an artificial mixture of the individual constituents of milk, of all the constituents, that is, which were then known, namely, the proteins, fats, carbohydrates, and salts. He found that upon such a mixture the animals failed to survive, and was led to conclude that '*a natural*

food such as milk must therefore contain besides these known principal ingredients small quantities of unknown substances essential to life' [1881]. Such a statement, already half a century old, when allowed to stand out clear and apart from a context which tended to bury it, seems to contain the essentials of what is believed to-day.

It will not be uninstructive to seek for an explanation as to how such a significant remark could remain for years almost without notice, instead of being an immediate challenge to further investigation. It may be noted first of all that the title of Lunin's paper [1881] 'Ueber die Bedeutung der anorganischen Salze für die Ernährung des Thieres', was one prone to conceal the important but incidental suggestion it contained. The thought of the Bunge school was, as a matter of fact, both then and long afterwards, concerned largely with the inorganic factors of nutrition. After an interval of ten years, during which, so far as one can discover, no effort had been made to follow up the pregnant suggestion of Lunin's work, another member of the school came again to Lunin's point of view. He, however, was once more concerned primarily with the study of an inorganic element in nutrition. Socin, the worker in question, though, as the title of his paper [1891] 'In welcher Form ist das Eisen resorbiert?' indicates, he had not set out to seek further for those wholly unknown substances essential to life the existence of which Lunin had predicted, was nevertheless, owing to the nature of his experiments, inevitably driven to a belief in them. Socin, it is true, was led to think that the ineffectiveness of the synthetic diets employed by him might be due to an inadequacy in the quality of the protein contained in them, a view not wholly unjustified and foreshadowing knowledge which only long afterwards became definite and proven. He became convinced, however, that other and unknown substances must be sought, substances which, he states, are certainly present in whole milk and egg yolk. He remarks, moreover, with conviction that to discover them was the first task to be faced before new feeding experiments were undertaken. Here was a strong enough challenge to investigate, yet we have again no knowledge of any attempt in Bunge's laboratory to follow up the challenge. Perhaps an explanation of this is to be found in the circumstance that Bunge, though in his well-known book he observes that it would be worth while to continue the experiments which had suggested the existence of unknown nutritional factors, was himself inclined to disbelieve in them. He thought that the real error in the synthetic diets used by his pupils (which was, so to speak, 'dissected' milk) was that the method of its preparation had involved the separation of inorganic constituents from certain organic combinations, in which latter form alone could they adequately subserve the purposes of metabolism. Other causes may have contributed to the long neglect, first of Lunin's and then of Socin's suggestions. It must be admitted, indeed, that no *experimentum crucis* was carried out in Bunge's laboratory. In Lunin's experiments the fate of six mice only (those placed by him upon a normal salt mixture—all others had been given deficient mineral supplies) really brought suggestions for the existence of unknown factors; and

no data are given as to their consumption of food. Neither Lunin nor Socin made any attempt to complete the evidence by making discriminate additions to the diets which had proved inadequate. Lastly, as already suggested, since the main intention of their work and the titles of their publications were remote from the special issue, their significant remarks might well have appeared as little more than *obiter dicta* when read without the light of modern developments.

Fourteen years after these observations were described an investigator distinguished in many fields was led to repeat and extend them. This was the late Professor Pekelharing, whose conclusions [published in 1905] unhappily remained wholly unknown to the majority till recently. It is, indeed, remarkable that the results of such significant statements as his, though published in the Dutch language alone, should not have become rapidly broadcast. After speaking of experiments carried out on lines similar to some of those done in Bunge's laboratory, and indicating that they pointed to the existence of some unknown essential, Pekelharing goes on to say: 'Till now my efforts, constantly repeated during the last few years, to separate this substance and get to know more about it have not led to a satisfactory result, so I shall not say any more about it. My intention is only to point out that there is a still unknown substance in milk which even in very small quantities is of paramount importance to nutrition. If this substance is absent, the organism loses the power properly to assimilate the well-known principal parts of food, the appetite is lost and with apparent abundance the animals die of want. Undoubtedly this substance occurs not only in milk but in all sorts of foodstuffs both of vegetable and animal origin.' Here we have a clear statement of the vitamin doctrine already a quarter of a century old. It is to be regretted that Pekelharing refrained from publishing any of his experimental data. There is little doubt that he was led to undertake his physiological experiments because of his intimate knowledge, not then shared by the majority of workers outside Holland, of the important researches now to be mentioned. For there was at this time other evidence available to indicate that orthodox teaching concerning nutrition was incomplete—evidence different in kind from that just discussed. It demonstrates for the first time the existence of what we have now learnt to call Deficiency Disease.

Following researches carried out from 1890 onwards Christiaan Eijkman, who was at the time a military doctor in the Dutch Indies and later became Professor of Hygiene at Utrecht, had, in 1897, come to the conclusion that the disease beri-beri resulted from the continuous consumption of decorticated (polished) rice. His view was more particularly supported by a study made with his collaborator Vorderman of diet and health in no less than 101 of the prisons in Java [Eijkman, 1897, 2]. The evidence so obtained was striking enough, but Eijkman's view was strengthened by his discovery that birds (fowls, pigeons, and ducks) when fed upon polished rice developed a disease, which he named *polyneuritis gallinarum*, the symptoms of which closely resembled those of human beri-beri. He found further that the avian disease failed

to develop when the birds were fed upon whole rice, or rice grains still associated with the pericarp or 'silver skin'. This observation made it possible to add the results of controlled animal experiments to those of statistical studies and greatly contributed to advance in the subject.

Eijkman himself, as was mentioned above, at first viewed the facts he had discovered from a standpoint more in accordance with the thought of contemporary pathologists than with our present conceptions. He concluded that foods such as rice, over-rich in starch, produced a substance in the intestine which acted as a poison to nerve cells, for which the outer layers of the rice grain contained an antidote. Somewhat later, however, another Dutch scientist, Grijns [1901], gave a different interpretation to the facts in putting forward the view that beri-beri, whether in birds or man, was due to a 'deficiency'—to the absence from the food of an essential nutrient. This, in rice, happens to be confined to the cortical parts of the grain which are removed by polishing. To Grijns belongs the credit of first picturing clearly what is to be justly called a deficiency disease, as well as that of first investigating the properties and distribution of a vitamin.

In 1907 Braddon further established the correctness of Eijkman's observations upon the influence of rice diets by widely extended studies upon rice-eating native races [1907]. They were fully supported, moreover, by the discriminating investigations of Fraser and Stanton [1909].

It is now difficult to understand how, even long before Eijkman's work, scurvy failed in practical medicine to obtain recognition as a disease due to a deficiency in food. The possibility of such causation was doubtless, just as in the case of beri-beri, against the trend of thought in current pathology. A better reason, perhaps, was the absence of definite evidence based upon controlled animal experiments to replace the vaguer impressions of clinical experience. The means for such experimental studies was first provided in 1907 by the researches of two Norwegian scientists, Holst and Frölich, who showed that typical scurvy could be produced in guinea-pigs by deficient diets and as easily cured by suitable additions. That this opportunity for experiment, like that given by Eijkman's discovery of polyneuritis in birds, has since greatly advanced the subject will be made clear in later sections of this monograph.

A survey of the literature of the first decade of the present century will show that the facts brought to light by the researches so far described had by then exerted little or no effect upon orthodox views and teaching concerning human nutrition. Calorie-supply, protein-minima and such factors still dominated the general conception of nutritional adequacy. We are now to see that researches described in the course of the next three years led to a change in the outlook.

Reference may first be made to the work of Stepp [1909; 1911; 1912]. This author's original concern was to decide whether fats and lipoids must be supplied in the food in order that life may be maintained. He found that whereas mice lived satisfactorily for several months upon certain foods, such as wheat bread made with milk,

they were unable to live longer than a month when fed upon the same diet after it had been subjected to prolonged extraction with alcohol and ether. That the extraction itself had only lowered the nutritive value of the foodstuffs by removing some essential component was demonstrated by restoring the extract to the extracted food, when it once again became adequate for the nutrition of the mice.

Stepp made a number of experiments in order to ascertain the nature of the essential factor removed by the extraction process. The addition of the ash of the extract, or of certain neutral fats in a pure condition, did not remedy the deficiency, but he found that milk and egg-yolk did. This led him to suggest the existence of an unidentified indispensable dietary unit, which he appears to have regarded as a member of the lipoid class. He was unable to identify this substance with cholesterol, lecithin, kephalin, or cerebrone, since all members of that class of substance failed to restore the nutritive value of the extracted foodstuffs. He remarks in one of his later papers: '*It is not impossible that unknown substances indispensable for life go into solution with the lipoids, and that the latter thereby become what may be termed carriers for these substances.*'

Attention should now be turned to the classic experiments carried out by Hopkins in this country. As early as 1906 he wrote as follows: 'But further, no animal can live upon a mixture of pure protein, fat, and carbohydrate, and even when the necessary inorganic material is carefully supplied, the animal still cannot flourish. The animal body is adjusted to live either upon plant tissues or other animals, and these contain countless substances other than the proteins, carbohydrates, and fats. Physiological evolution, I believe, has made some of these well-nigh as essential as are the basal constituents of diet; lecithin, for instance, has been repeatedly shown to have a marked influence upon nutrition, and this just happens to be something already familiar, and a substance that happens to have been tried. The field is almost unexplored, only it is certain that there are many minor factors in all diets of which the body takes account. In diseases such as rickets, and particularly in scurvy, we have had for long years knowledge of a dietetic factor, but though we know how to benefit these conditions empirically, the real errors in the diet are to this day quite obscure. They are, however, certainly of the kind which comprises these minimal qualitative factors that I am considering. Scurvy and rickets are conditions so severe that they force themselves upon our attention, but many other nutritive errors affect the health of individuals to a degree most important to themselves, and some of them depend upon unsuspected dietetic factors' [1906].

Some results of his experiments, which had extended intermittently over several years, were published in 1912 [Hopkins, 1912]. Young rats were fed upon an artificial food mixture containing caseinogen, starch, cane sugar, lard, and inorganic salts. When the animals were fed upon the diet composed of these constituents in the crude condition, they were able to live and to show a certain amount of growth. When, however, the components had been carefully purified, growth invariably ceased after a comparatively

short period, and the rats declined and died. In experiments of the kind it is of the utmost importance that the material of which the control synthetic diet is composed should be fully purified. Inadequate purification led earlier workers, and, as we are to see, later workers also, to claim that successful nutrition may be obtained on artificial dietaries. Hopkins employed diets in which very complete purification of the protein, carbohydrate, and (as the author then thought) of the fats administered was a special feature. A novel feature of these experiments was that Hopkins showed by estimations of the energy consumptions of the animals that this failure was not due to an insufficient food intake. It was found that growth ceased at a time when the animals were consuming food in more than sufficient quantity to maintain normal growth.

Another series of animals which received, in addition to the basal ration of purified foodstuffs, a very small daily allowance of milk afforded a great contrast to those just described. In all cases the milk addendum, although its total solids amounted to only 4 per cent. or less of the whole food eaten, induced normal growth, and greatly improved the general condition of the animals.

The extraordinary effect of this apparently insignificant addition to the diet upon the growth of the experimental rats is well illustrated by Figures 1 and 2, which have been reproduced from the original paper. A similar growth-stimulating action was exerted by the addition of protein-free and salt-free extracts of milk solids, or of yeast, to the basal diet. As the milk ration was administered separately and in advance of the administration of the main dietary, it could not have affected the palatability of the food or diminished its monotony. Some authors have stated their inability to obtain normal growth with amounts of milk so small as those used by Hopkins. It is true that when an animal depends entirely on milk for its vitamin supply growth cannot be maintained for long periods on so small an amount.

In discussing his results Hopkins remarked: 'It is possible that what is absent from artificial diets and supplied by such addenda as milk and tissue extracts is of the nature of an organic complex (or of complexes) which the animal body cannot synthesize. But the amount which seems sufficient to secure growth is so small that a catalytic or stimulative function seems more likely.'

After reference to possibilities which had more weight when his paper was written than now, he wrote in conclusion: '*If the attachment of such indispensable functions to specific accessory constituents of diets is foreign to current views upon nutrition, so also is the experimental fact that young animals fail to grow when they are absorbing daily a sufficiency of formative material and energy for the purpose of growth.*'

Attention should now be turned to the work and writings of Casimir Funk, to whom we owe the term Vitamine, which, slightly modified by omission of the final e, has now become indissolubly attached as a class name to the substances with which this monograph is concerned. This investigator has greatly helped to advance our knowledge of the subject, and at a time when

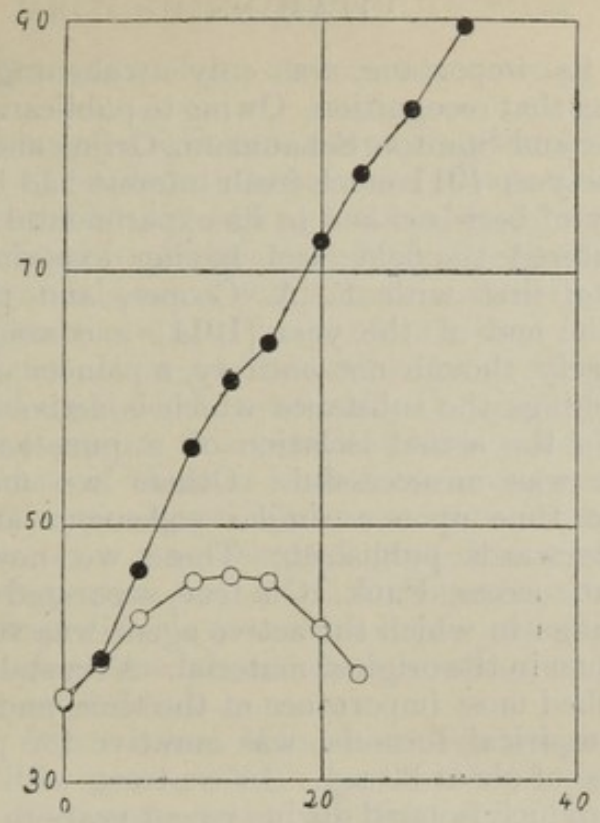

FIG. 1. Lower curve, six rats on artificial diet alone. Upper curve, six similar animals receiving in addition 2 c.c. of milk each per diem. Abscissae, time in days; ordinates, average weight in g. (Reproduced by permission from the *Journal of Physiology*, 1912, **44**, 432.)

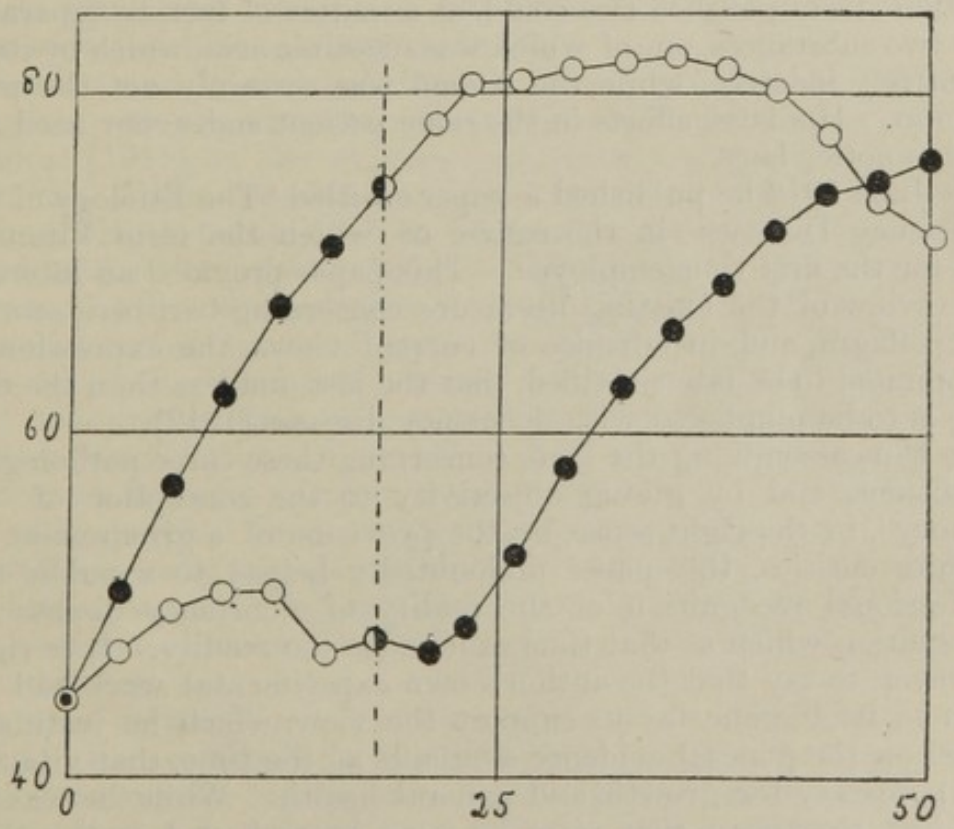

FIG. 2. Lower curve (up to eighteenth day), eight male rats upon pure dietary; upper curve, eight similar rats taking 3 c.c. of milk each a day. On the eighteenth day, marked by vertical dotted line, the milk was transferred from one set to the other. Average weight in g., vertical; time, horizontal. (Reproduced by permission from the *Journal of Physiology*, 1912, **44**, 433.)

recognition of its importance was only awakening he did much towards securing that recognition. Owing to publications by William Fletcher, Fraser and Stanton, Schaumann, Grijns and others during 1907–10, by the year 1911 much fresh interest had been awakened in the aetiology of beri-beri and in its experimental cure. In that year Funk entered the field, and having experimented at the Lister Institute, first with E. A. Cooper, and then alone, he described, at the end of the year [1911], a courageous attempt, which was largely, though not entirely, a pioneer effort, to isolate from rice polishings the substance which is active in the cure of the disease. In the actual isolation of a pure active substance the endeavour was unsuccessful. Others we may note were engaged at the time upon a similar endeavour and the results were soon afterwards published. These we now know were equally without success. Funk, it is true, separated fractions from the rice polishings in which the active agent was very much more concentrated than in the original material. A crystalline fraction to which he attached most importance at the time, and for which he provided an empirical formula, was curative for polyneuritis in pigeons in doses of about 20 mg. In contrast with this, however, a crystalline product, isolated during recent years in the laboratory where Eijkman did his fundamental work, which is possibly, though not certainly, the pure vitamin, has proved to be many thousand times as active as Funk's preparation. A year and a half after his first paper appeared Funk himself showed that his original 'vitamine fraction' from polished rice could, as a matter of fact, be separated into two substances, one of which was nicotinic acid, which by itself is entirely inactive, while the second was certainly not the pure vitamin. His later efforts in the same patient endeavour need not receive notice here.

In June 1912 he published a paper entitled 'The Etiology of the Deficiency Diseases' in the course of which the term Vitamine was for the first time employed. This paper provided an interesting review of the existing literature concerning beri-beri, scurvy, and pellagra, and, in advance of current views, the expression of an opinion, to be later justified, that the last, not less than the first two, is to be numbered with deficiency diseases [1912].

In thus assembling the facts concerning these three pathological conditions, and by giving objectivity to the conception of 'deficiency', in the right sense, by the provision of a group name for definite entities, this paper undoubtedly helped to a public and professional recognition of the reality of deficiency disease; a recognition which at that time came none too readily. It is right, however, to say that the author's own experimental work had not then in itself gone far to support the view which he justifiably based on the general evidence available at the time, that vitamins are necessary for growth and general health. While he was not the first to express that view he must be reckoned among those who first impressed it upon scientific opinion.

It is certain that publications which appeared in the years 1911–13 led ultimately, though with some delay, to the full beliefs of to-day. It is not easy or necessary to attempt

an estimate of the exact share of each in producing that desirable result.

A few words may be said here concerning Funk's choice of the term Vitamine. The prefix *vita* connotes of course the importance of the substances to life; the ending *amine*, owing to the special significance of the word in organic chemistry, implies that they are of the nature of organic bases, or compounds derived from ammonia by substitution. Funk separated bases of this kind in his attempted isolation mentioned above, and he believed that the nature of vitamines in general could be thus defined. Complete proof, however, that any one of them has this constitution is yet lacking, and we now know that two of them at least do not even contain nitrogen. Funk has nevertheless argued with some reason that the readiness with which the term was accepted proved its convenience and adequacy. The suggestion due to J. C. Drummond, that by dropping the final *e* the unjustifiable connotation just mentioned might be to a degree avoided, was nevertheless a wise one, and the name *Vitamin* is now established.

The work of Osborne and Mendel at Newhaven, Conn. [1911; 1912; 1913, 1, 2, &c.], and of McCollum and his colleagues in Wisconsin (later in Baltimore), published from 1912 onwards, yielded results of much importance. Alike at Newhaven and Wisconsin the conviction that vitamins are essential to life came, however, rather slowly. It will not indeed be out of place here to say a few words concerning the progressive experience of the American workers which ultimately led to that conviction. That experience when duly surveyed becomes very instructive.

Osborne and Mendel had, for some years previous to 1912, been carrying out experiments, of which the results have become classical [1911], on the relative nutritional value of various pure proteins. These experiments, carried out on rats, called for the use of synthetic dietaries in which the proteins to be tested were combined with carbohydrates, fats, and mineral salts. Of necessity it became of prime concern with these investigators to decide whether such diets when made of pure materials had defects other than those which might appertain to any particular protein under study. It is noteworthy that at one stage in their studies Osborne and Mendel decided that a synthetic diet of the above nature, if containing an adequate protein, could, as a matter of fact, support life and growth. They published curves demonstrating excellent growth in young rats, and remark: 'These are so far as we know the first successful feeding experiments in which lasting growth has been obtained with carefully purified foodstuffs and artificial salt mixtures.'

In view of the later experience of the authors themselves, and of that of a multitude of more recent investigators, it may seem difficult to explain the apparent success of these particular experiments. It was doubtless due in the main to the circumstance that the diet contained about one-fourth of the carbohydrate supply as milk sugar which had not been specially purified. It was pointed out a little later that when milk sugar was used in such experiments it was necessary that it should be carefully purified.

The sugar when first separated from its source adsorbs a considerable proportion of the milk vitamins. Using purified milk sugar Hopkins and Neville [1913] could obtain no growth with a diet otherwise exactly similar to that employed in the above experiments.

It should be understood, however, that the greater number of Osborne and Mendel's experiments, made both before and after the dates of those just discussed, were not carried out on purified foodstuffs alone. During the earlier stages of their studies they had met with experimental difficulties, especially (as they then believed) in the endeavour to secure an efficient salt mixture for their synthetic diets. Their chief interest, as we have seen, was in the nutritive value of proteins. Now whole milk had been shown to form by itself an adequate diet for rats ; it can support growth for long periods, if not indefinitely. Osborne and Mendel saw that if the proteins were removed from milk, and the proteins alone, all other substances necessary for proper nutrition should remain in the fluid. The milk protein could then be replaced by any individual protein under study. By a happy inspiration therefore such 'protein-free-milk ' was made a constituent of their experimental diets, and, except during the period when the above-mentioned experiments for a while deceived them, they looked upon it as probably essential for nutritional success. It was employed primarily as a means of obtaining a physiologically correct salt mixture, but the authors came to recognize that it might supply other constituents of importance. As a matter of fact their protein-free preparation contained, as we now know, some of the vitamins of the original milk.

During the following year, however, Osborne and Mendel endeavoured to repeat their experiments, but now taking care that all materials used should be of 'the highest degree of purity'. 'To our surprise,' they write when discussing the results, 'the artificial protein-free milk made with these purer chemicals failed in every case but one to promote more than slight growth.' They still supposed at first that some mineral deficiency was in question ; but, as appears later in the paper just mentioned, a critical examination of their data led them at last to conclude that some specific essential was present in the fats of natural milk. 'It would seem', they wrote, 'as if a substance exerting a marked influence upon growth was present in butter . . . ' [1913, 1, p. 319].

The experience of the investigators at the Wisconsin centre will next be described. McCollum and various colleagues had been engaged, from 1907 onwards, in scientific studies of nutrition, with special reference to the rationing of farm stock. Special attention had been given to the determination of nitrogenous and mineral deficiencies in individual cereals and in other food materials used in practical dietaries. McCollum apparently came to feel, as others have felt, that many nutritional problems would be simplified if the adequacy of dietaries could be defined in terms of their individual pure constituents, and during the year 1912 he was engaged with Davis in testing the ability of synthetic diets to maintain health and growth in rats—a line of inquiry which of necessity has already received frequent reference in this historical survey. Like

Osborne and Mendel, McCollum and Davis came at first to the conclusion that they could obtain adequate nutrition with such diets. They found later, however, that the growth, apparently promoted by these, ceased abnormally soon, and in a paper published in 1913 they describe an experience which first brought their investigations into the field of vitamin studies [1913]. They had found that when, on a synthetic diet of which the fat constituent was lard, growth had prematurely ceased, the administration of butter fat or ether extracts of egg yolk secured its immediate resumption. They were then led, like Osborne and Mendel, to recognize that a factor essential to nutrition was associated with certain (but not all) fats. It may be noted as of interest that their paper just mentioned was submitted for publication in the same month (June 1913) as Osborne and Mendel's paper also dealing with the specific influence of butter fat, to which reference has been made.

A year later McCollum and Davis [1914] demonstrated at once the objective nature of the factor associated with animal fats and its relative stability by showing that it could be transferred from saponified butter fat to olive oil which otherwise contains none of it.

In 1915 these authors published an important paper [1915, 2] in which it was made clear that at least two 'accessory' diet factors were essential for normal growth. Owing to the special interest which Eijkman's studies had conferred upon polished rice, McCollum, who had previously employed wheat-grain in his studies, decided at this time to make the former the basis of a synthetic dietary. McCollum and Davis had been convinced by previous experience that growth and well-being could be maintained in animals fed on a mixture of purified proteins (casein), starch, *milk sugar*, butter fat, and a suitable mixture of mineral salts. They held, therefore, that polished rice, if its low content of protein and certain mineral deficiencies were first corrected by suitable additions, ought when mixed with butter fat to form a completely adequate dietary. When submitted to trials, however, this diet entirely failed to induce any growth in young rats. The explanation soon came to hand. It will have been noticed that the above-mentioned synthetic diet, the apparent success of which led to the expectation that the rice diet would be adequate, contained milk sugar. Once more the circumstance that this sugar adsorbs the vitamins of milk had led to unreal success with an artificial dietary. Hopkins and Neville's earlier experience on this point had not till then impressed these later investigators. McCollum and Davis found, as did Osborne and Mendel, that purification of the commercially 'pure' lactose it contained could make all the difference between adequacy and inadequacy in a synthetic diet. Such experiences should not be looked upon as trivial in their bearings. They illustrate how exceedingly small may be the amounts in which accessory components of food can exert a potent action, a significant circumstance wholly unrealized by earlier students of nutrition and involving a lesson which had to be learned by all experimentalists. McCollum and Davis quickly saw the significance of the facts before them. They realized that what was associated with the unpurified sugar could not consist in the main at least of the sub-

stance associated with butter fat. They sought for this second substance elsewhere and found it widely spread in natural food materials, wheat germ being specially rich in it. They now suggested the names 'fat-soluble A' and 'water-soluble B' as labels for these two entities, so introducing the current alphabetical nomenclature of vitamins, which, though displaying some inconveniences owing to the increasing complexity of the field, has yet justified itself on the whole.

Evidence derived from the observed distribution of 'water-soluble B' and the antiberi-beri vitamin discovered by Eijkman pointed to an identity between these two dietary factors, which has since been disproved. For a considerable time from 1915 onwards the relatively simple conception held the field that only three vitamins, fat-soluble A, water-soluble B, and antiscorbutic vitamin C, were capable of demonstration by biological methods. Following E. Mellanby's discovery in 1918 of the existence of an antirachitic vitamin associated with certain fats, evidence for the subdivision of the fat-soluble vitamin into vitamin A and an antirachitic vitamin D gradually accumulated. More recently the existence of the fertility vitamin E has been discovered and laborious researches on water-soluble B have brought to light the complex nature of this factor and necessitated the recognition in it of at least five different principles. The historical sequence of the researches leading up to our present knowledge of the subject will be dealt with in detail in later sections of this monograph.

CHAPTER II

THE FAT-SOLUBLE VITAMINS. VITAMIN A.

Historical.

After the recognition by McCollum and Davis [1913] of the existence of a fat-soluble vitamin necessary for growth, observations gradually accumulated which indicated that more than one accessory food factor was present in some of the natural fats and oils.

A convenient starting-point for the history of the further development of the subject is found in the recognition by E. Mellanby [1918–19, 1, 2; 1919; 1920; 1921] of rickets as a deficiency disease. He was able to demonstrate that experimental rickets could be induced in puppies by withholding from the diet a substance (the antirachitic vitamin) which appeared to be similar as regards distribution and properties to the fat-soluble vitamin A. Although calcium and phosphorus deficiencies were naturally of importance, the potency of the antirachitic vitamin in effecting the calcification of bone was so pronounced as to imply that the substance held a key position in the aetiology of rickets. Cod-liver oil was much superior to all the other fats tested (e.g. beef suet, butter, lard, and various vegetable oils) in promoting the calcification of bone and in preventing rickets. Mellanby was inclined to identify this antirachitic vitamin with the fat-soluble A recognized by McCollum and Davis some five years earlier [1913]. The evidence was not complete, but further support to the idea was given by the observation that butter through which oxygen was bubbled at 120° C. lost both its growth-promoting [Hopkins, 1920] and antirachitic power [Mellanby, 1921].

The advance due to Mellanby was consolidated and extended to experiments on rats by Sherman and Pappenheimer [1920–21; 1921]; Korenchevsky [1922]; McCollum, Simmonds, Parsons, Shipley and Park [1921]; and other workers. The experimental procedure necessary for the production of rickets in rats emphasized the importance of the calcium/phosphorus ratio in the diet, as well as the deficiency of antirachitic vitamin. The following results gained widespread acceptance:

(1) A fat-soluble vitamin similar in many respects to vitamin A is necessary for the production of perfectly formed bones, even when the calcium and phosphorus content of the diet is adequate and well-balanced.

(2) Deficiency of either calcium or phosphorus or an unbalanced proportion of these two elements increases the importance of an adequate supply of antirachitic vitamin.

(3) The power of controlling calcification of bone and of promoting growth is most potently exerted by fish-liver oils [Goldblatt and Zilva, 1923]. Body fats exhibit some little potency with the exception of lard, which, like most vegetable oils, is usually devoid of both antirachitic

and growth-promoting activities. Egg yolk and milk usually contain both vitamins.

(4) Rickets is a disease associated with growth and affects susceptible animals at their most active period of development; with increasing age an animal becomes less dependent on the antirachitic vitamin.

The similarity in distribution of vitamin A and the antirachitic vitamin extends to lean meat, milled cereals, and yeast, all of which contain very little or none of either principle. The antirachitic vitamin, like vitamin A, is associated with the unsaponifiable portion of fat, and no connexion can be traced with fatty glycerides as such [Steenbock and Boutwell, 1920, 1; Steenbock, Nelson and Hart, 1921; Drummond and Coward, 1921; Zucker, Pappenheimer and Barnett, 1921–22; Zucker, 1922]. Both substances are resistant to heat alone, but in the presence of oxygen destruction of both occurs at elevated temperatures.

Separation of the two Fat-soluble Vitamins.

McCollum, Simmonds, Becker and Shipley [1922] showed that the antixerophthalmic action of cod-liver oil was destroyed by 12–20 hours' oxidation at 100°C., whereas its antirachitic action could be demonstrated after this treatment [see also Steenbock and Nelson, 1923]. Similarly it was found by Goldblatt and Zilva [1923] that the rates of destruction by heat and oxidation of these two properties in cod-liver oil differed markedly, the antirachitic principle being much the more resistant to oxygenation and to heat. Vitamin D was not unattacked but was only decomposed by a more prolonged or more drastic treatment than was necessary to effect the destruction of vitamin A.

In addition to this evidence differences were recorded, both qualitative and quantitative, in the distribution of these two principles in foodstuffs. McCollum and his co-workers made the very significant observation that butter, while much weaker than cod-liver oil in both its antixerophthalmic and antirachitic actions, was relatively much stronger in the former than the latter, thus indicating that these two different properties of butter depended on two different substances. These workers also found that coco-nut oil, which, like many other vegetable fats was lacking in fat-soluble vitamin A, unlike these contained 'a substance which stimulates the deposition of calcium salts in rickets in a manner similar to cod-liver oil' [McCollum, Simmonds, Becker and Shipley, 1922].

This conclusion was strengthened by the gradual accumulation of evidence from 1923 to 1925 that antirachitic (vitamin D) but not antixerophthalmic (vitamin A) properties could be produced by the action of certain radiations upon the animal organism or upon natural foodstuffs composing the diet (see pp. 69, 70). Since then it has been accepted that vitamin A (the antixerophthalmic vitamin) and vitamin D (the antirachitic vitamin) are distinct fat-soluble dietary factors, both of which are required by mammals for satisfactory growth and nutrition [Steenbock and Nelson, 1923; 1924–25; Steenbock and Black, 1924; Hess and Weinstock, 1924, 1, 2; 1924–25; 1925, 1, 2, 3].

Vitamin A.

The Effects of Vitamin A Deficiency.

Growth.

The first indication that a fat-soluble accessory food factor, not identical with any of the common triglycerides or any of the known "lipoid" substances, was necessary to ensure satisfactory health and growth in small laboratory animals fed on artificial diets, was provided by the work of Stepp [1909; 1911; 1912]. Stepp showed that mice fed on foodstuffs which had been previously extracted with alcohol and ether died after a few weeks. If the alcohol-ether extract was restored to the diet, the animals were enabled to live in good health. The unknown factor involved was shown not to be inorganic, since the addition of the ash of the extract to the diet did not remedy the deficiency. The addition of various triglycerides, or of cholesterol, lecithin, or kephalin to the extracted diet was equally ineffective. There is little doubt, however, that the dietary deficiency studied by Stepp was complicated by the removal by the alcohol of factors other than those which we now regard as fat-soluble, possibly of the water-soluble B vitamins, since butter was found to be deficient and skimmed milk relatively rich in the missing constituents.

More clear-cut results were obtained when McCollum and Davis [1913] discovered the 'fat-soluble A' vitamin and showed that rats failed to grow normally when fed on artificial rations in which lard or olive oil was the only source of fat. Good growth, however, followed the addition of an ether extract of egg yolk or of butter to the diet. Similar results were shortly after published by Osborne and Mendel [1913, 1, 2].

At this period and during the following few years no distinction was recognized between vitamin A and the antirachitic fat-soluble factor discovered later by Mellanby. It is certain, therefore, that the dietary deficiency studied by many early workers in this field was a deficiency of both vitamins A and D and not simply of vitamin A.

Young rats fed on a diet deficient only in vitamin A usually grow for a few weeks. This period is followed by one in which the body weight remains at an approximately constant level. The animals then show a sharp decline in weight and death rapidly follows. The timely administration of adequate supplies of vitamin A is soon followed by a resumption of growth.

The initial growth of animals fed on diets free from vitamin A is believed to correspond with the period during which they are utilizing their own reserves of vitamin A. The duration of this initial period of growth depends therefore largely on their previous dietary history [Smith and Chick, 1926].

Xerophthalmia.

Many years before the discovery of the vitamins the belief was widely held that certain types of conjunctivitis occurring in children were of dietary origin. M. Mori [1904] made the important observation that the disease known as 'hikan', prevalent among children in Japan and characterized by xerosis of the conjunctiva, could be prevented

by giving various fish-liver oils. He concluded that the cause of the condition was a deficiency of fat in the diet. Bloch [1917, 1, 2, 3] and Monrad [1917] as a result of studying cases of conjunctivitis occurring in Danish children also concluded that the disease was a dietary one. The former, while agreeing with Mori in believing that a fat deficiency was the main aetiological factor, suggested that a vitamin deficiency might be a contributory cause. Monrad, on the other hand, definitely inclined to the belief that a deficiency of accessory food factors was the cause.

Knapp [1908] described an infective condition of the conjunctiva of rats fed on inadequate dietaries. Osborne and Mendel [1913, 2; 1914; 1915] called attention to the frequency with which infected eyes in rats were associated with a deficiency of what was then known as the fat-soluble accessory factor, and they pointed out that the administration of fats rich in the factor resulted in a prompt alleviation of the symptoms. Similar observations were recorded by McCollum and Davis [1915, 1].

The analogy between the human disease and that occurring in rats as a result of a deficiency of 'fat-soluble A', was clearly pointed out by McCollum and Simmonds [1917], who held that 'xerophthalmia' should be regarded as a deficiency disease.

In rats, the first symptom observed is a swelling of the eyelids which is followed by an inflamed condition of the conjunctiva. Haemorrhagic and purulent discharges follow; the cornea becomes affected and ultimately blindness results. The first symptoms are usually observed shortly before or simultaneously with the slowing in the rate of growth [Osborne and Mendel, 1921].

The histological changes which occur have been studied in detail by Stephenson and Clark [1920]; Wason [1921]; S. Mori [1922, 1, 2]; Yudkin and Lambert [1921–22, 1, 2]; and G. M. Findlay [1925] among others. There have been some differences of opinion as to whether the bacterial invasion, which invariably accompanies the disease, is the primary cause of, or a secondary effect following, the changes that occur in the conjunctiva and cornea. Stephenson and Clark were unable to detect any histological change in the eyes preceding the bacterial invasion, and they therefore considered that the primary change was a lowering of resistance to infection. S. Mori stated that the primary change occurred in the lachrymal glands and that the resulting diminution of the secretion was the cause of the xerosis of the cornea and conjunctiva. Similar views have been advanced by Findlay, who has also made the important observation that the tears of rabbits fed on vitamin A-deficient diets are definitely deficient in lysozyme, thus providing an explanation for the extreme susceptibility to local bacterial infection in the eyes of these animals.

Hume [1922] and Goldblatt and Soames [1922] simultaneously showed that exposure of rats, fed on diets deficient in fat-soluble vitamins, to ultra-violet radiation was ineffective in preventing the appearance of the symptoms of xerophthalmia, although at first promoting growth. Since ultra-violet radiation was known to be specific in the cure of rickets, this fact suggested that the aetiological factors responsible for xerophthalmia and rickets were different. Further work by Bloch [1924, 1, 2], drawing attention to the infrequency with

which dietary conjunctivitis accompanied rickets in children, pointed to the same conclusion. It was realized, therefore, when a distinction was made between the two fat-soluble factors, vitamins A and D, that a deficiency of the former was the cause of xerophthalmia.

Xerophthalmia is not an invariable symptom of vitamin A deficiency. Emmett [1920] observed that 120 out of 122 rats developed the typical symptoms when fed on vitamin A-deficient diets. On the other hand, Osborne and Mendel [1921] recorded an incidence of only 50 per cent. and Green and Mellanby [1928, 2] of 38 per cent. With more highly purified diets Osborne and Mendel [1924] reported an 80 per cent. incidence. Stammers [1924, 1], and Sherman and Storms [1925] have shown that adult rats are less susceptible to the disease than the young. It seems probable, therefore, that variation in age and in the degree of purity of the diets employed may to some extent account for the irregularity in the occurrence of xerophthalmia.

The specificity of xerophthalmia to vitamin A deficiency was at one time questioned by McCollum, Simmonds and Becker [1922], who observed a similar condition in rats receiving adequate supplies of vitamin A, but whose diet was unbalanced with regard to its inorganic constituents. S. Mori [1923] concluded from histological studies that the anatomical changes occurring in the eyes of rats in this condition were identical with those observed in xerophthalmia caused by vitamin A deficiency. Subsequently [Simmonds, Becker and McCollum, 1927, 1, 2; J. H. Jones, 1927; McCollum, Simmonds and Becker, 1926–27] it was shown that the cause of this new type of xerophthalmia was the substitution of ferrous sulphate for ferric citrate in the diet and the destruction of the vitamin A in the diet by the ferrous sulphate. Jones regarded the destruction of the vitamin to be the result of an oxidation catalysed by the ferrous salt.

Night-blindness (Hemeralopia).

For many years ophthalmologists have recognized a connexion between the occurrence of conjunctivitis in human beings and certain types of hemeralopia or night-blindness (see p. 234). Fridericia and Holm [1925] have shown that vitamin A-deficient rats placed in the dark after exposure to light show a subnormal rate of regeneration of the visual purple. Holm [1925] tested the ability of rats to jump off a table in a dim light, after previous exposure to bright sunlight. In this way it was shown that vitamin A-deficient rats had developed a well-defined hemeralopia, 'at a stage where it was impossible to perceive any other signs of avitaminosis'. This condition did not develop unless the animals were previously exposed to bright light for considerable periods. The administration of vitamin A to hemeralopic rats resulted in a disappearance of the symptoms within two to three days.

These results have been confirmed by Tansley [1931], who followed the course of the regeneration of the visual purple in normal and vitamin A-deficient rats. The visual purple was extracted from the retinas by a dilute solution of digitonin and quantitatively estimated by a photographic method. She was able to show that the retinas of the deficient rats contained in every case subnormal amounts of the visual purple.

Susceptibility to Infection.

The history of the development of knowledge relating vitamin A to infection has been complicated by the slow differentiation of the fat-soluble vitamin complex. It is true that at a relatively early stage in the history of this work the absence of vitamin A from the diet had been associated with xerophthalmia, and the fact that irradiation of the animal did not cure xerophthalmia [Hume 1922] was interpreted by Steenbock and Nelson [1923] as evidence that vitamin A and the antirachitic vitamin were different entities. Since all the earlier work concerned with vitamin A deficiency also involved a vitamin D deficiency, the expression 'fat-soluble vitamin' deficiency will be used in describing this earlier work.

Early attempts to correlate immunity reactions with vitamin deficiencies failed to bring out any connexion between the two. Thus Zilva [1919, 2] found that animals fed on 'vitamin A'-deficient diets showed no diminution in agglutinin or amboceptor titres. Findlay and Mackenzie [1922] were unable to show any decrease in the opsonic activity of the blood-serum of 'vitamin A' deficient rats. Cramer, Drew and Mottram [1922] detected a marked thrombopenia in rats deprived of vitamin A, to lack of which they ascribed this increased susceptibility. Bedson and Zilva [1923], on the other hand, were only able to detect a slight decrease in the platelet count and did not regard the observed change as a specific lesion produced by the deficiency. Cramer, Drew and Mottram [1923], however, maintained their original findings, but in a later paper [Cramer and Kingsbury, 1924] the thrombopenia was regarded as a result of, rather than the cause of, the bacterial invasion. The observations of Falconer [1926] were in close agreement with those of Bedson and Zilva.

McCollum [1917] was the first to draw attention to the susceptibility of rats when fed on diets deficient in fat-soluble vitamin to respiratory infection [see also Drummond, 1919, 2]. Under similar dietetic conditions puppies also often developed broncho-pneumonia [E. Mellanby, 1919]. This question of the relation of diet to infection later attracted more interest [Steenbock, Sell and Buell, 1921] and was extended to the intestine by Cramer [1923]. Following up S. Mori's [1922, 2] observations on the hyperplasia of epithelium produced by diets deficient in fat-soluble vitamin, Wolbach and Howe [1925] discovered the metaplasia of epithelium and the keratinization changes produced by this dietetic defect. Although primarily concerned with the epithelial changes they also noted the local infective conditions often found at the seats of epithelial change. It is interesting to note that Wolbach and Howe remarked on the 'cysts' found under the tongue in these animals, a condition later alluded to as abscesses by Sherman and Munsell [1925], one of the commonest forms of infection to be found in rats fed on diets deficient in fat-soluble vitamins. Cramer and Kingsbury [1924] no doubt meant the same lesion, or a similar one of the submaxillary glands, when they referred to a 'subcutaneous abscess in the neck' in animals eating diets defective in this respect.

As regards the differentiation of the parts played by vitamins A and D respectively in susceptibility to infection, E. Mellanby [1926, 2]

analysed the dietary of a large number of puppies which had died from, or escaped, an epidemic of broncho-pneumonia and found that the susceptibility to this disease was correlated with a deficiency of fat-soluble vitamin in the diet, but that this susceptibility was not related to the calcification of the bones. Dogs, for instance, whose diet included butter, although often developing defective bone and even local atelectasis (collapse) of the lungs, escaped broncho-pneumonia: when the butter was heated and oxygenated, broncho-pneumonia often developed as well as badly calcified bones. Cod-liver oil produced perfect bones and also protected against broncho-pneumonia.

Goldblatt and Benischek [1927] repeated the work of Wolbach and Howe on the dietary factor responsible for hyperplasia and metaplasia of epithelium, but they took the added precaution of giving vitamin D in the basal diet in the form of irradiated cotton-seed oil, so that the diet was deficient only in vitamin A. The epithelial changes were thus proved to be due to deficient vitamin A intake, and the infective lesions also found, such as keratomalacia, broncho-pneumonia, and enteritis, could now be definitely ascribed to lack of this particular vitamin.

Green and Mellanby [1928, 2] reinvestigated this problem on a large scale with the object of finding out why animals ceased to grow and died on diets deficient in vitamin A. They found that rats on such diets almost invariably died with multiple infective foci of the type described by previous workers, and that these lesions could be readily observed macroscopically. The addition of vitamin D in the form of irradiated ergosterol did not increase the resistance to bacterial infection—in fact, this addition often appeared to hasten the onset, possibly because of the increased rate of growth. The incidence of the infective foci in 92 rats fed on diets deficient in vitamin A was as follows:

Xerophthalmia, 38 per cent.
Abscess at base of tongue, 72 per cent.
Infections of lungs, 9 per cent.
Infections of genito-urinary tract (including renal and bladder calculi), 44 per cent.
Middle-ear abscess and septic nasal sinuses, 20 per cent.

Other seats of infection were prostate glands, seminal vesicles, spleen, intestine, and ovaries. Fifty control rats on the same diet with the addition of some source of vitamin A, as for example dried cabbage, butter or cod-liver oil, developed no sign of any of these infections. Green and Mellanby therefore gave the name 'anti-infective vitamin' to vitamin A to signify that animals deprived of this substance died of bacterial infective lesions.

A further development of this work was the demonstration by Green and Mellanby [1930] that carotene (see p. 39) also has the specific effect of preventing the local infections above described as well as the hyperplastic changes of the epithelium. A point of interest about this work is the quantitative relationship between the amount of carotene ingested and the degree of infection. Thus a daily dose of 0·08 mg. of the specimen of carotene used prevented the development of all infective foci in the young rat; 0·01 mg. allowed a moderate degree of infection; while 0·005 mg. had no significant protective effect.

Two important problems in this connexion remain for future investigation :

(1) The specificity of vitamin A in its relation to bacterial resistance.
(2) The significance of this relationship in the case of man and other animals.

There is a general consensus of opinion that in the case of other vitamins deficiency of intake also lowers the resistance of animals to infection, but there is no evidence that the relationship is as direct as in vitamin A deficiency. For instance, the work of Grant, Suyenaga and Stegeman (1927, 1, 2), and of Eichholz and Kreitmair [1928] has shown that a lowered resistance to certain types of bacterial infection results from vitamin D deficiency.

To what extent these results are applicable to some human infections remains to be established. The fact that some of the infective conditions observed in rats are commonly found among human beings, especially children, suggests that a deficiency of vitamin A intake is a factor of importance in the aetiology of these human diseases. This might well apply, for instance, to broncho-pneumonia, middle-ear disease, and septic nasal sinuses. Several attempts have been made to see whether vitamin A therapy, either curative or prophylactic, can influence certain cases of infection. For instance, Mellanby and Green have treated puerperal septicaemia with diets specially rich in vitamin A, and their results indicate an increased resistance in cases of this disease, except in those of the fulminating type and in those complicated with peritonitis and septic thrombophlebitis [1929 ; Green and Mellanby, 1930]. The same workers have also recorded the results of treating women late in pregnancy with vitamin A preparations and have compared the amount of puerperal sepsis which developed after parturition in these cases with that in an equal number of women who received no such addition to the diet. Here again the results showed a definite increase in resistance to infection in the former group [1930] (see p. 270). Donaldson and Tasker [1930] at the Crown Gold Mines, Johannesburg, tested this form of treatment in cases of pneumonia among the native workers. To 100 cases of pneumonia they gave daily ½ lb. of liver, to a second 100 radiostoleum (a preparation containing vitamins A and D), and a third 100 received no additional treatment. The untreated cases had a mortality of 13 per cent., that of the first and second groups was 8 per cent. On the whole, therefore, although the evidence is at present no more than suggestive, it seems possible that the animal results may prove to have some bearing on certain types of human infection. It is of interest to note that Bloch [1921] in an analysis of 64 cases of vitamin A deficiency in human beings, diagnosed on the basis of xerophthalmia, found a high rate of other infective conditions. There were 15 cases of pneumonia, 12 of bronchitis, 15 of otitis media, 27 of pyuria, and 14 of pyodermia.

The animal experimental method has also been used to see whether vitamin A deficiency influences the resistance to infections transmitted to the animals and not developed autogenously. Thus Lassen [1930] has found that rats inoculated by mouth and by subcutaneous injection with Breslau bacilli (paratyphoid) succumb more readily when the diet is deficient in vitamin A than when rich in this substance. An

obvious difficulty in this type of work is that animals deprived of vitamin A have much less vitality than animals on complete diets and therefore die more readily when exposed to the strain of a septicaemia. Topley, Greenwood and Wilson [1931] determined the mortality of mice consuming diets respectively rich and adequate in vitamin A when associated with other mice infected with *B. aertrycke*. They found no increase in resistance in the mice fed on the richer diet.

The subject is one of great practical importance and is attracting much attention among laboratory and clinical investigators. For the time being it is impossible to assess the significance or the limitations of the animal results so far as human disease is concerned. It may be, as claimed by Cramer and Kingsbury [1924], that a deficiency of this substance in the body simply brings about a breakdown of the local defences of such organs as the intestine, eyes, and lungs against bacterial invasion and has no effect on the general humoral defences. Even if this is its mode of action, the prophylactic and therapeutic uses of vitamin A in human disease ought to be of great value.

Susceptibility of the Central Nervous System to Toxic Influences.

The experimental production of a syndrome resembling subacute combined degeneration of the spinal cord has recently become a problem of some interest and promises to open up to scientific study from a nutritional aspect a number of human nervous diseases which have hitherto been almost entirely subject to clinical study alone.

Hart, Miller and McCollum [1916] described changes in the anterior horn cells of pigs' spinal cords caused by diet. No changes in the conducting fibres were described and the authors decided that they were dealing with a form of beri-beri, not, however, due to a deficiency of vitamin B, which was present in abundance in the wheat germ contained in the diet. Indeed the pathological condition was ascribed rather to a toxic action of the wheat germ. The changes in the cord could be prevented by adding alfalfa meal and meat scraps to the diet and the protective action of these foodstuffs was attributed to a combined action of additional proteins, fat-soluble vitamins, and a better salt mixture, but not to any one of these substances alone. Hughes, Lienhardt and Aubel [1929] observed degeneration of fibres in the spinal cord, and in some peripheral nerves, of pigs after feeding them from the time of weaning on white corn, tankage and bone-ash. They also found that these changes could be prevented by cod-liver oil, butter fat, yellow corn, and alfalfa leaf meal, and suggested that the preventive dietary factor was probably vitamin A

Some years previously E. Mellanby [1926, 1] described the production in dogs of a condition resembling combined system disease with degenerated nerve fibres in tracts of the cord by the use of diets rich in cereals, especially in wheat germ, and deficient in fat-soluble vitamins. He ascribed this effect to a nerve toxamin in the cereals, which like the de-calcifying toxamin also contained in cereals could only operate in the absence of a fat-soluble vitamin.

In 1929, in a lecture in Kenya, E. Mellanby [1930, 1, see also 1931, 1, 2] gave further results of the experimental production by dietary measures of subacute combined degeneration of the cord, which he

found could be hastened and intensified by adding a small quantity of ergot (2–5 g. daily) to the diet. Vitamin D which was found to be a constituent of ergot [Mellanby, Surie and Harrison, 1929] played little or no part in the processes. The addition of vitamin A-containing substances such as mammalian-liver oil or butter prevented the neuro-toxin contained in ergot from being effective. Carotene (about 5 mg.) or carotene-containing substances (e.g. carrots or cabbage) also prevented degeneration of the spinal cord, and this condition, if permitted to develop, was rapidly improved by the addition of carotene or vitamin A to the diet, and the degeneration of the central nervous system was arrested.

It is not yet possible to make any definite statements as to the significance of these facts with regard to nervous disease in man. E. Mellanby suggests, however, that they probably explain the appearance in epidemic form of convulsive ergotism, a condition which was common at one time in rye-eating countries. These epidemics appear in times of famine and drought and are no doubt due partly to the consumption of ergotized rye and partly to the fact that butter, vegetables, and other sources of vitamin A and carotene are unprocurable. If the diet is rich in vitamin A dogs can eat large quantities of ergot continuously without causing any affection of their nervous system [E. Mellanby, 1931, 2].

Lathyrism is another disease associated with degenerative changes in the spinal cord similar to those occurring in dogs fed on vitamin A-deficient diets with addition of ergot. This disease which is often widespread in some Indian provinces is also a disease of poverty and develops in persons eating largely of peas (*Lathyrus sativus*). In this case also it is possible that the absence of vitamin A and carotene from the diet allows a neuro-toxin in the peas to exert its harmful effect on the nervous system [E Mellanby, 1931, 2].

In pellagra somewhat similar degenerative changes are found in the nervous system. In view of the fact that pellagra occurs commonly, but not exclusively, among maize-eating populations, it is of interest to note that yellow maize not only does not produce the characteristic demyelination of the cord in dogs when it forms the cereal element of diets deficient in vitamin A, but actually prevents ergot from producing this effect [E. Mellanby, 1931, 2]. Yellow maize apparently contains sufficient carotene to give the necessary protection against the neuro-toxin of ergot. White maize has been found to possess little or none of such a protective property.

Whether administration of vitamin A or of carotene results in any great improvement of the nervous manifestations of pellagra has not been tested. Clinical evidence suggests that vitamin A may play a significant part in preventing the development of certain other conditions characterized by nerve demyelination which are more commonly found in this country. Pernicious anaemia, for instance, and disseminated sclerosis have been treated with mammalian liver, and good results have been claimed. Whereas it is now recognized that the abnormal condition of the blood in pernicious anaemia can be successfully treated with watery extracts of mammalian liver [Minot and Murphy, 1926], this substance has no curative or preventive action on the lesions in the central nervous system, often present in

the later stages of that disease. It is reported, however, that whole liver has been found to be curative both of the blood condition and of the nervous lesions [Ungley and Suzman, 1929]. In this case, the beneficial effect on the cord may be due to the vitamin A contained in the liver, as suggested by E. Mellanby's experiments. Similar curative effects of whole liver in disseminated sclerosis have been described by Goodall and Slater [1931]. Owing to the well-known fact that remissions often occur normally in cases of disseminated sclerosis, it is too early yet to accept liver therapy as a specific treatment in this disease, but the evidence so far is promising. The argument based on the frequent occurrence of remissions in this disease is, however, double-edged and may imply unrecognized dietetic influences, as was proved to be the case in pernicious anaemia.

Keratinization of Epithelial Cells.

Closely linked with the question of susceptibility to bacterial infections are the pathological changes in epithelial surfaces that have been repeatedly observed as a symptom of vitamin A deficiency. S. Mori [1922, 1, 2] was the first to draw attention to these changes which he observed in the larynx, trachea, and in the ducts of various glands. Wolbach and Howe [1925, 1928] showed that the substitution of stratified keratinizing epithelium for normal epithelium in the alimentary tract, the respiratory tract, the genito-urinary tract, the eyes, the parocular glands, and the pancreatic duct was a characteristic effect of vitamin A deficiency in rats and guinea-pigs. They claim [1925] 'that the substitution of keratinizing epithelium in all locations is not secondary to infections, and presumably is a primary effect of the withdrawal of factors essential for the chemical activities or maintenance of differentiation of the epitheliums concerned'. As Green and Mellanby [1928, 2] have stated, the susceptibility to infection may be 'due to the favourable medium which obstruction by desquamated keratinized cells provides for bacterial growth'.

The continued appearance of keratinized epithelial cells in the vaginal tract of the rat has been observed by several workers and used as a criterion of vitamin A deficiency [Evans and Bishop, 1922, 1, 2; Macy, Outhouse, Long and Graham, 1927; Coward, 1929, 1]. Since in the normal rat the presence of keratinized epithelial cells indicates the period of oestrus, Evans and Bishop at first wrongly assumed that the prolonged keratinization observed was due to a prolongation of oestrus, but later experiments [Evans, 1928, 3] clearly showed that this could not be so since the same phenomenon was observed even after double ovariectomy. Coward [1929, 1], and Coward, Morgan and Dyer [1930], on the other hand, found this symptom of little use for the diagnosis of vitamin A deficiency, since not only did they fail to find constantly a 'cornified' vaginal smear after cessation of growth, but in certain cases the vaginae of the young rats did not open until after the other symptoms of vitamin A deficiency had appeared.

Reproduction.

Failure in reproduction by rats deprived of vitamin A has been observed for many years [Drummond, 1919, 3; Sherman and MacLeod, 1925]. Parkes and Drummond [1926] tried to determine the nature

of this sterility by histological examination of the gonads of animals in different stages of vitamin A deficiency. No degeneration or other abnormalities of structure were found even in rats which had ceased to grow when far below adult weight. When similar rats were changed to a vitamin A-rich diet, they grew to maturity and reproduced freely. Since, however, it is now realized that these rats were probably also suffering from a shortage of vitamins D and E, the effects cannot be ascribed simply to a deficiency of vitamin A.

Sure [1928, 1] demonstrated that a deficiency of vitamin A leads to resorption of the foetus, as does a deficiency of vitamin E.

Properties and Attempted Isolation of Vitamin A.

Properties.

In the early literature on the general chemical properties and stability of fat-soluble A, many apparent contradictions are to be found. These can partly be ascribed to the difficulties encountered in even roughly quantitative estimations of the vitamin by animal growth tests. On the other hand insufficient care to exclude more than one factor influencing the stability of the vitamin has led in many cases to confusion. A striking example of the latter is seen in the question of the stability of vitamin A to heat. McCollum and Davis [1914] originally pointed out that since the fat extracted from boiled eggs was highly active, the vitamin must be resistant to heat. Osborne and Mendel [1915; 1920, 1] found that the vitamin in butter fat was highly thermostable, while Steenbock and Boutwell [1920, 2] were able to show that the vitamin from vegetable sources was also stable to heat. On the other hand Steenbock, Boutwell and Kent [1918], and Drummond [1919, 2] reported experiments which indicated that the vitamin in butter fat was destroyed to a large extent by heat.

The explanation of these discrepancies became clear when it was shown by Hopkins [1920] that although the vitamin in butter was resistant to temperatures up to 120° C. it was readily destroyed by aeration at all temperatures. Hopkins's results on butter fat were independently confirmed by Drummond and Coward [1920], while Zilva [1920, 1; 1922] showed that the vitamin in fish-liver oils was also destroyed by aeration at ordinary temperatures and more rapidly if ozone were employed.

Attempts to isolate Vitamin A.

The important observation that vitamin A was resistant to alkaline saponification was made by McCollum and Davis [1914]. This fact, confirmed by Steenbock and Boutwell [1920, 3], Steenbock, Nelson and Hart [1921], and Drummond and Coward [1921], formed the basis of many of the subsequent attempts to concentrate and isolate the vitamin from various sources. Provided that adequate precautions against oxidation are observed, the vitamin in cod-liver oil is stable to treatment with a hot solution of alcoholic potash or sodium ethoxide, and may be quantitatively recovered in the unsaponifiable fraction by extraction of the soaps with ether or some other suitable solvent. Since the unsaponifiable matter present in many natural fats and oils containing the vitamin forms only a small fraction of the total bulk,

a considerable degree of concentration may be accomplished by this process.

The technique of the process [Steenbock, Sell and Buell, 1921; Drummond, Channon and Coward, 1925] was modified by Takahashi *et al.* [1925], who converted the soaps into calcium salts and obtained the unsaponifiable matter from the alcoholic extract of these, and by Marcus [1928] who extracted the semi-solid mass of soaps with ethylene dichloride and claimed to have obtained quantitative extraction of both vitamins A and D. Dubin and Funk [1923] extracted cod-liver oil with acetic acid and saponified the extract in the usual way.

The cholesterol in the unsaponifiable fraction of cod-liver oil can be precipitated by digitonin without removing any of the vitamin A activity [Drummond and Coward, 1922]. Since approximately 50 per cent. of the unsaponifiable matter of many natural oils containing the vitamin consists of cholesterol, a still further degree of concentration can be obtained by such a process, and the cholesterol-free unsaponifiable matter has been the starting-point for several attempts to isolate this vitamin. This fraction has been shown to contain carbon, hydrogen, and oxygen, but no nitrogen, phosphorus, iodine or other halogens [Takahashi, 1922; Drummond, Channon and Coward, 1925]. The absence of iodine is of particular interest since at one time the suggestion was put forward that the therapeutic action of cod-liver oil was due to traces of this element [Kopp, 1836, 1, 2].

Takahashi and his colleagues [1922; 1923; 1925] made extensive studies of these active concentrates and by fractional distillation under reduced pressure isolated material which they regarded as the pure vitamin [1925]. This material, termed by them 'biosterin', was described as a reddish-yellow transparent viscous oily substance, and was assigned the formula $C_{27}H_{46}O_2$. The suggestion that this substance was an unsaturated alcohol related to the sterols was advanced by them.

Drummond, Channon and Coward [1925], following up earlier work [Drummond and Coward, 1924] on the chemical nature of the cholesterol-free unsaponifiable fraction of cod-liver oil, were able to show that the claim of Takahashi to have isolated the vitamin was unfounded. They succeeded in effecting some degree of concentration by distillation in a high vacuum, but the most active fraction, of the same order of activity as 'biosterin', proved to be a complex mixture containing, amongst other substances, the unsaturated hydrocarbon squalene, and a solid saturated alcohol. These two substances were found to be devoid of growth-promoting activity. Since the remainder of the most active fractions appeared to consist largely of a complex mixture of unsaturated alcohols, physiological tests on oleyl alcohol, selachyl alcohol, and phytol were carried out. All these substances were inactive. The inactivity of phytol was also demonstrated by Javillier, Baude and Lévy-Lajeunesse [1925].

A further unsuccessful effort to isolate vitamin A from liver oil was made by Drummond and Baker [1929], using the colour test (p. 37) as criterion almost exclusively. Attempts to concentrate the cholesterol-free unsaponifiable fractions of both cod-liver oil and sheep-liver fat by distillation at low pressures met with no success, since the extensive decomposition which occurred led to considerable losses

in the total vitamin activity. On the other hand, distillation of the unsaponifiable matter of shark-liver oils yielded certain fractions possessing a higher activity than the original material. The most active fractions were found to consist very largely (90–95 per cent. or more) of selachyl, batyl, chimyl, and oleyl alcohols, each of which was physiologically inactive. The vitamin was probably present only to the extent of less than 1 per cent. in such unsaponifiable fractions, and the difficulties in the way of the isolation of vitamin A from these sources would appear to be great.

The physiological activity of vitamin A preparations does not appear to be impaired by chemical treatments which involve reactions with hydroxyl groups, e.g. acetylation, benzoylation, etc. On the other hand, processes which affect double bonds, e.g. oxidation, hydrogenation, bromination, lead to complete destruction of the activity [Drummond, Channon and Coward, 1925].

Karrer and his co-workers [Karrer, Morf and Schöpp, 1931, 1, 2; Karrer *et al.*, 1931] have confirmed the observation first made by Rosenheim and Webster [1927, 6], that the liver oils of certain fishes, such as the halibut, are much richer sources of vitamin A than cod-liver oil. They used livers of halibut (*Hippoglossus*) and of mackerel as starting materials, and the colour reaction with $SbCl_3$ was relied on for controlling the progress of purification. This consisted mainly in saponification of the liver oil and removal of the sterols by cooling to –60° C. a methyl alcoholic solution of the unsaponifiable portion. The final purification was effected by fractional adsorption on fibrous alumina and furnished a faintly yellow viscous oil, which, it is claimed, contains at least 50 per cent. of vitamin A. The oil (obtained from halibut) had a colour value of 10,000 (cod-liver oil = 1) and a biological activity of 0·5γ (i.e. the smallest amount producing distinct increase in growth). It still contained about 5 per cent. of vitamin D. A molecular weight estimation in camphor (Rast) gave mol. wt. 320, a figure in agreement with the mol. wt. 333 calculated by Bruins, Overhoff and Wolff [1931] on the basis of diffusion experiments and colour tests.

The oil obtained from the unsaponifiable fraction of the liver oil of mackerel had similar properties to that from halibut, but was free from vitamin D (vitamin A activity not stated). Its elementary analysis gave figures agreeing with the formula $C_{20}H_{30}O$ (or $C_{22}H_{32}O$) and it yielded uncrystallizable esters with acetic and *p*-nitrobenzoic acids, the elementary composition of which again agreed with the above formula. On ozonization both preparations furnished geronic acid.

On the basis of this work Karrer, Morf and Schöpp [1931, 1, 3] make the interesting suggestion that both vitamin A and carotene contain the ring-system of β-ionone, since geronic acid is obtained by similar treatment from both β-ionone and carotene. A constitutional formula containing the β-ionone ring-system with an aliphatic side-chain, similar to that present in carotene, is tentatively advanced.

Heilbron, Heslop, Morton, Drummond and Rea [1932] have obtained results which in many respects confirm those of Karrer and his co-workers. Specially selected halibut oils were used and the extraction was followed spectroscopically. The final stages of the

concentration were effected by fractional vacuum distillation at pressures below 0·00001 mm. The product resembled that described by Karrer, namely a poly-ene alcohol, of molecular weight *circa* 300. The degree of unsaturation appears to correspond with 4 or 5 double bonds, whilst the carbon content (*circa* 82 per cent.) is lower than would be expected for $C_{20}H_{30}O$. The distilled preparations promote growth in rats in daily doses of 0·1γ.

The Colour Reactions and Absorption Spectrum attributed to Vitamin A.

Attention was drawn to the possible significance of the colour reactions of cod-liver oil when it was recognized by Rosenheim and Drummond [1920] that the purple colour developed with sulphuric acid is due to a chromogen which is also a normal constituent of all liver fats, whether derived from fish, bird, or mammal. The distribution and general properties of this chromogen, such as resistance to saponification, solubilities, lability towards oxygen, suggested to them a relationship with vitamin A [cf. Drummond and Watson, 1922]. The extremely unstable nature of the reaction, however, prevented its use for quantitative comparison of different oils.

The discovery that arsenic trichloride and similar reagents give with cod-liver oil, and other liver fats, a brilliant blue colour reaction, which is sufficiently stable for colorimetric purposes, paved the way for the quantitative application of this reaction [Rosenheim and Drummond, 1925]. It was found that the distribution of this chromogen is similar to that of vitamin A, whilst the positive reaction given by carotene strengthened the previous suggestions of a relationship between this pigment and vitamin A. There is an essential difference between the absorption spectra (see later) of the colour produced in the reaction with carotene and of that produced from the cod-liver oil chromogen, but the fact that the cod-liver oil chromogen appears in the livers of vitamin A-deficient rats after administration of carotene [Moore, 1930, 1931] provides additional evidence of the specificity of this reaction for vitamin A.

By substituting antimony trichloride for arsenic trichloride and using Lovibond's graded colour glasses, Carr and Price [1926] made it possible to measure accurately and to compare the colour value of different oils. The reaction is carried out by mixing chloroform solutions of the oil and the reagent. The rapid matching of the transient blue colour is greatly facilitated by the use of the colorimeter described by Rosenheim and Schuster [1927]. In order that different observers should obtain concordant results it is essential that standardized conditions should be adhered to. These have been defined by the Pharmacopoeia Commission [1931], as it was considered that this colour test might prove of value for indicating the degree of deterioration of pharmaceutical cod-liver oils. The Pharmacopoeia Commission deprecates the use of the term 'blue unit' in expressing the colour value of cod-liver oil and points out that 'the figures given to a glass on the Lovibond scale represent merely a grading in a series of glasses, and do not represent an amount of biological activity'.

The application of the test is limited, as pointed out originally by Rosenheim and Drummond [1925], to medicinal cod-liver oils and similar liver fats, which are usually only slightly pigmented. Since other carotenoids as well as carotene, such as xanthophyll, lycopin, and crocetin, give colours with $AsCl_3$ and $SbCl_3$ which are superficially similar to those given by cod-liver oil, the blue colour alone cannot be regarded as a specific test for vitamin A. The colour given by the yellow pigment carotene can, however, be differentiated from that of the colourless vitamin A contained in cod-liver oil by its specific absorption at 590 $m\mu$ [Dulière, Morton and Drummond, 1929].

Whilst the maximum intensity of the absorption band of the product of the $SbCl_3$ reaction of liver oils was stated to be at 614 $m\mu$ by Wokes [1928] and at 608 $m\mu$ by Drummond and Morton [1929], it has been found by Gillam and Morton [1931] that the blue colour is complex, and possesses two absorption bands with maxima at 572 $m\mu$ and 606 $m\mu$. The relationship of these bands to each other and to vitamin A remains undefined, although certain evidence (see later) suggests that the chromogen giving rise to the band at 572 $m\mu$ is probably related to vitamin A, and that the band at 606 $m\mu$ is due to the product of slow oxidation of another substance present in liver oils [Heilbron, Gillam and Morton, 1931].

Fish-liver oils show selective absorption in the ultra-violet region of the spectrum at about 320–328 $m\mu$ [Takahashi *et al.*, 1925; Schlutz and Morse, 1925; Heilbron, Kamm and Morton, 1927; Woodrow, 1928], which has been brought into relationship with vitamin A by Morton and Heilbron [1928]. Their results showed a striking parallel between the depth of the blue colour given by the $SbCl_3$ reagent and the intensity of the selective absorption at 328 $m\mu$ in various fish-liver oils, etc. Later work led these authors [Gillam and Morton, 1931; Heilbron and Morton, 1931] to the view that the substance responsible for the ultra-violet absorption band at 328 $m\mu$ is probably identical with the chromogen giving rise to the 572 $m\mu$ band in the $SbCl_3$ reaction.

Coward, Dyer, Morton and Gaddum [1931] found that the intensity of the colour produced by the action of antimony trichloride on the unsaponifiable fraction of an oil [see also Norris and Church, 1930, 1, 2, 3, 4; Smith and Hazley, 1930] was in better agreement with the result of their biological test for vitamin A than the intensity of the colour produced with the oil itself. It seems therefore advisable in all cases to make the colour test on the unsaponifiable fraction rather than on the fat itself. Tests based on measurement of the intensity of absorption of the oil at 328 $m\mu$ gave slightly better agreement with the results of the biological tests, but certain of the oils examined showed serious discrepancies between the results of the biological tests and both of these physical tests, so that the latter cannot yet be regarded as trustworthy measures of vitamin A. Until the specificity for vitamin A of the chemical and physical tests has been fully established by the isolation of vitamin A itself, biological evidence must obviously remain indispensable as final proof for the presence of vitamin A.

Other colour reactions. A red colour reaction with pyrogallol and trichloroacetic acid was considered characteristic for vitamin A by Fearon [1925; cf. Willimott and Moore, 1926; Euler, Myrbäck and

Karlson, 1926], but was found to be non-specific by Rosenheim and Webster [1926, 3, 4].

A blue colour reaction with $AsCl_3$, $SbCl_3$ etc., superficially resembling that shown by cod-liver oil with the same reagents, is given by 'oxycholesterol' [Rosenheim, 1927], a mixture of chemically undefined substances obtained by treatment of cholesterol with oxidizing agents [Lifschütz, 1913]. The reaction differs from that given by cod-liver oil in its absorption spectrum and other characters. When tested biologically, the product was found to be devoid of vitamin A activity [Rosenheim, 1927]. As Seel and Dannmeyer [1930; 1931] and Seel [1931] ascribed antixerophthalmic and growth-promoting properties to 'oxycholesterol', the subject was reinvestigated and the results showed that three different preparations of 'oxycholesterol', when tested in doses up to 1 mg. daily, failed to prevent the death of rats deprived of vitamin A or to cure xerophthalmia [Rosenheim, Hume and Crawford, unpublished results, 1931.]

The Relation of Vitamin A to Carotene.

The close parallel that exists between the vitamin A potency of many foods of vegetable origin and their content of yellow fat-soluble pigments has frequently been commented upon (see p. 45). Steenbock and his colleagues [1919; 1920, 1, 3; 1921] emphasized this relationship and suggested that the vitamin was identical with, or closely related to, one of these pigments. Similar views were expressed by Rosenheim and Drummond [1920] and by Coward [1923], who, working with the white and yellow petals of flowers, stated that 'some lipochrome—generally carotene—is always associated with the vitamin in plant tissues'.

Steenbock and Boutwell [1920, 3] showed that on fractionating the unsaponifiable matter of pigmented vegetable oils with light petroleum and aqueous alcohol, the vitamin A activity was associated with the carotene fraction, soluble in light petroleum, rather than with the xanthophyll fraction. Furthermore, Steenbock, Sell, Nelson and Buell [1921] claimed that carotene which had been recrystallized several times, when fed to animals on vitamin A-deficient diets, caused a resumption of growth. Steenbock suggested that vitamin A might be the leuco-compound of a lipochrome [1919]. Palmer and Eckles [1914, 1, 2, 3, 4, 5] and Palmer [1915] had previously shown that the plant carotenoids are the source of the so-called animal lipochromes. Since the animal organism was known to depend upon vegetable foods for its supplies of vitamin A, this fact seemed to supply additional support for the view that vitamin A was closely related to the carotenoids.

On the other hand, it was soon found that the parallel between vitamin A potency and yellow pigmentation did not extend to fats of animal origin. Steenbock, Sell and Buell [1921] found that although highly pigmented butters tended to possess a high vitamin activity, the relationship was not constant, and in the case of cod-liver oil the activity was vastly greater than would have been expected from the colour.

Palmer and his colleagues [1919, 1, 2, 3; 1921] were unable to agree with the views expressed by Steenbock and his co-workers, and by

experiments in which chickens and rats were successfully reared on diets free from carotenoids, but containing pig's liver, claimed to have demonstrated the non-identity of the vitamin with any of these pigments. No vitamin A potency was demonstrated in pure crystalline carotene by Drummond [1919, 2], Rosenheim and Drummond [1920], Stephenson [1920], or Drummond, Channon and Coward [1925], while Stephenson [1920] found that the yellow pigment could be removed from butter fat without causing loss of vitamin activity.

The weight of evidence against the theory that vitamin A was identical with one of the carotenoid pigments thus appeared overwhelming and for some years the theory was abandoned.

The feeding tests with pure carotene were carried out by the above-mentioned authors at a time before it was clearly understood that vitamins A and D were separate principles and that vitamin D itself was also necessary to maintain growth in young rats. Attention was drawn to this point by Euler, Euler and Hellström [1928]. These workers were struck by the fact that the colour reactions supposed to be due to vitamin A were also positive for carotene, as had already been pointed out by Rosenheim and Drummond [1925]. Euler and his colleagues therefore subjected crystalline carotene to a further feeding test, with the result that this pigment was found to supplement satisfactorily a vitamin A-deficient diet in doses of about 0·005 mg. per rat per day. These findings were confirmed in a later communication [Euler, Euler and Karrer, 1928]. Xanthophyll [cf. Willimott and Moore, 1927]; lycopin, α-crocetin, capsanthin, bixin, and norbixin were found to be inactive.[1] The suggestion was tentatively advanced by Euler and his colleagues that vitamin A activity was associated with a certain system of double bonds present in the molecules both of carotene and of the vitamin A contained in liver oil.

The vitamin A-like activity of crystalline carotene was shortly afterwards confirmed by Collison *et al.* [1929], and by Moore [1929, 1, 2], although the fact that pure carotene became decolorized by oxidation in ethyl oleate solution [Hume and Smedley-MacLean, 1930; Drummond, Ahmad and Morton, 1930], whilst impure specimens were more stable, led at first to some confusion [Dulière, Morton and Drummond, 1929].

Although it was apparent that the classical vitamin A of cod- and other fish-liver oils (nearly colourless) was not identical with carotene (orange-red), the possibility still remained that the activity of carotene might be due to the presence of traces of the former. Carotene is less potent than some liver-oil concentrates; if therefore the activity of the pigment were due to traces of the liver-oil vitamin, the absorption band at 328 $m\mu$ attributed to vitamin A might be visible in carotene solutions. Capper [1930, 1] was unable to detect this band in such solutions, although the absorption due to the pigment itself was insufficient to obscure it if present.

The blue colours given by carotene and the liver-oil vitamin A with antimony trichloride have been shown to differ appreciably. The colour given by carotene is characterized by an initial band at 590 $m\mu$ [Euler, Euler and Hellström, 1928; Dulière, Morton and Drummond, 1929; Moore, 1929, 2]. The blue colour due to the liver-oil vitamin on the

[1] The claim that dihydro-α-crocetin was active has not been sustained.

other hand shows a band at about 610 $m\mu$. Moore [1929, 3; 1930] has put forward evidence that carotene is converted into the 'liver-oil vitamin' *in vivo*. The rat proved to be an exceptionally suitable animal for this experiment, since as Palmer and Kennedy [1921] showed, this animal normally contains little carotene in its body. The liver fat of rats suffering from vitamin A deficiency was found [Rosenheim and Webster, 1926, 3] to give no blue colour with antimony trichloride but, after feeding with large amounts of purified carotene, the liver fat was found by Moore to show the characteristic absorption band at 328 $m\mu$, and to give an intense blue colour with antimony trichloride with a band at 610–630 $m\mu$. These findings were shortly afterwards confirmed by Capper [1930, 2] and by Drummond, Ahmad and Morton [1930].

These results suggest either that carotene is converted into vitamin A *in vivo*, or else that the growth-promoting potency both of the pigment and of liver-oil concentrates is due to the presence of traces of the same intensely active substance. In this connexion the possibility should not be lost sight of that photo-synthetical production of both vitamin A and carotene might be the cause of their association in the plant. The statement, based on spectroscopic observations only, has been made that carotene is converted in the liver into vitamin A by the agency of an enzyme, provisionally called carotenase [Olcott and McCann, 1931].

The relation of vitamin A to carotene is conveniently summarized in the following table:

Carotene	*Vitamin A of liver oils*
Synthesized in the plant.	Stored in animal.
Orange-red	Almost colourless.
328 $m\mu$ absorption band absent.	328 $m\mu$ absorption band present.
Greenish-blue $SbCl_3$ reaction showing absorption at 590 $m\mu$.	Vivid blue $SbCl_3$ reaction showing absorption at 572 and 606 $m\mu$.

It has been shown that 'carotene' from carrots and other sources is a mixture of isomerides of widely differing optical activity [Kuhn and Lederer, 1931, 1, 2; Karrer, Euler *et al.*, 1931; Rosenheim and Starling, 1931]. Specimens of carotene prepared by the usual method [Willstätter and Stoll, 1913; for details see Escher, 1909] from carrots or leaves of spinach, chestnut, nettle, etc., possess the same elementary composition, agreeing with the formula $C_{40}H_{56}$, but vary in their optical activity from $[\alpha]_{cd} < 5°$ to $+60°$. The dextrorotatory component of the mixture has been called α-carotene and is slightly more soluble than the optically inactive β-carotene. The existence of a laevorotatory form is indicated by the earlier work of Kohl [1902] and the recent observations of J. H. C. Smith [1931], and of Karrer *et al.* [1931].

The highest recorded rotations of α-carotene are $_{cl}[\alpha] + 380°$ [Kuhn and Lederer, 1931, 2] and $[\alpha]_{6075} + 458°$, the latter having been observed by Karrer and Morf [1913, 2] in a specimen prepared by Kuhn, presumably from carrots. Red palm oil appears to be a good source of α-carotene [Kuhn and Brockmann, 1931, 2], whilst carotene prepared from paprica [Zechmeister and Cholnoky, 1927] seems to consist mainly of β-carotene. The most easily accessible product from carrots is a mixture of α-carotene and β-carotene, containing from 75 to 90 per cent. of the latter.

The methods for the separation of 'carotene' into its constituents

are as yet far from satisfactory, and depend primarily on the relatively slight differences in solubility of the isomerides in such solvents as light petroleum, pyridine, etc. Fractional recrystallization is followed by fractional precipitation with iodine and removal of the latter with sodium thiosulphate. For the final purification of α-carotene fractional adsorption on suitable adsorbents (fibrous alumina) is essential.

On account of the difficulties of the separation process, it has not yet (December 1931) been ascertained whether α- and β-carotene have equal growth-promoting activity [See Karrer *et al.*, 1931; Kuhn and Brockmann, 1931, 1].

The Biological Estimation of Vitamin A.

The biological estimation of vitamin A is generally based on its growth-promoting property, though some American workers claim to have obtained good results by estimating its antixerophthalmic potency.

Any test which depends on the growth-promoting property of the vitamin under examination demands that (*a*) the basal diet should be uniformly free from that vitamin, and (*b*) all growth-promoting factors other than the one under test should be abundantly supplied. Much of the early work on the distribution of 'vitamin A' was done before there was proof of the separate existence in the fat-soluble complex of the antirachitic vitamin D. Steenbock and Nelson [1923] in the course of work on the differentiation of vitamins A and D recognized the growth-promoting action of the latter, and demonstrated the necessity of supplying it in the basal diet of all experiments dependent on the growth response of test animals. They suggested providing vitamin D in tests for vitamin A either by the irradiation of the test animals or by supplying aerated cod-liver oil which exhibited no antixerophthalmic potency; the exact technique of the method was described later [Steenbock, Nelson and Black, 1924–25]. Drummond, Coward and Handy [1925] proposed to administer irradiated cholesterol and Steenbock and Coward [1927] suggested the irradiation of the whole basal ration as a means of supplying this factor. Much of the earlier work on vitamin A has therefore been repeated with the tests modified so as to include adequate supplies of vitamin D in the basal diet and many of the early results have been found still to hold good. This is probably due either to the fact that vitamin D was being administered along with the vitamin A, as was the case with butter and cod-liver oil, or to the fact that the vitamin D reserves of the animals were large. On the other hand, some later work has given different results from the early work, which must presumably have been done on animals whose reserves of vitamin D were exhausted. A notable example of this is shown in the work on carotene, which was repeatedly found to be inactive when a basal diet was used which was devoid of vitamin D, but its activity is readily demonstrated when the basal diet contains vitamin D.

The necessity for an adequate ration of the vitamin B complex and for increasing this as the test animals get older is also stressed for all basal diets used in the estimation of vitamin A.

Technique of the Biological Test.

Standard of reference. Much work has been carried out with the aim of discovering for vitamin A (as for other vitamins) some stable substance which could be used as a standard of reference in estimations of this vitamin and in terms of which a unit could be defined. The International Conference on Vitamin Standards held by the Health Section of the League of Nations (London, June 1931) adopted as provisional standard of reference a sample of crystallized carotene, the unit being defined as the vitamin A activity of 1γ (0·001 mg.) (see Appendix II).

Experimental animals. Young rats immediately after weaning, of about 40 g. weight, are suitable. The breeding stock should be so fed that the young rats are not endowed with excessive reserves of vitamin A and, though healthy, cease to grow at about 100 g. weight when fed upon a vitamin A-free diet.

Experimental diets. Seeing that the estimations of vitamin A potency are based on a growth response, the basal diet should contain optimal amounts of all other essential constituents. The different basal diets employed usually consist of:

Caseinogen	15–20 per cent.
Starch (sometimes partially dextrinized) about	75 per cent.
Dried yeast or yeast extract, about	8 per cent.
Salt mixture—Osborne and Mendel's or McCollum's or Steenbock's, about	4 per cent.
A 'hardened' vegetable oil (vitamin A-free) about	15 per cent.

Coward, Key, Dyer and Morgan [1930; 1931] found that 'light white' caseinogen was more satisfactory than other preparations. Some workers consider the provision of fat in the basal diet unnecessary.

An adequate amount of vitamin D must also be given. About 4 units (International Standard, see p. 83) twice a week are required for each rat. The vitamin D may be given as irradiated ergosterol or by irradiation of the rat or of the oil or yeast contained in the diet.

Arrangement of the test. The test may be either prophylactic or curative. In the former, doses of the material to be tested are given either from the beginning of the feeding with the experimental diet or from about three weeks after, when the rat's reserves of vitamin A have been partially exhausted. The doses are given daily until consistent differences between negative controls and test animals are obtained. Comparisons are made between doses of different test materials (or of test material and standard) which cause equal increments in weight when the averages are compared for groups of about ten animals on each dose. The rats from each litter used must be so distributed among the groups that equal numbers occur in each group, and the sexes should be represented as equally as possible in each group. A test with the standard material should be included in each estimation.

In a curative test, the animals are allowed to exhaust their reserves of vitamin A, as indicated by cessation of growth, after which doses of the test substance are administered for about five weeks. In this test comparisons are also drawn between doses which give equal average increments in weight. Owing to the large margin of error

in estimations of this type it is often difficult to decide how closely these average increments in weight should coincide in order that the doses causing them may be considered equal in vitamin A potency. Some workers find it more satisfactory to determine the doses which just maintain weight in the test animals [Collison *et al.*, 1929].

An economy in animals and labour is said to be effected by the method described by Coward, Key, Dyer and Morgan [1930; 1931]. They have drawn up a 'curve of response' in which the mean increase in weight during five weeks of groups of rats, which had previously become steady in weight by deprivation of vitamin A, is related to the daily dose of a standard sample of cod-liver oil. The curve covers a range of daily doses from 0·25 mg. to 7·5 mg. and the most useful part of it is that dealing with a mean weight increase of about 20 g. in five weeks. The curve is used in the following way. About 10–12 animals similarly prepared receive the same daily dose of the test substance (e.g. 1 or 2 mg. of cod-liver oil, 0·1 g. butter) for five weeks. The mean increase in weight of members of the group is determined. The dose of the standard cod-liver oil which brought about the same increase is read from the curve, and the ratio of this to the dose of test substance measures the vitamin-A potency of the unknown substance relative to that of the standard oil. In this way substances may be compared either at the same time, or at different seasons of the year, by determining their potency in terms of that of the original oil.

If further research should confirm the general applicability of such reference curves to vitamin testing, it would obviously be necessary to construct such curves for the standard materials suggested for international adoption.

Until such are available and proved to be trustworthy it is preferable to retain the usual principle employed in the biological estimation of vitamins—that of equating the vitamin potency of doses of test material and standard which produce an equal biological effect.

The Distribution of Vitamin A in Naturally Occurring Foodstuffs.

The important question of the distribution of vitamin A among the commonly used foodstuffs of both vegetable and animal origin has for many years received much attention. Of necessity much of the work has been largely qualitative in nature, making anything but a rough comparison between such foodstuffs difficult. Many of the results obtained will be found in Table XXI, Appendix I.

In assessing the relative values of different foodstuffs as sources of vitamin A in human dietaries, it must be borne in mind that the real dietetic value of any particular foodstuff depends not only upon its vitamin-A content calculated on a weight basis, but also upon the amount which may with convenience be included in a normal diet. Thus milk and butter, while being relatively poor in vitamin A when compared on a weight basis with cod-liver oil, are nevertheless extremely valuable sources of this factor, owing to the prominent position which they occupy in a normal human diet.

Foodstuffs of Vegetable Origin.

Although it seems possible that the vitamin-A potency of vegetable tissues may be due *solely* to the presence of the yellow lipochrome pigment carotene (see p. 39), the fact has not been proved. The total amount of carotene present in a vegetable foodstuff may nevertheless be a valuable indication of its value as a source of vitamin A [Euler *et al.*, 1930]. The relationship between yellow pigmentation and vitamin-A content as determined by biological tests has been shown to hold for a variety of vegetable foodstuffs. Thus Steenbock and his collaborators [Steenbock, Boutwell and Kent, 1919; Steenbock and Boutwell, 1920, 1, 3] pointed out the marked superiority of yellow maize over white maize as a source of vitamin A. This observation has since been confirmed by several workers, notably Hauge and Trost [1928; 1930], Hauge [1930] and W. C. Russell [1930]. Hauge has pointed out that the vitamin-A value of maize is related to the degree of yellow pigmentation in the endosperm and bears no relationship to the colour of the pericarp. The superior vitamin-A content of the outer green leaves of lettuce to that of the inner white ones [Dye, Medlock and Crist, 1927], can, in a similar manner, be correlated with the larger amounts of carotene which are contained in leaves in which photosynthesis is active. Crist and Dye [1929] have shown the same relationship to hold between green and bleached asparagus.

Green vegetables are rich sources of vitamin A owing to their high carotene content. Spinach, cabbage (green outer leaves), lettuce, Brussels sprouts, green peas, the French artichoke [Morgan and Stephenson, 1923], and watercress [Coward and Eggleton, 1928], all contain large quantities of this factor. Among root vegetables, carrots, and yellow sweet potatoes are rich in vitamin A, while white potatoes, turnips, onions, parsnips, mangolds, radishes, and beetroot contain little or none.

Some fruits, such as the tomato, banana, and date, contain amounts of this factor comparable with those present in many green vegetables. Morgan and Smith [1928–29] have shown that much greater amounts are present in the ripe tomato than in the green fruit. The tropical fruit papaya is reported to be exceptionally rich in vitamin A [H. G. Miller, 1926–27] and the mango contains about as much as an average sample of butter [Perry and Zilva, 1932]. Apples, figs, oranges, lemons, grapefruit, pineapple, and peaches contain small but appreciable amounts. Morgan [1923] has shown that the oil of orange peel contains vitamin A, whereas the oils from lemon and grapefruit peel are inactive.

Cereals are in general rather poor in this factor, but, as has already been mentioned, yellow maize contains appreciable amounts. Nuts, while containing large amounts of fat, are usually poor sources of the vitamin [Coward and Drummond, 1920]; Rose and MacLeod [1921–22], however, reported fairly large amounts in almonds. Many vegetable oils such as arachis oil, linseed oil, cotton-seed oil, olive oil, and coco-nut oil contain little or no vitamin A; red palm oil and some samples of maize oil [Stammers, 1924, 1] are rich sources.

Foodstuffs of Animal Origin.

Animal organisms possess the power of storing reserves of vitamin A which have been obtained directly or indirectly from vegetable sources. The amounts of vitamin present in the different tissues of an animal will therefore depend not only upon the power of that tissue to store reserves, but upon the previous dietary history of the animal. Thus the amounts of reserve vitamin A stored in the tissues of a grass-fed animal are usually much higher than those stored in the tissues of a stall-fed animal. The importance of this fact in the consideration of the vitamin A content of milk and butter is obvious (see p. 219).

By far the greater proportion of the reserve vitamin A in the animal body is contained in the liver. Sherman and Boynton [1925] studied the distribution of vitamin A among the tissues of the rat. It was shown that the lung and kidney contained more than forty times, and the liver two hundred to four hundred times, that present in the muscle. Significant quantities were also shown to be present in the blood. As might be expected therefore, the liver fats of grass-fed animals, such as the sheep and ox, are among the richest sources of vitamin A known, being often much richer than cod-liver oil [Rosenheim and Webster, 1927, 6].

Cod-liver oil. Osborne and Mendel [1914] were the first to show that cod-liver oil, the therapeutic value of which had long been known, contained what may now be termed the 'fat-soluble complex'. A few years later Zilva and Miura [1921, 2] in the course of the elaboration of their quantitative method demonstrated the extremely high vitamin content of cod-liver oil, 2·2 mg. being sufficient to induce growth in rats previously deprived of 'vitamin A', while over 200 times the quantity of butter was required to produce the same effect. They therefore concluded that the high therapeutic value of cod-liver oil was due to its high vitamin content. Other fish-liver oils were found to be as potent as cod-liver oil [Zilva and Drummond, 1921]; examples are the liver oils of the herring, salmon, hake, pollock, shark, halibut, and haddock.

It is important to realize that different samples of cod-liver oil may differ by as much as 30-fold in their vitamin-A potency [Coward, Dyer, Morton and Gaddum, 1931]. This may be due partly to a difference in the potency of the original oil [Zilva and Drummond, 1922] and partly to the effect of storage. Zilva, Drummond and Graham [1924] found that neither the sexual condition nor the age of the fish was responsible for the variable potency of cod-liver oil, and they suggested that possibly variation in the food might be the cause of this.

It had already been shown by E. Mellanby [1918–19, 1; 1921] that cod-liver oil was rich in the antirachitic vitamin.

That the ultimate origin of the fat-soluble complex present in the liver of the cod was probably to be found in the marine algae which are capable of effecting a synthesis of this factor was suggested by the researches of Drummond, Zilva and Coward [1922]. In this connexion it has been shown by Ahmad [1930] following up earlier work by Jameson, Drummond and Coward [1922] that a marine diatom (*Nitzschia closterium*) can synthesize vitamin A when grown under

artificial conditions. The whole of the activity present in the diatom could be accounted for in the supposition that 3 per cent. of the yellow pigment present in the ether-soluble material obtained was carotene. Ahmad suggests that such marine diatoms are the ultimate sources of the vitamin A in fish-liver oils, the carotene which they synthesize being converted into vitamin A in the body of the fish which feed upon them. No vitamin D however was detected in *N. closterium* (see p. 87).

In view of the widespread use of cod-liver oil medicinally, the question of the effect of the different methods of preparation and refining on the vitamin content is of considerable importance. Work bearing on this problem has been carried out notably by Holmes and by Drummond and Zilva. The monograph by Drummond and Hilditch [1930] summarizes much of the more important work done in this direction and presents a large amount of new and valuable data.

It has been known for some years that exposure of cod-liver oil to light results in a loss of its vitamin-A activity [Peacock, 1926]. The ease with which vitamin A is destroyed by oxidation has already been pointed out in preceding paragraphs. Since, therefore, oils such as cod-liver oil contain a large number of autoxidizable substances (e.g. the unsaturated fatty acids), it might be expected that loss of vitamin would occur in such oils on exposure to the air. The problem of the storage of cod-liver oil for medicinal use, under conditions which will ensure a minimum destruction of vitamin A by light and oxidation, is a question of great practical importance. The matter has been investigated by Drummond and Hilditch [1930]. Experiments in which various bottled samples of cod-liver oil were stored in the dark or exposed to the light, clearly showed the necessity of storing such oils in dark coloured containers or in the dark.

Huston, Lightbody and Ball [1928] have extended the work of Moureu and Dufraisse [1928] on antioxidants to the question of retarding the destruction of vitamin A in milk fat and cod-liver oil. Loss of vitamin activity was decreased by the addition of relatively high concentrations (0·05–0·1 per cent.) of quinol (hydroquinone). Drummond and Hilditch have studied the effects of different amounts of quinol on the oxygen uptake of cod-liver oil, with the result that although the oxygen uptake is lessened, 'amounts of hydroquinone that might be regarded as practicable on the industrial scale are of little or no value in protecting the oil from oxidation'.

CHAPTER III

VITAMIN D (THE ANTIRACHITIC, CALCIFYING VITAMIN).

In the previous chapter vitamin D has been described as a fat-soluble substance having the specific function of controlling the deposition of calcium and phosphorus in tissues. Since bones and teeth are largely composed of calcium phosphate it is evident that the structure of these tissues is largely determined by the adequacy of the vitamin D supplied to the body during their period of development Although these are the tissues most prominently influenced by this factor it is certain that calcium-phosphorus metabolism is a process affecting other organs of the body, and to this extent, therefore, these organs are influenced by vitamin D. Knowledge on this point is still, however, meagre. Like vitamin A, vitamin D can also be regarded as necessary for growth (Steenbock), since young animals deprived of it ultimately cease to increase in weight but resume growth on its addition to the food. This property of growth-promotion is also probably related to the regulation of calcium-phosphorus metabolism by vitamin D.

Before considering the properties and function of vitamin D in greater detail two points of general interest may be stated.

(1) The relation of vitamin D to calcium and phosphorus metabolism is the only instance in the present knowledge of vitamins where it is possible to state the function of a vitamin in terms of chemistry. This, of course, does not mean that we know how the vitamin acts, but the biological problem can be presented in terms of known chemical entities.

(2) The extent of defective bone formation, as seen in the high incidence of rickets in countries in the temperate zone, the widespread defect in the structure of teeth and the high incidence of dental caries suggest that a deficient intake of vitamin D may be the most important dietetic defect at present known.

Although our knowledge of the chemistry of vitamin D has in recent years been greatly increased, all fundamental studies of this substance are still dependent on its biological properties and the only methods of estimation at present available are biological. In the present chapter, therefore, the part played by this vitamin in the animal body is first described and its chemical and physical properties are dealt with later.

Biology of Vitamin D.

Vitamin D in relation to Rickets and Allied Disorders.

While some bone defects develop independently of the vitamin D intake, those most commonly found in man, namely rickets, osteoporosis, and osteomalacia, are directly related to this vitamin. These bone conditions are alike evidence of an abnormal calcium-phosphorus metabolism and this fact explains many of the difficulties which faced the earlier workers interested in the antirachitic vitamin. It is true

FIG. 3. Children 6 years of age showing severe rachitic deformities compared with normally grown child (centre) of the same age.

Vienna, 1920. Amerikanische Kinderheilstätte.

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that there is still much to be learnt as to the factors which determine the development of one or other of these bone defects apart from the deficient supply of vitamin D. It is known, however, that the type of animal under investigation, the rate of growth, the age and the composition of the defective diet all influence the form taken by the defects in calcification of the bones. Some of these subsidiary influences will be described more fully in later sections.

The Diagnosis of Rickets.

Simple clinical examination will often enable a diagnosis of human rickets to be made when a combination of such signs as beading of the ribs, deformity of the limbs, head, and chest wall, and enlargement of the epiphyses is present (see Fig. 3). But even in such cases it may not be possible to decide at once whether the changes found in the bones are due to defective calcification still proceeding (active rickets) or are the result of such a process which was formerly active but has now ceased (healed rickets). The deformities in the bones may long outlast the period of active rickets as shown radiographically. In such cases radiography is of great service and usually enables a satisfactory diagnosis of the state of activity of the disease to be made. Eliot [1925] and M. G. Wilson [1926] consider that the very earliest stages of rickets can be recognized in radiograms of the infant's forearms long before any clinical symptoms are apparent. In their experience radiographical evidence of very slight rickety changes in the bones may be recognized even in the majority of apparently healthy infants, though these changes do not progress if measures are taken to provide some source of the antirachitic factor for the infants.

Estimations of the concentration of inorganic phosphorus in the blood have been employed, particularly in the United States, both as a means of diagnosing active rickets and as an indication of the progress of the disease following treatment. Although in general the blood-phosphorus is low in clinical as in experimental rickets and is raised towards the normal figure shortly after the institution of therapeutic measures, several workers have expressed doubt as to the reliability of blood-phosphorus estimations as an indication of the healing of rickets. Koch and Cahan [1927] obtained perfect calcification of bones in rats which showed persistently low blood-phosphorus values. Hess, Weinstock, Rivkin and Gross [1930] found that giving very small doses of irradiated ergosterol to rats raised their inorganic phosphorus to a normal level without healing the rickets. On the other hand, small doses of cod-liver oil might cause rickets to heal in rats without raising the inorganic phosphorus of their blood. These observers occasionally saw cases of active rickets in infants in which there was a high level of inorganic phosphorus in the blood, and consider this test not infallible, although at times useful, in helping to establish a diagnosis.

An attempt was made by Redman [1928] to apply to children suffering from rickets the test originally described by Zucker and Matzner [1923–24], namely, the determination of the reaction (pH) of the faeces, which in dogs and rats is changed from alkaline to acid when active rickets is controlled by any therapeutic measure [Jephcott and Bacharach, 1926; Grayzel and Miller, 1928]. The results

in children, however, were too variable to give the test any practical value.

For all ordinary purposes clinical examination supplemented by radiography suffices to establish the diagnosis, and a series of radiographs taken of any individual at regular intervals should enable a sufficiently accurate estimate to be made of the efficacy of any particular treatment adopted. (See Fig. 6.)

Experimental Rickets and Osteoporosis in Animals.

Most of our knowledge of the aetiology of rickets and allied disorders has developed as the result of the application of the experimental method to animals. In some, e.g. the dog, rickets is a naturally occurring and commonly found disease just as it is in children, and the deformities produced are of the same kind (see Figs. 4 and 5). In others, for instance the rat and the rabbit, the disease is probably unknown in nature but can be easily produced under laboratory conditions.

The detection of these diseases in animals and the determination of their degree of intensity depend upon a technique similar to that used in the case of human beings. Whereas, however, accurate clinical examination in children is usually restricted for obvious reasons to radiography, histological and chemical examinations of the bones are also greatly used in experimental work on animals. In animal experiments each of these methods has its own particular value. For instance, the radiographic method will at once reveal the presence of rickets by the characteristic changes at the epiphyseal ends of the growing bones. This mode of examination has the merit of being possible during the life of the animal and may be repeated at will. Slight degrees of osteoporosis may, however, be undetected by this method.

Histological diagnosis involves the preparation of the bones for microscopic examination, but allows the determination of both rickets and osteoporosis and their differentiation. In rickets the abundant uncalcified osteoid tissues, present at the epiphyses in varying amount according to the intensity and duration of the disease, can be distinguished from the normally calcified bone. In osteoporosis, a condition in which there is absorption of old bone and defective formation of new bone, the bony trabeculae and periosteal bone are small in amount and osteoid tissue may be deficient or absent. Often rickets and osteoporosis are present in the same bone.

The chemical examination of bone in these diseases in animals has the advantage of giving a numerical answer as regards the degree of calcification, but does not allow any differentiation between rickets and osteoporosis.

Further consideration of these methods will be given in a later section (p. 84) where methods of determining vitamin D are discussed.

The Aetiology of Rickets.

Prior to the experimental work on puppies [E. Mellanby, 1918–19, 1, 2; 1919, 1920, 1921, etc.] in which it was shown that the antirachitic vitamin was the corner stone of the aetiology of rickets, many

FIG. 4. Photograph of two puppies after being fed for 18 weeks after weaning on the same diet deficient in vitamins A and D. Puppy 1 (left) was the offspring of a mother fed during pregnancy and lactation on a diet rich in A and D and containing bread as the cereal, whereas the mother of puppy 2 (right) was fed on a diet deficient in these vitamins and having oatmeal as the cereal. It will be noted that puppy 1 shows little sign of rickets, while puppy 2 has developed severe rickets.

FIG. 5. Rickets following a diet of 175 c.c. whole milk, white bread ad lib., and 10 c.c. linseed oil per diem. Time of experiment, $5\frac{1}{2}$ months. Increase in weight during period of experiment 2,670 g.

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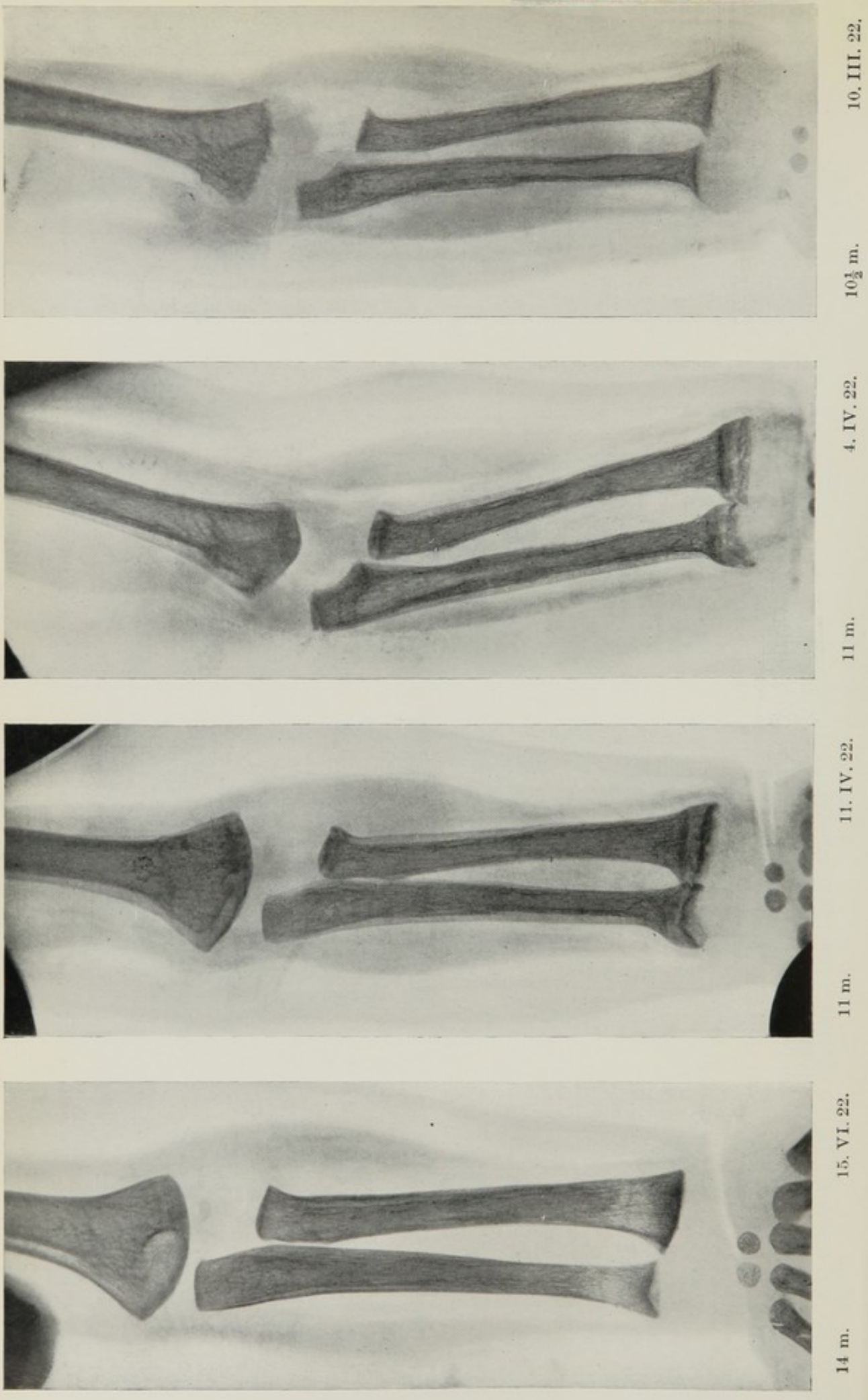

FIG. 6.

(Reproduced by permission from Special Report Series, N

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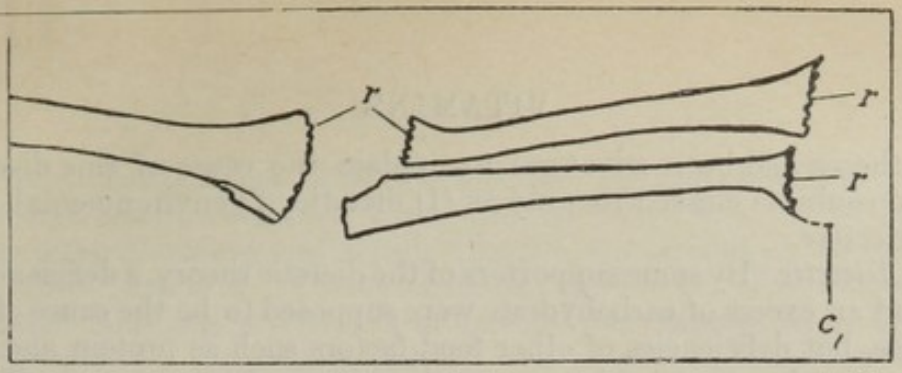
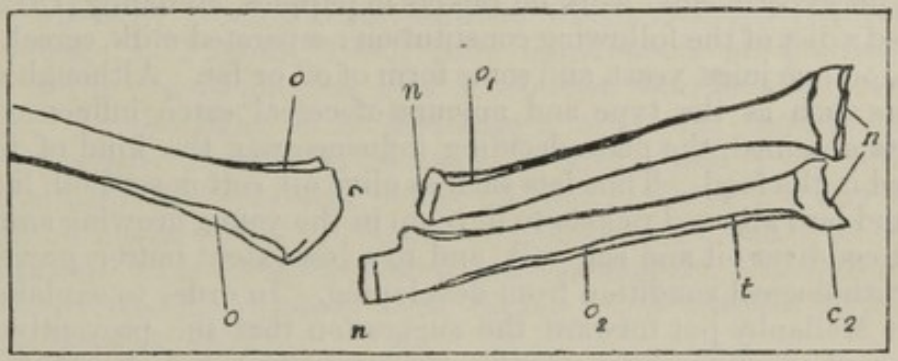
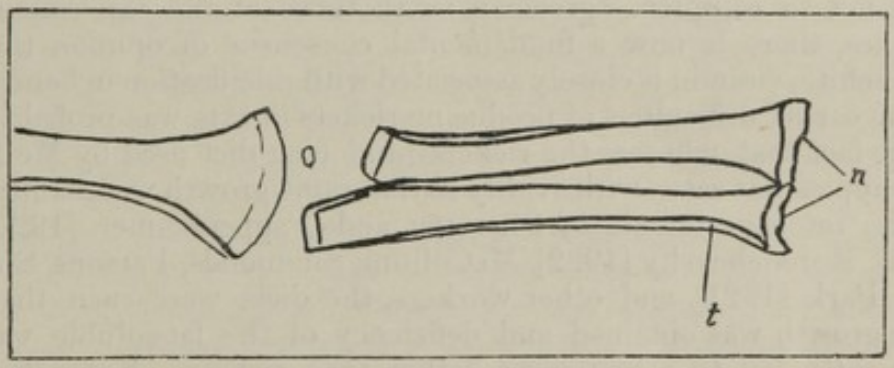


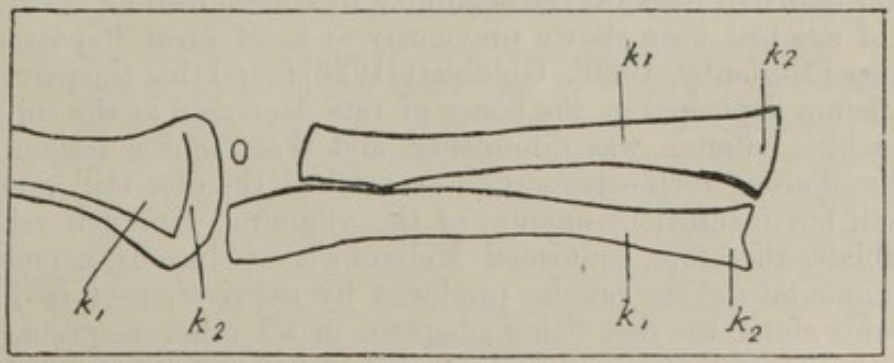
1. (10.3.22.) Florid rickets, no evidence of healing. Osteoporosis present. The structure of the bones is coarse, and the compact bone of the shafts ill-defined and irregular. The metaphyseal margins are frayed (r). The distal end of the ulna is slightly cupped, and there is a faint shadow indicating the margin of the expanded, uncalcified zone of osteoid tissue (c_1) which can be detected clinically as an enlargement.



2. (4.4.22.) Healing in progress after 27 days' treatment, showing (1) the deposition of calcium along the shafts of radius (o_1), ulna (o_2), and humerus (o); (2) calcium deposition at the metaphyseal margins and in the osteoid zones previously uncalcified and, therefore, scarcely visible (c_2). These areas are bounded on the side towards the epiphysis by irregular lines of shadow which represent the newly formed zone of preparatory calcification (n).



3. (11.4.22.) Rapid progress in healing. Note (1) the line of dense calcification at the metaphyseal margins (n) corresponding to the new preparatory calcification zone, and (2) the increase in periosteal calcification (osteophyte layer) showing calcified trabeculae (t) running at right angles to the long axis of the layer, (3) calcification of the centre of ossification at the distal end of the humerus.



4. (15.6.22.) Healing well advanced after 3 months' treatment. The contour of the shafts is nearly normal, and the coarse structure has become more finely meshed. A band about 0·5 cm. wide of newly formed bone (k_2) is clearly visible at the distal end of the humerus. At the distal end of the radius and ulna the corresponding band is also to be seen. It can be distinguished by its fine homogeneous structure from the portion of the shaft (k_1) formed before the onset of rickets.

showing X-ray photographs illustrating severe
recovery after treatment with cod-liver oil.

hypotheses had been advanced to explain the cause of this disease. These could be classed roughly as (1) dietetic, (2) environmental, and (3) infective.

1. *Dietetic.* By some supporters of the dietetic theory, a deficiency of fat and an excess of carbohydrate were supposed to be the cause of the disease, but deficiencies of other food factors such as protein and calcium were also regarded by some authorities as of importance. These views were mainly based on clinical observation; the small amount of experimental evidence available seemed to point against the dietetic explanation [L. Findlay, 1908].

In his experimental work on rickets in puppies, Mellanby [1918–19, 2] used a diet of the following constitution: separated milk, cereal, lean meat, orange juice, yeast, and some form of oil or fat. Although other factors such as the type and amount of cereal eaten influenced the results obtained, the main deciding influence was the kind of fat included in the food. Thus fats such as olive oil, cotton-seed oil, linseed oil, and lard allowed rickets to develop in the young growing animals, while cod-liver oil and egg yolk, and to a less extent butter, prevented the pathological condition from developing. In order to explain this result Mellanby put forward the suggestion that the preventive fats contained an antirachitic vitamin, the properties and distribution of which appeared at the time to correspond fairly closely with those then recognized for vitamin A.

Some years later [1921] experimental work on rickets was extended by other workers to rats, and although the results obtained at first were not in complete agreement with those of the experiments on puppies, there is now a fundamental consensus of opinion that an antirachitic vitamin is closely associated with calcification in bone. One of the earlier difficulties of producing rickets in rats was probably due to the fact that, whereas the rickets-producing diet used by Mellanby for puppies was eaten with avidity so that rapid growth was maintained, in the rat experiments of Sherman and Pappenheimer [1920–21; 1921], Korenchevsky [1922], McCollum, Simmonds, Parsons, Shipley and Park [1921], and other workers, the diets were such that but poor growth was obtained and deficiency of the fat-soluble vitamin frequently led to osteoporosis rather than rickets. Korenchevsky, however, obtained the definite result [1922] that deprivation of fat-soluble vitamins alone, the diet containing adequate amounts of all other elements, produced rickets in rats provided the age was not greater than 3–6 weeks at the beginning of special feeding. The question of age had been shown previously to be of great importance in puppies [Mellanby, 1920]. Goldblatt [1923] found that the percentage of calcium deposited in the bones of rats decreased as the supply of antirachitic vitamin was diminished, and that rachitic lesions were produced along with osteoporosis even when the diet still contained a small but insufficient amount of the vitamin. Korenchevsky and Goldblatt, therefore, confirmed Mellanby's conclusions, namely that experimental rickets can be produced by deprivation of fat-soluble vitamins alone, the diet being adequate in all other respects. It is clear, however, that in order to produce rickets in rats with certainty it is usually necessary to alter the calcium-phosphorus ratio as well as to exclude the antirachitic vitamin (see p. 59). In puppies

Mellanby [1921] found that although the calcium-phosphorus ratio influenced the development of rickets, this effect was not of significance when abundant antirachitic vitamin was present in the diet.

2. *Environmental.* The effect on the production of rickets of bad hygienic conditions and of lack of exercise was emphasized by Lataste [1884] and by Hanseman [1906] who developed the Domestication Theory of Rickets. This view arose mainly from observations on the common occurrence of rickets in wild animals confined in cages. It is a matter of interest, however, to note that Bland-Sutton [1889] had succeeded in raising lion cubs in the Zoological Gardens free from rickets by feeding them on diets which included cod-liver oil and bones. L. Findlay [1908] from experiments on puppies also came to the conclusion that lack of exercise was the main cause of rickets. In the light of E. Mellanby's work this conception was re-examined by Findlay in conjunction with Paton, from both the experimental and statistical points of view. These workers found puppies brought up in the country and properly exercised to be free from rickets, whereas others reared in the laboratory, although receiving a diet containing much more butter fat, developed the disease [see L. Findlay, 1922].

Fergusson and L. Findlay [1918] made an inquiry into the dietaries and home conditions of rachitic and non-rachitic families in Glasgow and drew the conclusion that there was little difference in the diets of the two classes, but that the onset of rickets was favoured by imperfect maternal care, by improper housing, and by the absence of facilities for open-air life, the child being thus deprived of the desire to exercise its muscular system. A more careful scrutiny of the diets on the basis of Mellanby's work with puppies showed, however, that the diets of the rachitic families were such as might be expected to result in more rickets than those of the non-rachitic families [E. Mellanby, 1918–19, 2; 1919].

Observations made in India by Hutchison and Shah [1922] supported the 'confinement' hypothesis and dealt with the incidence of the disease in India among the Mahomedans and Hindus of the labouring classes, as compared with those of higher caste who adopt the practice of 'purdah'. The higher caste women marry when twelve years old and thereafter are confined to houses which admit but a minimum of light and fresh air. Their diet was found better than that of the labouring inhabitants in that it contained more milk, eggs, and butter, but, in spite of this, rickets was much more commonly found in the children, and late rickets in the women, of the higher caste.

The Glasgow School thus held the view that rickets was due primarily to confinement and lack of exercise and that diet was of little or no significance. Mellanby [1920] however, in experiments devised to test the point, found that confined puppies did not develop rickets on an adequate diet, whereas those allowed complete freedom on a defective diet acquired the disease: nor did confinement prevent the cure of the disease when suitable diets were administered containing, among other things, substances rich in the antirachitic vitamin such as cod-liver oil, milk, and egg yolk. Mellanby concluded that exercise played a subsidiary part in the aetiology of rickets and that diet was of prime importance.

During this controversy as to the relative importance in the aetiology of rickets of the antirachitic vitamin and of lack of exercise, important evidence from other quarters was accumulating which finally settled the issue.

As long ago as 1890 Palm [1890] drew attention to the relation between the geographical distribution of rickets and the incidence of sunlight. The significance of these observations remained for long unrealized, and interest was centred on the alternative views that rickets arose from either faulty diet or unsuitable environment. It gradually became clear, however, from the results of more recent work that a lack of sunlight was the dominating factor in the environmental conditions conducive to rickets. Huldschinsky [1919; 1920] and other workers found that infantile rickets could be cured by exposure of the patient to the ultra-violet rays of the mercury-vapour quartz lamp, whilst Hess and Unger [1921, 1, 2,] showed that a similar result was obtained by exposure to sunlight. The latter workers have pointed out that the seasonal incidence of this disease is in all probability closely related to the prevalence and active power of sunlight, the great majority of cases developing between November and May. The extended investigations of Chick and her colleagues in Vienna [1922; 1923] afforded conclusive evidence of the therapeutic value of sunlight and ultra-violet rays in infantile rickets.

By 1922, therefore, it was established that both the dietetic and environmental hypotheses were required to elucidate the aetiology of rickets and that the specific factors influencing this disease were (1) the antirachitic vitamin supplied in the food and (2) the action of sunlight and ultra-violet radiation on the skin. The extraordinary story describing the correlation of these two effects is related elsewhere (p. 74).

3. *Infective.* The hypothesis that rickets is due to an infection was advanced by many workers including Morpurgo [1902; 1907], J. Koch [1912], Marfan [1911], Paton [1920], and Bull [1918] and received wide support from clinicians. In recent years the idea has been discarded, although it is probably true that after suffering from an infection children are more prone to rickets, and also that rachitic children are often more susceptible to infection.

Deficiency of Vitamin D in the Diet.

Since the methods that have been shown to prevent or cure rickets in experimental animals are equally efficacious in human rickets, it is highly probable that vitamin D plays the same part in the calcification of bone in the human subject as in experimental animals. It has long been observed that infants fed at the breast are much less liable to contract rickets than those fed on cow's milk, and it would therefore be expected that human milk would contain a greater amount of vitamin D than cow's milk. Hess, Weinstock and Sherman [1927], and later Outhouse, Macy and Brekke [1928], were not able to demonstrate by rat-feeding tests that this was true. No satisfactory explanation of this discrepancy has been forthcoming. It is well known that breast-feeding itself is by no means a certain safeguard against the development of rickets, whereas giving vitamin D to infants affords a much more reliable degree of protection.

The vitamin D content of cow's milk is a variable quantity, the antirachitic potency depending on the environment and diet of the cow. In summer the potency of the milk is influenced by the seasonal effect of sunlight and in winter the vitamin D content can be raised considerably by including cod-liver oil or irradiated yeast in the diet of the animal (see p. 220).

The known poverty of some samples of cow's milk in respect of vitamin D may well be a factor in determining the incidence of human rickets. Milk is at no time a rich source of this vitamin and skim milk contains mere traces of it. The fact that rickets is common in some districts among infants whose diet consists largely of cow's milk is therefore compatible with the view that the absence of a sufficient supply of vitamin D is responsible for the occurrence of human rickets.

The only natural products which are known to be rich in vitamin D are egg yolk and cod-liver oil and other similar fish oils. Cream and butter contain small amounts and certain animal fats, such as beef suet, have a definite antirachitic effect on laboratory animals. Fresh vegetables, fruit, and meat have no appreciable antirachitic activity, and cereals, as will be seen later, may actually favour the development of rickets. Children who, on account of poverty or for any other reason, cannot be supplied with adequate amounts of dairy produce, including eggs, and consume vegetable fats (in the form of margarine) containing no vitamin D, in place of animal fats, are obviously liable to suffer from a deficiency of this vitamin unless its supply is ensured in some special way as by administration of cod-liver oil or by exposure to natural or artificial ultra-violet radiation.

Relation of Rickets to Sunlight and other sources of Ultra-violet Rays.

It is an interesting fact that the stimulating effect of ultra-violet rays on calcification was first discovered in the case of children (p. 54), and that only at a later stage were the observations confirmed by investigations made on animals.

Using a diet upon which young rats in the laboratory invariably contract rickets, Hess, Unger and Pappenheimer [1921–22, 1] kept six rats in complete darkness and found that they all contracted rickets in 22–34 days. Seven other rats, on the same diet, exposed daily to sunlight in the open air for 15–20 minutes, but otherwise kept under the same conditions, failed to develop rickets in the same period. These investigators also observed [1921–22, 2, 3, 4] that the curative power of the sunlight was lost when it passed through a flint glass window, and that the rays from the mercury quartz lamp and the carbon arc lamp were also effective. When the time of exposure was reduced to the minimum sufficient to protect white rats, black rats were not protected, probably owing to the greater absorption of the rays by the pigment. This suggests that a similar function of the pigment in negro infants may be a factor in the abnormal susceptibility of these children to rickets in northern climates.

Similar results were simultaneously obtained by Powers and his colleagues [Shipley, Park, Powers *et al.*, 1921–22; Powers, Park, Shipley, McCollum and Simmonds, 1922, where a detailed historical

account of the discovery of the action of light waves in rickets is given] with a diet containing twice the optimal amount of calcium but low in phosphorus and fat-soluble vitamins.

The exact position of the active rays of the spectrum was investigated by Hess and Unger [1922] and it was found that the curative rays were confined to that part of the spectrum having wave-lengths about 300 $m\mu$ or shorter. Huldschinsky [1926; see also Luce, 1926] stated that the active rays were of wave-length 289–320 $m\mu$, and finally Hess and Weinstock [1926–27] fixed the wave-length as 303 $m\mu$, or possibly 313 $m\mu$ on the basis of experiments with filters. Rays of wave-length less than 290 $m\mu$ are more effective than those between 290 and 313 $m\mu$. These observations accounted for the negative results with sunlight filtered through glass, since window glass is opaque to the radiations noted.

The penetrating power of ultra-violet rays of this order of wave-length is very small and necessitates the assumption that the primary process in the antirachitic action of sunlight is restricted to a very thin layer of skin. The transmission of epidermis for a 0·08 mm. layer falls from 55 per cent. at 313 $m\mu$ to 44 per cent. at 300 $m\mu$, 26 per cent. at 294 $m\mu$ and then very sharply to 2·9 per cent. at 289 $m\mu$ [Lucas, 1931; cf. Glitscher and Hasselbach, 1926]. The spectrum of sunlight includes a considerable range of ultra-violet rays, the intensity falling off rather rapidly in the region of 330 to 296 $m\mu$. The shortest wave-lengths detectable vary somewhat with the seasons, and the intensity of ultra-violet rays is subject to wide variations [see Luckiesh, 1930]. From December to February the shortest rays have been given as 312 $m\mu$; from September to November, 308 $m\mu$; from March to May, 301 $m\mu$; and from June to August, 296 $m\mu$. The summer intensity of ultra-violet rays may often be ten times that recorded in winter.

The effects of ultra-violet rays striking the skin are not confined to a favourable effect on the calcification of bone. For instance, latent and actual tetany have been cured by these rays [Huldschinsky, 1920]. This is of special interest in view of the fact that tetany, at least in children, is nearly always related to rickets, and many facts point to a common factor in the aetiology of the two diseases (p. 64). Powers, Park, Shipley, McCollum and Simmonds [1922] observed that sunlight brought about a greater consumption of food in rats, stimulated activity, improved the appearance, and increased their reproductive capacity.

There is no doubt that a deficiency of sunlight can play a very important part in determining the incidence of human rickets. The geographical distribution of the disease indicates that this is the case. Certainly the disease occurs in all parts of the world and affects all races of mankind, but it is particularly frequent among the urban populations of temperate climates, where it has a seasonal incidence corresponding to the months of the year when there is least available sunshine [Hess and Unger, 1921, 2; Chick *et al.*, 1922; 1923]. A high incidence of rickets in children has been observed in manufacturing centres, no doubt partly on account of the screening effect of a smoky atmosphere on the ultra-violet radiation from the sun. Climates noted for their long hours of bright sunshine are not, however, incompatible

with a high incidence of rickets. The disease has been reported as prevalent in certain districts in Southern Europe, North Africa, China, Australia, and Central America. It is possible for children brought up in tropical countries to be largely protected from the direct rays of the sun during their early years. The fact that such children may develop rickets does not therefore indicate that sunshine is a negligible factor in the aetiology of the disease. When the skin is deeply pigmented the protective effect of ultra-violet radiation is lessened, as was noted by Hess, Unger and Pappenheimer [1921-22, 4] in rats. It is probably for this reason that negro children are peculiarly susceptible to the disease when they live in temperate climates and are habitually clothed in the European style.

The relation between vitamin D and sunlight was studied in connexion with human rickets by Chick *et al.* [1922, 1923] at the University Kinderklinik, Vienna. Observations on prophylaxis were made upon young infants receiving two types of diet in the same wards. Diet I consisted of undiluted fresh milk with added sugar ; diet II of standardized full-cream dried milk with the addition of cod-liver oil. Between the months of May and October no case of rickets was detected among a total of 42 infants. Between November and April, 13 out of 51 infants developed rickets, and all these cases occurred among the 25 infants receiving diet I. During the summer months the infants spent a great part of their time out of doors and the authors ascribe their protection from rickets to the action of sunlight. During the winter the infants receiving diet II were protected by the vitamin D contained in their cod-liver oil. Further observations demonstrated that cases of active rickets could be cured in the summer by exposure to sunlight, no matter whether the subjects received diet I or diet II. In the winter rickets could be cured by diet II but progressed on diet I.

Systematic radiographic studies were made during the cure of cases of well-developed rickets by administration of cod-liver oil and by exposure to sunshine or the radiations of a mercury-vapour lamp respectively. The types of healing in these three cases, as shown in X-ray photographs, were found to be indistinguishable from one another [Wimberger, 1922].

The mechanism by which ultra-violet radiations from the sun or artificial sources exert their antirachitic action has not been fully explained, but it seems probable that vitamin D is produced in the skin itself or in the blood circulating immediately below the surface by activation of a precursor already present (see p. 75).

Such an assumption is strengthened by the fact that the presence of ergosterol has been demonstrated in animal and human skin [Rosenheim and Webster, 1927, 5 ; Hentschel and Schindel, 1930]. Hess and Weinstock [1925, 2] showed that excised human skin fed to rats conferred an absolute protection against rickets after it had been irradiated by a mercury-vapour lamp, but no protection without such irradiation. More recently Falkenheim [1928] found that parts of the skin which had not been directly irradiated, but which had been contiguous to areas exposed to ultra-violet rays, developed an appreciable antirachitic activity. It is therefore probable that irradiation of the skin compensates for a deficiency of preformed vitamin D in

the diet by activation *in situ* of the precursor of vitamin D (i.e. ergosterol) the activated product then being available for carrying out its specific activities in the tissues generally in the same way as if it had been absorbed from the alimentary tract. The fundamental cause of rickets is therefore a deficiency of vitamin D, but this deficiency can be supplied either by the ingestion of the pre-formed vitamin or by the exposure of the surface of the body to radiations which have the power of producing it in the tissues.

Relation of Rickets to the Mineral and other Constituents of the Diet.

Mellanby's conclusion that a deficiency of a fat-soluble vitamin in the diet resulted in the appearance of rickets in puppies could not at first be confirmed in the case of young rats [Sherman and Pappenheimer, 1920–21; 1921; McCollum, Simmonds, Parsons, Shipley and Park, 1921; Korenchevsky, 1922]. It was found that rickets could only be produced in these animals by giving diets deficient in calcium and phosphorus, or containing them in unbalanced proportions. The disease could then be cured by correcting the balance of these elements without any addition of fat-soluble vitamin, in which the diets were deficient. Deprivation of fat-soluble vitamin alone with a normal supply of calcium and phosphorus resulted in osteoporosis rather than rickets. Korenchevsky [1922], however, was able to produce rickets in rats by depriving them of fat-soluble vitamin alone, the diet containing adequate amounts of all other known requirements, provided their age was not greater than 3–6 weeks at the beginning of the period of special feeding. Other workers have also succeeded in producing rickets in rats simply by depriving them of fat-soluble vitamin [Goldblatt, 1923]. There is no doubt, however, that a deficiency of either calcium or phosphorus, or a faulty balance of these elements, in a diet containing inadequate supplies of the calcifying vitamin will intensify the rickets-producing effect of the diet.

Deprivation of Calcium Alone.

Korenchevsky [1922] found that young rats kept on a diet containing very small amounts of calcium, but otherwise complete, developed osteoporosis and in some instances mild rickets. If the mothers of the young rats had been kept on a calcium-deficient diet during lactation, the changes produced in the bones of the young rats were more pronounced, resembling those of rickets of moderate severity. This latter pathological condition was not prevented by moderately large doses of cod-liver oil given both to mothers and offspring.

Deprivation of Calcium and Vitamin D.

A combined deficiency of calcium and fat-soluble vitamin has been shown to produce rickets in rats more effectively than the deficiency of either factor alone. As with calcium deficiency alone, the intensity of the effect was greatest when the defective diet was begun at an early age [Korenchevsky, 1922]. Mellanby [1921] found that moderately severe rickets could be produced in dogs that were receiving butter, which contains only small amounts of

vitamin D; when calcium salts were added to the diet containing butter, rickets was either prevented or appeared in a much less severe degree.

Deprivation of Phosphorus and Vitamin D.

Reference has already been made to the work of Sherman and Pappenheimer [1920–21; 1921] in which rickets was first produced in rats by a diet deficient, among other things, in phosphorus and fat-soluble vitamin, and then cured by the addition of basic potassium phosphate. For a time this and similar work of other American investigators resulted in a widespread belief that phosphorus deficiency rather than any vitamin deficiency was the most important aetiological factor in the production of rickets. Mellanby's experiments on the rickets-producing effect of certain cereals [1922; 1925; Green and Mellanby, 1928, 1], which will be referred to again in a later section, demonstrated, however, that both in dogs and in rats a severe type of rickets could be produced with an adequate supply of phosphorus in the diet.

Balance between Calcium and Phosphorus.

McCollum, Simmonds, Parsons, Shipley and Park [1921] suggested that the relative proportions of calcium and phosphorus in the diet were of greater importance than their absolute amounts in determining the occurrence of rickets in rats. In the absence of an adequate supply of vitamin D rickets may be produced in the rat by diets containing either an abnormally high or an abnormally low ratio of calcium to phosphorus. It is known that excess of calcium in the diet diminishes the amount of phosphate absorbed [see Forbes and Keith, 1914]. Mellanby [1921] has suggested that excess of phosphorus as compared with calcium, leading to a loss of calcium from the body, may be the determining cause of the greater rickets-producing effect of acid-caseinogen, which contains phosphorus but no calcium, than of casein, which contains calcium as well as phosphorus. Mellanby's work referred to above (p. 52), in which the diets were composed of natural foodstuffs including cereals and small but inadequate supplies of vitamin D, made it clear that for dogs the ratio of calcium to phosphorus does not necessarily determine the rickets-producing action of a diet. Moreover, this ratio can be varied within very considerable limits without the appearance of rickets provided an adequate amount of vitamin D is supplied. The usual modern technique of testing foods and other substances for vitamin D depends upon the fact that this vitamin can compensate for the imperfect calcium-phosphorus ratios generally provided in experimental rickets-producing diets (McCollum No. 3143; Steenbock No. 2965).

Relation of Mineral Supply to the Occurrence of Human Rickets.

The fact that rickets occurs in infants who are receiving the bulk of their food in the form of milk suggests that neither calcium nor phosphorus deficiency plays any determining rôle in the causation of the human disease. Human milk contains much less phosphorus than cow's milk, so that if phosphorus deficiency were as important a factor in determining the occurrence of rickets in infants as it appears to be in young rats, rickets should be much commoner in

infants fed at the breast than in those receiving cow's milk. Actually the reverse is found to be true, so that the conclusion seems inevitable that phosphorus deficiency is not the determining factor. It is, however, possible that a deficiency of calcium or phosphorus, or of both elements, plays a part in causing rickets in older children who may receive little or no milk and an insufficient supply of vitamin D. Direct evidence on this question is, however, difficult to obtain.

Relation of the Acid-Base Balance in the Diet to the Occurrence of Rickets.

The suggestion has frequently been made that a disturbance in the acid-base balance of the diet plays a part in determining the occurrence of rickets [Aub, 1928–29; Elliot, Crichton and Orr, 1922; Jones, James and Smith, 1923–24; Zucker, Johnson and Barnett, 1922–23]. The marked discrepancies in the results obtained by these workers, however, suggest that the acid-base ratio of a diet can be of relatively little importance in determining the development of rickets.

Relation of Vitamin D to Cereals.

Excess of carbohydrate in the food has long been regarded as a factor predisposing to rickets in infants [Cheadle, 1906]. This hypothesis has now been placed upon a definite scientific basis by Mellanby's experimental work with puppies [Mellanby, 1922; 1925]. He showed that when the diets of puppies were deficient in the anti-rachitic vitamin, the intensity of the rickets which they developed was related to their intake of cereal. When all other factors in the diet and environment were kept constant, rickets was most strongly developed in those animals which ate the most bread. The explanation seemed to be that the consumption of extra quantities of bread induced a greater rate of growth, which necessitated an increased supply of calcifying vitamin to promote normal calcification of the bone. Since this supply was not forthcoming the bones were less efficiently calcified than in those animals which grew at a less rapid rate. It appeared most likely that it was the carbohydrate in the bread which was responsible for the more intense rickets, and in the few instances in which it was possible to induce puppies to eat large amounts of pure starch or sugar for a period of several months, it was found that the calcification of the bones was less perfect than in puppies that had not received such additions. The whole effect produced by increasing the bread in a diet did not, however, appear to be explained by these experiments. Further investigations on the rickets-producing effect of different cereals showed that the carbohydrate moiety of a cereal was not the only factor concerned with intensifying the severity of rickets in the absence of a sufficient supply of vitamin D. Of the cereals studied oatmeal produced the most severe rickets, then maize and barley, and finally rice and wheat flour, which interfered least with bone calcification. The germ of wheat added to a diet also intensified its rickets-producing effect. Oatmeal contains 67·5 per cent. of carbohydrate, while flour contains 76·4 per cent. [Sherman, 1920, 1], so that it cannot be an excess of carbohydrate which endows oatmeal with its greater rickets-producing effect.

The nature of the offending substance or substances has been sought in a long series of feeding experiments [Mellanby, 1925]. It could not be definitely identified with the protein fraction of the cereal, nor with the absolute or relative amounts of calcium and phosphorus. Oatmeal contains about three and a half times as much calcium, and four times as much phosphorus, as white flour, so that giving oatmeal in place of white flour certainly does not lead to any quantitative deficiency of either of these elements. Moreover, a consideration of the ratios of calcium to phosphorus in the various cereals fails to support the view that imperfect balancing of these elements is responsible for their rickets-producing effect. The ratio of calcium to phosphorus in oatmeal is 1 : 5·7, and in white flour 1 : 4·6. These figures are not very different, but the two cereals differ widely in their power of interfering with normal calcification. Wheat germ has a calcium-phosphorus ratio of 1 : 14·8, and rice of 1 : 10·7 ; these figures are of the same order, though differing widely from those of oatmeal and white flour and yet wheat germ interferes greatly with calcification, while rice interferes much less. Mellanby [1926, 1] has suggested that the name *toxamin* might be employed for the agent in question to indicate its unfavourable action and the fact that it can be neutralized by a sufficiency of vitamin D. Efforts to trace the chemical identity of this factor associated with cereals have so far not been successful.

If oatmeal is boiled with 1 per cent. hydrochloric acid until all the starch is converted to sugar and the solution is achromic to iodine, the 'toxamin' action of the cereal is destroyed [E. Mellanby, 1925]. On the other hand Holst [1927] found that the rickets-producing effect of oatmeal could be demonstrated in the filtrate from the cereal after it had been boiled with 0·5 per cent. hydrochloric acid. Thus, whereas a short period of boiling with dilute acid appears to bring the rickets-producing substance into solution, more prolonged boiling of the cereal destroys its action.

The antagonistic effect of cereals, and especially oatmeal, to bone-calcification, has been demonstrated with rats as well as with dogs [Green and Mellanby, 1928, 1 ; Holst, 1927]. How far this effect of cereals is of practical significance in relation to the production of human rickets remains to be proved. The animal-feeding experiments showed that the cereal effect could be obtained only when the diets were deficient in vitamin D. Even when large quantities of oatmeal were given, normal calcification could be brought about by giving cod-liver oil at the same time, and a considerable degree of protection against rickets could be secured by exposing the animals to ultra-violet radiation. It is probable that when the diets of young children contain a bare minimum of vitamin D, and when at the same time the amount of exposure to sunshine is strictly limited, the consumption of large quantities of cereals may determine the onset of frank rickets.

Vitamin D and Maternal Feeding in Relation to Rickets.

It has been the experience of those studying rickets, both in rats and dogs, that when mothers receive in their diets during pregnancy

and lactation some potent source of vitamin D, their young are distinctly less susceptible to rickets when themselves subjected to vitamin D deficiency than other young animals whose mothers have not been well supplied with this vitamin. Thus both Korenchevsky [1922] and Goldblatt [1923] found that when rats were given cod-liver oil during pregnancy and lactation their young did not develop rickets when later receiving diets totally devoid of antirachitic vitamin. Grant and Goettsch [1926] kept rats during pregnancy and lactation on diets deficient in vitamin D but adequate in other respects, and found that their first two or three litters did not develop rickets when diets deficient only in vitamin D were subsequently given to them, but the fourth and fifth litters developed severe rickets under these conditions. In this instance it appeared that the mother's original stores of vitamin D took a considerable time to become depleted and that until the birth of the fourth litter she was able to pass on sufficient vitamin D to her young to protect them subsequently from rickets. When the mothers' diets were deficient in calcium as well as vitamin D, severe rickets developed in the first litter.

Mellanby observed early in the course of his experiments with dogs that if the mother had received cod-liver oil during pregnancy and lactation it was much more difficult to induce rickets in her puppies when they subsequently received diets deficient in vitamin D (see Fig. 4). He demonstrated, moreover, that the influence of the maternal feeding lasted for a considerable period in the life of the offspring [1926, 2]. Two bitches were allowed to become pregnant at the same time and to one was given cod-liver oil during pregnancy and lactation, while to the other was given olive oil. After weaning the puppies were divided into six pairs, each containing one from each mother, and received diets some of which were designed to have a rickets-producing action. In each case the puppy from the mother which had not received cod-liver oil developed severe, or moderately severe, rickets, while the corresponding puppy from the other mother developed no rickets or only slight rickets, depending on the rickets-producing potency of the particular diet which it received. Cod-liver oil was given to both members of one pair and their bones developed quite normally. After the lapse of four months when the animals were well grown, the diet of this pair was changed to one containing minimal quantities of vitamin D and a rickets-producing cereal. After six further months of such feeding the animal whose mother had received cod-liver oil during pregnancy remained free from any suggestion of bone abnormality, while the other whose mother had not received cod-liver oil developed definite bony changes which resembled those found in late rickets in man.

It is not yet certain what degree of protection against human rickets can be obtained by the consumption of adequate quantities of vitamin D by pregnant and nursing women (see p. 269). Hess and Weinstock [1924, 1] tried the effect of giving cod-liver oil to mothers during the last two months of pregnancy, but concluded that it had no appreciable prophylactic effect, for almost all the babies developed rickets during the subsequent winter. Weech [1927] found that administering large doses of cod-liver oil to women during lactation

definitely diminished the severity of rachitic manifestions in their infants, although it could not be relied on to afford absolute protection (see also p. 273).

In the light of animal results it would certainly be expected that at least some degree of protection would be afforded to infants by ensuring liberal supplies of vitamin D to the mothers during pregnancy and lactation. In any case, as will be seen in the later section dealing with osteomalacia, for the mother's own sake it is advisable that she should receive ample supplies of vitamin D during this period.

If any considerable degree of protection is afforded to a young animal by antirachitic measures applied to the lactating mother, it might be expected that such measures would produce a demonstrable increase in the vitamin D content of the mother's milk.

Much work on this point has yielded conflicting results, but the balance of evidence supports the view that the young receive at least some measure of protection from rickets if the mothers are supplied liberally with vitamin D during pregnancy and lactation.

Special Conditions favouring the Occurrence of Rickets in Children.

According to our present knowledge of the aetiology of rickets the supply of vitamin D is the most important factor, and this can either be ingested ready-made in the diet or can be produced in the skin under the influence of ultra-violet radiation. As far as the production of vitamin D in the skin is concerned the usual limiting factors are the intensity of the solar ultra-violet radiation, the duration and extent of exposure of the body surface to light, the degree of pigmentation of the skin, and the nature and amount of the clothing worn. Under ordinary circumstances efficient protection against rickets can be secured by supplying vitamin D in the diet; nevertheless, cases are sometimes met with in which rickets develop in spite of what appears to be an adequate supply of vitamin D.

Prematurity. Premature infants are notoriously liable to develop rickets. It has been suggested that the normal infant receives from its mother during the last few weeks of pregnancy important stores of vitamin D which help to protect it from rickets during its post-natal career. Whether failure to receive this supply explains the increased incidence of rickets in premature children or not, there are reasons for supposing that administration of vitamin D has the same effect in these as in normal children. Gerstenberger and Nourse [1926] state that cod-liver oil can protect premature infants against rickets, but it must be given almost immediately after birth and in rather large doses. Hess [1930] records cases in which rickets appeared in premature infants receiving cod-liver oil, but irradiated ergosterol caused healing of the rickets.

Coeliac disease. In this disease which is associated with infantilism one prominent and constant feature is the passing of bulky stools containing abnormally large amounts of undigested fat. There is a general failure of growth and an osteoporotic condition of the bones, which may or may not show typical rickety changes in addition. The main disturbance appears to consist in an inability to absorb fat from the

intestinal canal, and seeing that vitamin D in food is associated with fats, there is likely to be simultaneous deficient absorption of this vitamin, even though the supply in the food may be adequate for protection against rickets in a normal child. It is stated by Parsons [1927] that 'coeliac rickets' can be cured by exposing the patient to ultra-violet radiation and giving vitamin D by mouth in the form of irradiated ergosterol.

Renal rickets. There is a form of chronic nephritis in children which leads to infantilism and often to rickets. In this condition the concentration of inorganic phosphorus in the blood may be raised far above the normal level. The appearance of rickets in these cases is not associated with any deficient supply of vitamin D, for there is no healing response to foods rich in vitamin D, even when well digested by these patients, and no response to irradiated ergosterol or to irradiation with ultra-violet light.

Infantile Tetany.

The association of laryngismus stridulus, carpopedal spasms, or generalized convulsions with rickets in infancy has been commented upon for many generations. These symptoms occasionally appear in infants who do not exhibit the classical signs of rickets, and those suffering from obvious rickets sometimes exhibit an increased excitability of their nerves (latent tetany) without showing any of the more severe manifestations of tetany. The incidence of infantile tetany is similar to that of rickets. Both show a pronounced seasonal variation [Japha, 1905 ; Moro, 1926] with exacerbations in the winter and early spring, and both are particularly liable to affect premature infants ; in the United States negro infants are more frequently affected than white infants. There is one point of difference. Florid rickets is seldom met with in poorly nourished infants, for this condition is essentially one associated with good growth, but tetany, especially the latent form, is by no means uncommon in cases of malnutrition.

A pronounced lowering of the concentration of calcium in the serum is one of the more constant findings in tetany, but not in rickets, unless tetany is also present. A lowering of the serum calcium, associated with rickets, can be produced in rats by feeding them on diets deficient in vitamin D and in calcium, but relatively rich in phosphorus [Shipley, Park, McCollum and Simmonds, 1922]. Shohl, Bennett and Weed [1928] produced tetany in rats by giving them alkaline phosphates after they had been on a rickets-producing diet ; the tetany appeared in spite of the fact that the rickets showed histological evidence of being cured. This type of tetany is probably similar to that found in the alkalosis produced by forced breathing.

The fact that infantile tetany and rickets are so closely associated suggests that the lowering of the blood-calcium in the former is connected with a deficiency of vitamin D. This view is strongly supported by observations on vitamin-D therapy in infancy. Adequate doses of cod-liver oil are found to prevent tetany just as they prevent rickets. Cod-liver oil is also effective as a curative agent, although its effect is sometimes delayed. Irradiated ergosterol has a more rapid action and may raise the serum calcium to a normal level in 10 or 14 days.

The acute symptoms may be rapidly alleviated by giving large doses of calcium chloride or of ammonium chloride, which latter salt is stated by Gamble, Ross and Tisdall [1923] to increase the proportion of ionized calcium in the blood ; but a real cure can only be assured by supplying in some form sufficient vitamin D to restore to normal the disturbed calcium metabolism.

The Relation of Vitamin D to the Parathyroid Glands.

MacCallum and Voegtlin [1909] discovered that when the parathyroids of dogs were removed the calcium in the blood was lowered, an observation which indicated that these glands played a part in calcium metabolism.

Erdheim [1914] on the basis of pathological anatomical studies of human osteomalacia and of experimental work with rats traced a connexion between osteomalacia or rickets and abnormalities in the parathyroid glands.

In recent years, largely through the work of Mandl [1926 ; 1929], Collip [1925], and Aub and his collaborators [1928–29], the subject has again become one of great interest, and it is now recognized that the parathyroid secretion is an important factor in calcium-phosphorus metabolism. Anybody who has seen the dramatic effects produced by removal of a parathyroid cyst on the bone and blood condition in osteitis fibrosa cystica, or the effect of Collip's extract (parathormone) in tetany, must have been impressed by this relationship. The administration of vitamin D is also effective in the treatment of tetany, so that it is a matter of interest to know how parathormone and vitamin D are related.

The relation of the parathyroids to calcium-phosphorus metabolism has been elucidated by three types of observation :

(1) By determining the effect of removal of the glands.
(2) By observing the effect of injecting extracts of the glands [Collip, 1925 ; 1926 ; Greenwald and Gross, 1925 ; 1926].
(3) By observing the effect of the removal of parathyroid tumours in cases of osteitis fibrosa cystica [Mandl, 1926].

Removal of the glands from normal animals brings about a reduction of the calcium in the blood. The tetany which also results may be relieved temporarily by giving milk and other foods rich in calcium and abolished by repeated subcutaneous or intramuscular injection of extracts of the parathyroid glands. Parathormone raises the serum calcium in these animals to normal. Large doses produce hypercalcaemia, a condition which in normal animals especially is associated with illness and sometimes death.

After parathyroidectomy dogs excrete smaller quantities of calcium than the normal. On the other hand, the injection of parathormone increases the excretion of both calcium and phosphorus (Greenwald and Gross). In hyperparathyroidism the excretion of calcium and phosphorus is abnormally high, whether this condition is produced artificially in normal animals by the injection of parathyroid extract or is due to a tumour of the parathyroid glands in clinical cases. In hyperparathyroidism associated with osteitis fibrosa cystica, the blood has a high calcium and a low phosphorus content [Harrison, Shorr,

McClellan and Du Bois, 1930]. Removal of the tumour lowers the calcium of the blood greatly and increases the phosphorus but to a less extent. The excretion of both elements is greatly reduced by this form of treatment.

The effect of the secretion of the parathyroid is to maintain or raise the calcium and phosphorus excretion at the expense of the bones. Administration of vitamin D, however, in ordinary amounts brings about the incorporation of calcium and phosphorus in bones. Their actions are therefore in opposite directions. The fact that both vitamin D and parathyroid extract relieve tetany might suggest that they had a similar type of action. Vitamin D seems to raise to normal the calcium of the blood in cases of tetany by increasing the retention in the body of calcium which may be available in the food supplies. Parathyroid extract, on the other hand, raises the calcium in the blood by withdrawing it from the great tissue reserve of this substance, namely the bones.

Experiments have been made to ascertain whether vitamin D and the parathyroids are related by administering the vitamin to parathyroidectomized animals. Some observers have found that vitamin D acts just as well under these conditions as in the intact animal, large doses of irradiated ergosterol (400 to 800 times the therapeutic dose) producing hypercalcaemia even in the absence of the parathyroids. There is also evidence that these large doses of vitamin D (in the form of irradiated ergosterol) raise the blood calcium at the expense of the bones [Kreitmair and Hintzelmann, 1928; Baumgartner *et al.*, 1929; Hess, Weinstock and Rivkin, 1928–29; 1929–30], an action which is similar to that of the parathyroid extracts.

Taylor, Weld, Branion and Kay [1931, 1, 2] have presented evidence to show that the action of large doses of irradiated ergosterol in producing a rise in serum calcium takes place through the intermediary of the parathyroid glands, and have shown for example that large doses had no effect in relieving the tetany produced in dogs after an intensive operation in which thyroid and parathyroid glands and all accessory parathyroid tissue had been removed.[1]

Vitamin D in the Prophylaxis and Treatment of Rickets and Allied Disorders.

Rickets, osteomalacia, and infantile tetany have been shown to be due primarily to a deficiency of vitamin D, although other factors such as the supply of cereals and mineral salts may play an important part in their causation when there is a coincident partial deficiency of vitamin D. The prevention and treatment of these diseases should therefore not present any great difficulty, for there is a considerable choice of methods available for supplying vitamin D. Many of these methods have already been tested on a large scale, and though they may all have been found efficacious in achieving their immediate purpose, there may be advantages and disadvantages associated with some of them which require a somewhat detailed examination.

[1] For a review of work on the parathyroid glands, see Hunter [1931].

General Adjustment of Diet.

Much may be done to prevent rickets and osteomalacia by including in the diet a regular supply of natural foods containing vitamin D and by cutting down the amount of cereal. There are few natural foods rich in vitamin D, unless cod-liver and similar fish oils are considered natural foods. Egg yolk is powerfully antirachitic and may be given even to young infants. Milk, cream, and butter may contain appreciable quantities of the vitamin and the amount can be increased by feeding cows in the open air on good pasture. Milk is of considerable value also on account of its relative richness in calcium. Animal fats such as suet and dripping contain small amounts of vitamin D which give them a better nutritive value than vegetable fats which usually contain none.

The problem of cutting down the cereal content of a diet without increasing its cost is by no means easy to solve. It is quite true that the anti-calcifying effect of cereals can be neutralized by adequate supplies of vitamin D, but some evidence has been obtained in connexion with the work of M. Mellanby [1924; Mellanby and Pattison, 1926], on carious teeth in childhood, that even small supplies of vitamin D may be more efficacious in promoting normal calcification if the diet contains minimal quantities of cereals [see also Boyd *et al.*, 1928; 1929]. As regards cereals, it seems advisable to choose those which have the least anticalcifying action, such as white bread and rice. It is possible, as will be mentioned below (p. 70), to render cereals such as oatmeal antirachitic by irradiation. Greater use may be made of fresh vegetables and potatoes with little increase in the cost.

Insistence upon the breast-feeding of infants is strongly advised whenever possible.

Cod-liver Oil Therapy.

The specific properties of cod-liver oil as an antirachitic agent were first proved experimentally by E. Mellanby [1918–19, 1]. Since then it has been abundantly shown that cod-liver oil can almost always be relied upon to cure rickets if it is given in adequate doses. It contains large amounts of vitamin A as well as vitamin D and it appears likely that some of the beneficial effects observed after giving it to children are due to this fact. As a prophylactic agent it should be given early in the infant's career, especially to premature infants and during the winter months. Many experiments have been carried out on a large scale to test its prophylactic effect. Hess and Unger [1917] demonstrated that giving cod-liver oil to negro infants was more effective than breast-feeding in protecting them from rickets. Gerstenberger and Nourse [1926] found that 3·5 c.c. a day protected premature infants. Eliot [1925] could not altogether check what she interpreted as radiographic evidence of early rickets in infants by giving cod-liver oil, although those receiving it were protected from obvious clinical manifestations of the disease. Similarly M. G Wilson [1926] considered that cod-liver oil had no great influence in preventing the radiographic changes in the bones which she considered to indicate very early rickets. According to these observers a very slight degree of rickets may be regarded as physiological and almost universal in apparently healthy young infants even when they receive cod-liver oil.

In climates where there is little available sunshine the prevention of rickets depends on supplying liberal quantities of vitamin D by cod-liver oil administration. There are no serious objections to its routine use in infancy and childhood. Most young children not merely tolerate it but grow to like it. It may be given as a prophylactic measure after the second or third week of life in doses of one quarter of a teaspoonful, and be increased so that the dose at the age of three months is one-half teaspoonful twice a day, and at eight or nine months two teaspoonsful a day. There appears to be no danger whatever of these doses causing untoward symptoms in infants corresponding to those described by Agduhr [1926, 1, 2] in experimental animals.

The healing effect of cod-liver oil can often be detected radiographically within two or three weeks, but the treatment should be continued for at least a year, during which time most of the growth deformities, even in severe cases, will become rectified. Cases are sometimes met with in which vitamin D concentrates, such as irradiated ergosterol, promote healing when ordinary doses of cod-liver oil given for many weeks have had no appreciable effect.

The toxicity of cod-liver oil. It is a matter of common experience that cod-liver oil, especially in the adult, may produce anorexia and gastric discomfort. Much of this effect is psychological and results from its disagreeable taste and oily nature. A part is undoubtedly due to its physiological effect in inhibiting gastric secretion and motility, for, as has been shown by Roberts in the treatment of hyperchlorhydria [1930], cod-liver oil is much more effective in this respect than more highly saturated fats such as olive oil and butter fat. In these tests the various oils and fats were placed directly in the stomach so as to eliminate any psychological effect due to flavour.

Apart from these detrimental effects there is some evidence that cod-liver oil can do harm under certain conditions after absorption from the alimentary canal.

Normal adults taking large quantities of cod-liver oil (say 2 ounces daily) occasionally develop some degree of tachycardia and praecordial discomfort, even when there is no question of gastric disturbance [E. Mellanby, 1924]. The subject has been brought into a position of prominence by the investigations of Agduhr [1926–29], who found that cod-liver oil, when given continuously to animals, may produce toxic effects on the heart and the development of the following abnormalities—pigment degeneration and fatty degeneration of the heart-muscle, transformation of the muscle-cells into connective tissue, and calcareous incrustations. Parallel experiments with other oils, e.g. olive oil, rape oil, and cocoa fat, did not show alterations in the heart comparable with those produced by cod-liver oil.

It is undoubted that under certain experimental conditions cod-liver oil produces degenerative changes in the heart and the problem is to decide to what extent such results may be extended to man. Agduhr's results were undoubtedly magnified by the type of diet chosen for his work. Just as vitamin D in excessive doses produces much greater toxic effects when added to synthetic diets than when added to diets of natural foodstuffs [see Harris and Innes, 1931], so also the toxic effects of cod-liver oil are much greater under these same experimental conditions. The inhibition of the toxic effect of excessive cod-liver

oil by increasing the supply of the vitamin B complex, as shown by Harris and Moore [1929, 2], indicates the importance of testing the action of cod-liver oil in a well-balanced diet.

Another criticism of Agduhr's results applies especially to those experiments in which he obtained the greatest effects, namely in calves and herbivorous animals. These animals take cod-liver oil or any form of fat very badly, and indeed rabbits die soon after the addition of small quantities of fats to the food unless green vegetables are also given liberally [M. Mellanby and Killick, 1926]. It seems desirable that some of Agduhr's work should be repeated with animals eating well-balanced diets and receiving quantities of cod-liver oil more comparable to the relatively small amounts taken by an average human being. At the same time the results should probably be used as a warning that constant administration of large quantities of cod-liver oil to children and adults on ill-balanced diets may have an insidious and untoward toxic effect especially on the muscle-cells of the heart.

There is no reason to believe that any of the toxic actions ascribed to cod-liver oil are due to its vitamin content. Norris and Church [1930, 3] have shown that small doses of *iso*amylamine, as found in cod-liver oil, and also choline, given continuously, may produce paralysis, convulsions, and lack of growth, and that these symptoms can be prevented by giving more yeast. They suggest that these bases may be the toxic agents in cod-liver oil.

Exposure to Sunshine or Artificial Sources of Ultra-violet Radiation.

When the composition of the diet is such that it is neither powerfully rickets-producing nor powerfully protective, exposure of the skin to ultra-violet radiation from the sun, even in an unfavourable climate, may suffice to protect the individual from rickets or allied disorders. Chick and co-workers [1922; 1923] demonstrated the protective value of natural sunshine in Vienna; rickets could be both prevented and cured in the summer by allowing infants to remain in the open, although this effect could not be obtained in the winter. Huldschinsky's [1919] observations on the curative effect of artificial ultra-violet irradiation mark an epoch in the study of rickets and have been repeatedly confirmed. In many parts of the world Light Clinics have been established for the purpose of providing protection against rickets in districts where natural sunshine is not available during a considerable part of the year. This method of prophylaxis is undoubtedly effective, but is costly in operation and not free from danger, and must entail considerable time and expense on the part of the mothers who have to attend regularly with their children. It has, moreover, the disadvantage, as compared with the administration of cod-liver oil, of not supplying vitamin A, which is frequently also deficient in the diets of children suffering from a lack of vitamin D.

Concentrated Preparations of Vitamin D.

Since the discovery of the extremely powerful antirachitic activity of irradiated ergosterol, it has been possible to give much larger doses

of vitamin D than could previously be given in the form of cod-liver oil. The efficacy of this substance, both in the prevention and cure of rickets, has been proved by numerous observers in all parts of the world. It has also been shown to be effective in the treatment of tetany and osteomalacia [Hottinger, 1927, 1; Starlinger, 1927]. Several interesting comparisons have been published between the effects of cod-liver oil and irradiated ergosterol in preventing or curing rickets. Barnes, Brady and James [1930] found that one drachm of cod-liver oil given three times a day to children prevented or cured rickets in 95 per cent. of cases. Daily doses of irradiated ergosterol of equivalent potency as tested on rats prevented or cured rickets in 44 per cent. of cases only. De Sanctis and Craig [1930] gave three drachms of cod-liver oil daily to 103 infants and found that 97 were protected from rickets; ten minims daily of the American preparation of irradiated ergosterol known as 'viosterol', which, according to rat dosage, should afford good protection, prevented rickets in only 77 out of 123 infants.

These results show the desirability for enforcing standardization of commercial preparations of vitamin D sold for therapeutic use and the need which existed for the efforts which have been successfully made to secure international agreement as to a standard substance to be adopted for purposes of comparison in vitamin D tests and as to the unit in which the results are to be expressed (see Appendix II).

Irradiation of Foods.

The observation of Steenbock and Black [1924] that foods previously without antirachitic value were capable of preventing or curing rickets after they had been exposed to ultra-violet radiation (p. 74), led to clinical trials of the antirachitic effect of irradiated foods [Steenbock and Daniels, 1925]. The fact that vegetable oils develop high antirachitic potency after irradiation suggests that they might be irradiated with advantage before being incorporated into margarines.

Mellanby [1925; see also Green and Mellanby, 1928, 1] has shown that the anticalcifying effect of oatmeal is neutralized by exposure to the radiation from a mercury-vapour lamp for thirty minutes. If the oatmeal is freed from fat, irradiation does not increase its calcifying action. On the other hand, irradiated oatmeal loses its calcifying action when its fat is extracted and the fatty moiety can be shown to stimulate calcification. These facts indicate that the ergosterol present in the oatmeal is changed to vitamin D by the ultra-violet rays. When this substance is removed the anticalcifying effect of the cereal remains [M. Mellanby, 1929].

Toxicity of Irradiated Ergosterol. Hypervitaminosis D.

This subject has become a question of practical interest, especially to those concerned with the care of children. While, on the whole, it would appear that the danger to children of this form of therapy is very small, especially if their diet is otherwise balanced, it is not possible at the present time to lay down any clear cut rules as to the limits of safety.

Pfannenstiel [1927; 1928] observed in rabbits and Kreitmair and

Moll [1928] in rats, cats, rabbits, dogs, and guinea-pigs, that large doses (from 1–40 mg. daily) of irradiated ergosterol brought about death of the animals in periods ranging from 15–40 days. The symptoms were rapid loss of weight, anorexia, diarrhoea, and roughening of the coat, while examination of the tissues revealed large deposits of calcium in the arteries, myocardium, lungs, kidneys, stomach, and intercostal muscles.

These results have been confirmed by numerous workers and the pathological lesions have been described by Huckel and Wenzel [1929, 1, 2], Varela *et al.* [1929], Spies and Glover [1930], Duguid [1930], Hoyle [1930, 1, 2] and others. The predominating lesion is in the arteries and appears to be primarily a degenerative change in the muscular layer followed by calcification. The calcifying process extends from the larger to the smaller arteries, especially the renal vessels, until almost all are involved. A premature calcification of growing cartilage was observed by Collazo *et al.* [1929] and Schmidtmann [1928]. Klein [1929] and Harris and Stewart [1929] recorded a large increase in the calcium and inorganic phosphorus content of the blood after massive doses of irradiated ergosterol. There is an increased urinary excretion of both calcium and phosphorus, and this loss has been shown by Kreitmair and Hintzelmann [1928] and Baumgartner *et al.* [1929] to be associated with a decalcification of the bones particularly affecting the ribs.

There is, however, an extremely wide margin between the therapeutic and toxic doses. Kreitmair and Moll [1928] obtained toxic effects with 5,000–10,000 times the curative dose; Harris and Moore [1928] used 100,000 times the prophylactic dose; Bills and Wirick [1930] state that 100 times the prophylactic dose is harmless in rats, 1,000 times is just perceptibly harmful over a long period, whilst 4,000 times is definitely toxic.

Some workers have been unable to obtain these toxic effects even when very large doses of irradiated ergosterol were given. There is a great lack of uniformity in experimental conditions, including the species of animal used, the nature of the diet and the mode of irradiation of the ergosterol, all of which appear to be important factors. These divergent results have raised the question as to whether the toxic effect was actually due to the antirachitic principle or to some toxic by-product produced during the irradiation of ergosterol. The opinion that the antirachitic and toxic actions are the effects of different substances [Holtz and Schreiber, 1930] has been rendered very improbable by the observation that calciferol (p. 77), the purest form of vitamin D yet known, is also toxic in large doses [Askew *et al.*, 1932]. Most workers find ergosterol itself to be non-toxic though Kreitmair and Moll [1928] produced calcification in the vessel walls by its administration, but only after large doses for a very long period.

Many of the negative results mentioned above have been obtained by workers using natural diets rather than laboratory synthetic diets. Schmidtmann [1928] and Rabl [1929] have shown that a high calcium diet favours the early production of lesions, while according to Herzenberg [1929] a low calcium diet delays the appearance of lesions; Harris [1930] notes that a high Ca, low P diet definitely increases the toxic effect. Duguid *et al.* [1930] found that irradiated ergosterol was much

more toxic to rats living on a synthetic high Ca, low P diet than to rats fed on a mixed diet of bread and potatoes. The work of Hoyle [1930, 1,2] also showed that the nature of the diet was a very important factor: 80,000 times the usual therapeutic daily dose of ergosterol irradiated in alcohol caused death of rats on a synthetic diet in 2–3 weeks, while rats on bread and milk, apart from retarded growth after 30 to 40 days, showed no lesions except occasional urinary calculi. Neither bread alone nor milk alone, nor a synthetic diet constituted to resemble the bread and milk diet prevented the toxic action. When the dose was increased to 200,000 times the normal dose the bread and milk diet lost its protective effect.

There are other conflicting observations. Harris and Moore [1929, 1] noted a loss of toxicity when irradiation of the ergosterol in alcohol was prolonged sufficiently to destroy the antirachitic factor, whilst Hoyle [1930, 1, 2] finds no such loss under similar conditions. The nature of the solvent in which ergosterol is irradiated possibly influences the toxicity, though Harris and Moore [1929, 1] found that a pure specimen of ergosterol irradiated in air could still, in adequate doses, produce typical lesions. Dixon and Hoyle [1928] using ergosterol irradiated in oil found that a dose as high as 17 mg. daily caused poor growth, but apart from the presence of calcium phosphate calculi in the urinary tract there were no morbid lesions. Hoyle and Buckland [1929] with a larger dose confirmed this result in the main, while Hoyle [1930, 1] found that ergosterol, after irradiation in alcohol, caused intense tissue calcification although urinary calculi were rare.

Very few instances of toxic effects due to irradiated ergosterol have been described in human beings, which is not surprising in view of the enormous doses required to produce these effects in animals. Large doses of irradiated ergosterol have, indeed, been given to infants without the appearance of toxic symptoms. Hess, Poncher *et al.* [1930] in a prophylactic investigation, observed no toxic symptoms after daily administration of 20–52 times the prophylactic dose of irradiated ergosterol ('viosterol') for long periods and of 250 times for short periods. The children were lively, the appetite was increased; constipation was the rule. Bamberger [1929] records loss in weight, vomiting, anorexia, with blood, albumin, and hyaline casts in the urine and a raised blood calcium and phosphorus content in 10 of 11 tuberculous children receiving large doses of 'vigantol' (0·2–0·25 mg. irradiated ergosterol per kg. body-wt.). No such effects were observed with a cod-liver oil concentrate given in amounts equivalent in antirachitic potency; it would therefore appear that the toxic effect of 'vigantol' was not due to its vitamin D content. György [1929] observed anorexia, vomiting, renal disturbances, and hypercalcaemia in tuberculous children on daily doses of 5–10 mg. irradiated ergosterol, but normal children did not show such symptoms or hypercalcaemia on a dose of 6 mg. Hess and Lewis [1928] report toxic effects in 9 out of 22 normal and rachitic children receiving doses of 0·5–6·6 mg. per kg. body-wt. Klausner-Cronheim [1930] records the appearance of albumin, red cells and casts in the urine of 3 rachitic children receiving from 1 to 5 mg. of irradiated ergosterol daily. Putscher [1929] found abnormal calcification similar to that seen in animals in a child that had received 6 drops of 'vigantol' daily.

Bamberger and Spranger [1928] describe parenchymatous nephritis in a child dead from 'vigantol' poisoning.

Although there appears to be a wide margin of safety in the use of irradiated ergosterol in human therapeutics, it is clearly desirable that it should be prescribed on a standardized basis and that the possibility of toxic effects, particularly as revealed by urine examination, should be borne in mind. Animal experiments suggest that there is more danger when the diet is deficient or faulty in some respect.

General Conclusions.

A deficiency of vitamin D is the principal factor which determines the occurrence of rickets, defectively formed teeth and infantile tetany. The same deficiency is the cause of osteomalacia, a disease which may be regarded as the pathological analogue of rickets, occurring in adult life under special conditions of deprivation and often associated with pregnancy (see p. 239).

Deficiency of vitamin D arises either from a lack of vitamin D in the diet or from a deficient exposure of the skin to those radiations of short wave-length which are capable of producing vitamin D at the surface of the body. When the total amount of vitamin D supplied from either or both of these sources is large, rickets and the allied conditions will be prevented, although normal bones cannot be formed in the absence of the necessary minimum quantities of mineral elements. When the amount of vitamin D supplied is small, the occurrence of rickets may depend on other factors in addition to vitamin D deficiency. Thus rapid growth, a large intake of cereals, deficiency of calcium or phosphorus, an unsuitable proportion of the one to the other, or a defective supply of vitamin D to the mother during pregnancy and lactation may then determine the onset in individual cases. It is unlikely that all the factors which may influence the occurrence of rickets in the presence of limited amounts of vitamin D are at present recognized; nevertheless, except in rare pathological cases, liberal supplies of vitamin D can apparently counteract them all.

Chemistry of Vitamin D.

A remarkable result of the work on rickets was the discovery that light rays—either in the form of direct sunlight or of the radiation from the mercury-vapour quartz lamp—exert a profound influence on the disease. The first observations were clinical (see p. 55) and not only failed to clarify the problem, but tended to complicate the issue by raising the apparent paradox that rickets could be cured by two such dissimilar agents as ultra-violet rays and dietary factors. The attack on the problem by the biochemist, which finally led to its solution, only became possible when means were found to study the question experimentally in the laboratory on animals, rats in particular, kept on a rachitogenic diet. Since the sunlight effects could not be satisfactorily explained in terms of calcium and phosphorus metabolism alone and were similar to those produced by including cod-liver oil in the diet, the idea arose that sunlight striking the skin might cause a photosynthetic production in the skin of the antirachitic

vitamin, which was then liberated into the blood-stream. Its production would thus be analogous to that of vitamin-A potency in green leaves, which probably occurs as a result of photosynthesis.

Activation of Synthetic Diets by Irradiation.

A valuable clue, directing the experimental attack into the right channel, was unexpectedly supplied by the discovery that the effect of irradiation on the animal could be equally well evoked by irradiation of its rachitogenic diet. The experimental work which led up to this discovery was carried out by Hume [1922], Hume and Smith [1923], and by Goldblatt and Soames [1923, 1, 2].

Hume [1922] had observed that ultra-violet irradiation could replace cod-liver oil up to a point as far as the promotion of growth was concerned, but that it could not permanently supply the growth-promoting function of the oil, nor could it prevent xerophthalmia. Hume and Smith [1923] reported that rats eating a diet deficient in fat-soluble vitamins, when kept in glass jars which had been exposed to radiation from a mercury-vapour lamp for ten minutes on alternate days, maintained growth longer than control rats not so treated. The implication that the air in the jars could be activated by the rays was negatived by Webster and Hill [1924], who only obtained growth effect by exposing the animals themselves to the radiation [cf. Chick and Tazelaar, 1924; Nelson and Steenbock, 1924–25; 1925, 2; Hughes, Nitcher and Titus, 1925]. Hume and Smith [1924] then observed that rats housed in previously irradiated glass jars containing sawdust and fed on a diet deficient in fat-soluble vitamins grew as well as those undergoing direct irradiation, and that the degree of bone calcification was directly proportional to the growth. The growth-promoting and calcifying properties resided in the irradiated sawdust consumed by the rats, and were later shown to be due to the sterols of the sawdust which had become antirachitic by irradiation (see p. 75).

About the same time Goldblatt and Soames [1923, 2] found that the livers of irradiated rats on a diet deprived of fat-soluble vitamins had acquired growth-promoting properties, whilst the livers from non-irradiated rats were entirely inactive.

Steenbock and Black [1924] confirmed this observation and extended it by irradiating liver and the deficient diet directly, and they offered what is now known to be the correct explanation. The observed effects are due to the production, not of vitamin A, but of vitamin D, and both growth-promoting and bone-calcifying properties are imparted by irradiation to animal tissues and to synthetic diets deficient in the antirachitic vitamin. In confirmation of this view they discovered that various foodstuffs, incapable in themselves of preventing or curing rickets, became endowed with antirachitic power on exposure to ultra-violet rays. Hess and Weinstock [1924, 2; 1924–25; 1925, 1] arrived at similar conclusions. On further analysis it was found that the capacity to become activated resided only in those constituents of the diet which contained fats. The fats themselves, i.e. edible vegetable oils such as olive oil, cottonseed oil, arachis oil, &c., although inactive by themselves, became antirachitic after irradiation. It was also noticed that this activity was completely lost again on

prolonged irradiation. This remarkable property of fats is not due to their main constituents, the fatty acids and glycerol, but is restricted entirely to that small moiety of substances composing the 'unsaponifiable' fraction.

Subsequently three distinct groups of investigators [Steenbock and Black, 1925; Hess, Weinstock and Helman, 1925; and Rosenheim and Webster, 1925] discovered independently and simultaneously that the animal sterol, cholesterol, and the plant sterol, phytosterol, could be converted under the action of ultra-violet rays into very rich sources of vitamin D, although neither substance possessed any antirachitic action prior to irradiation. Even when cholesterol (obtained from brain) was subjected to purification by recrystallization more than twenty times, it retained the power of acquiring antirachitic potency under the action of ultra-violet rays.

The Recognition of Ergosterol as the Parent Substance of Vitamin D.

It was found by Rosenheim and Webster [1926, 1] that after irradiation of cholesterol more than 99 per cent. remained unchanged. By taking advantage of the insolubility of the digitonin-cholesterol compound, the antirachitic factor could be accumulated in a small amount of an amorphous substance not precipitable by digitonin. Such a behaviour is analogous to that of the vitamin D of cod-liver oil, which is also not precipitable by digitonin [Nelson and Steenbock, 1925, 1]. The preparation obtained from irradiated cholesterol sufficed to meet the antirachitic requirements of rats when administered at what was then considered the extraordinarily small dosage of 0·01 mg. per rat per day. Rosenheim and Webster also observed that the sterol of fungi, ergosterol (obtained from ergot of rye or from yeast fat) when irradiated became 'highly protective even in doses of 1 mg.'

A very large number of substances allied to cholesterol (isomerides, hydrocarbons, oxidation products, &c.) were then investigated [Rosenheim and Webster, 1926, 5; 1927, 2; Hess and Windaus, 1926–27]. The negative results obtained in this work, together with other considerations, led Rosenheim and Webster [1926, 5] to recognize that cholesterol contained a significant impurity which could not be eliminated by recrystallization. Cholesterol, in view of its single ethenoid linkage, readily forms a dibromide, from which the sterol can be regenerated by reduction with sodium amalgam and acetic acid. It was found that the cholesterol, purified by way of the dibromide, no longer possessed the power of becoming antirachitic after irradiation with ultra-violet rays. Such cholesterol is very transparent in the ultra-violet, no trace of selective absorption being shown. From this work of Rosenheim and Webster, it seemed probable that the precursor substance (provitamin D) was more readily attacked by bromine than cholesterol, or, at any rate, that it was destroyed in the purification process. It seemed legitimate therefore to assume that, since it resisted separation from cholesterol and was precipitated by digitonin, the provitamin was either an unsaturated sterol or a cholesterol derivative. From this point progress proved

to be rapid, and spectroscopic investigations afforded valuable contributory evidence.

The first preliminary spectroscopic investigations were carried out by Hess and Weinstock [1925, 2, 3] who recorded a qualitative change in absorption on irradiation. The activated material absorbed ultra-violet rays somewhat less strongly than the non-irradiated cholesterol, a difference in the integrated absorption over a range of wave-lengths being detected by means of a thermopile and galvanometer. Schlutz and Morse [1925] and Schlutz and Ziegler [1926] carried the matter farther when they recorded that highly purified cholesterol (M.P. 148·5°) showed 'two characteristic absorption bands at about 294 and 279 $m\mu$ wave-length'. They further suggested that the absorption might be due to a small amount of a highly absorptive impurity.

A quantitative study of the absorption spectrum of cholesterol was carried out by Heilbron, Kamm and Morton [1926; 1927]. Large quantities (2 kg.) of cholesterol were subjected to fractional crystallization from ethyl acetate, and the various fractions examined spectrographically. It was established that ordinary cholesterol contained a small quantity of another compound, capable of being accumulated in the least soluble fraction. The intensity of absorption in the original cholesterol was only one-fourth of that obtained with this fraction. A sample of the concentrated product was subsequently irradiated and tested biologically by Rosenheim and Webster [1927, 3] and found to possess an antirachitic potency 3–4 times as great as that obtained by irradiating the original sample of cholesterol. The 'impurity' in cholesterol was characterized by three well-defined absorption maxima in the ultra-violet at 293·5, 281·5, and 270 $m\mu$ respectively. Since these maxima disappeared on irradiation and at the same time the material acquired antirachitic activity, it became evident that the bands provided criteria for a photosensitive precursor substance. Pohl [1926] published similar data, arrived at independently and by a different technique. The spectroscopic observations thus effectively strengthened the evidence of the biological and chemical work and led to a search for the 'impurity' (provitamin D), which proved successful in a short time.

Attention was directed again to the fact that Rosenheim and Webster [1926, 1] had obtained positive results with ergosterol. This trebly unsaturated sterol is destroyed by bromine, it is precipitated by digitonin, and can be activated. When Rosenheim and Webster first examined irradiated ergosterol they obtained complete protection with 1 mg., but did not test smaller amounts. By progressively reducing the dosage, it was found that the antirachitic potency was of a much higher order than that obtained with any other substance. The daily dose of irradiated ergosterol needed to protect a rat against rickets is exceedingly small, 0·0001 mg. or less sufficing. It was also found that ergosterol possessed a highly characteristic absorption spectrum, showing the same maxima as those recorded by Pohl and by Heilbron, Kamm and Morton, but with enormously increased intensity as compared with cholesterol. The identification of ergosterol with provitamin D was jointly announced by Rosenheim and Webster [1927, 3, 4] and by Windaus and Hess [1927].

The laboratory results obtained by animal experiments were rapidly confirmed by extensive clinical experience and enabled the dietetic and environmental theories of the aetiology of rickets to be at last reconciled (p. 52). It is obvious that among the factors of hygiene advocated at different times as influencing rickets, sunlight and ultra-violet rays are of pre-eminent importance. *Diet and sunlight both influence the calcification of bone, the former by virtue of its content of vitamin D, sunlight by generating vitamin D in the skin from ergosterol.* Thus, many observations which formerly could not be brought into intelligible relationship are now seen to be consistent.

The Isolation of Crystalline Vitamin D (Calciferol).

When ergosterol is irradiated a complex resinous mixture of at least six substances is formed, either simultaneously or consecutively, one of which is the antirachitic vitamin. In spite of the persevering attempts of chemists in many countries, this intractable resinous mixture could not be brought to crystallization. Success was more than once announced [Jendrassik and Kemenyffi, 1929; Reerink and van Wijk, 1929; 1931], but the claim that the crystalline products represented vitamin D itself was not substantiated. It seems, however, as if the final stages in the isolation of the vitamin had now been reached, concurrently in England and in Germany, and fittingly in the same laboratories in which the original discovery of the parent substance of vitamin D was simultaneously made in 1927.

The first step towards success was taken when Bourdillon and his co-workers [Askew *et al.*, 1930, 1] produced from the irradiation mixture small amounts of a crystalline substance of high antirachitic activity by the use of an ingenious new method, distillation *in vacuo* combined with fractional condensation. Later they were able to obtain several grams of this substance, termed by them calciferol, which retained its biological and physical properties unchanged on repeated recrystallizations [Angus *et al.*, 1931]. This fact did not, in their opinion, exclude the possibility that the substance was a mixture or a molecular compound of a highly active substance with an inactive isomeric one. It was then found that calciferol, as obtained by distillation, yielded a well-characterized crystalline ester, the 3 : 5-dinitrobenzoate [Callow, 1931], by means of which it could be freed from an inactive isomeric admixture of very high dextro-rotation. The latter substance, pyrocalciferol, is formed by thermal effects during the process of distillation of the irradiation product. Pure calciferol has an antirachitic activity of 40,000 international units (see p. 83) per mg., M.P. 115-117°, $[\alpha]_{5461}$ + 122°, and has been isolated as the dinitrobenzoate directly from the undistilled crude mixture of irradiation products of ergosterol [Askew *et al.*, 1931; 1932].

Meanwhile Windaus [1931] also obtained a crystalline substance, differing from calciferol in its optical activity, by applying the reaction of Diels [1929] with maleic or citraconic anhydride to the products of long-wave irradiation of ergosterol. This substance had an antirachitic activity of 20,000 international units per mg. and the subsequent isolation by Linsert [1931] of a second crystallized product of apparently equal activity led Windaus and Lüttringhaus [1931] to assume the exis-

tence of two vitamins, 'vitamin D_1' and 'vitamin D_2'. They subsequently recognized the identity of their vitamin D_2 with calciferol, since it furnished a dinitrobenzoate identical with that of calciferol previously described by Askew *et al.* In addition they stated that 'vitamin D_1' is a molecular compound of calciferol (their vitamin D_2) with another inactive, but isomeric alcohol. Reerink and van Wijk's [1931] crystalline product, 'substance L', appears to be an unstable mixture of various substances, of which calciferol is probably a constituent.

It would therefore seem probable that calciferol (vitamin D_2 of the German workers), a stable crystalline substance of the highest antirachitic activity yet obtained, is vitamin D in a state of purity.

The Chemical Changes occurring on Irradiation of Ergosterol.

One of the most striking chemical changes induced by the action of ultra-violet rays on ergosterol is the loss of precipitability by digitonin [Rosenheim and Webster, 1927, 5], which indicates a steric inversion connected with the secondary alcohol group. The hydroxyl group, however, seems to be only indirectly involved in the activation process, since the irradiated esters of ergosterol are inactive as such, but acquire high activity after hydrolysis [Windaus and Rygh, 1928]. The acetate, benzoate, and palmitate form the only exceptions, their activity after irradiation probably being due to the nature of the acid radical which may easily be split off by the esterases of the animal organism. The fact that ergosterol methyl ether cannot be activated, although spectroscopically almost identical with ergosterol [Heilbron *et al.*, private communication], is further evidence for such a view.

Reduction of the irradiation products with metallic sodium destroys the antirachitic activity, and a crystallized isomeride of dihydroergosterol can be obtained from the reaction mixture [Windaus, 1930, 1]. The inactive products resulting on prolonged irradiation yield on treatment with cyanic acid a mixture of allophanic acid esters, from which two crystallized isomerides of ergosterol, suprasterol I and suprasterol II, have been separated by Windaus *et al.* [1930].

The strong laevo-rotation of ergosterol decreases slowly under the influence of irradiation and gives place to a slight dextro-rotation [Windaus and Linsert, 1928]. According to the same authors the hydroxyl group itself and three double bonds are still present in the reaction mixture freed from ergosterol, and the molecular weight and composition remain the same as those of ergosterol.

Irradiation apparently induces a stereoisomeric change or a rearrangement of ethenoid linkages, or a combination of changes of these types, the nature of which will remain unknown until the elucidation of the constitution of calciferol has been achieved. Calciferol is an alcohol, isomeric with ergosterol, and, like the latter, yields well-crystallized esters. Two of these have been described, the *p*-nitrobenzoate and the 3 : 5-dinitrobenzoate, the latter of which led to its isolation [Askew *et al.*, 1931]. Like ergosterol it possesses a system of conjugated double linkages, as demonstrated by the Diels reaction (condensation with maleic or citraconic anhydride), a fact made use of by Linsert in the alternative method of isolation [1931].

A specific relative position, as yet unknown, of the three double

bonds in the ring system of ergosterol is probably essential for its photo-chemical conversion into calciferol, giving rise to the specific groupings to which antirachitic activity is due.

Colour Tests for Vitamin D.

Shear [1925–26] found that cod-liver oil and irradiated cholesterol gave a characteristic red colour with a solution of aniline hydrochloride in excess of aniline. The suggestion that this reaction might serve for the detection of vitamin D was disproved by Rosenheim and Webster [1926, 1] and by Sexton [1928]. Bezssonoff's [1924, 1] phosphomolybdotungstic acid reagent was also found to be unspecific by the same observers. A number of other colour reactions have been proposed from time to time [Jendrassik and Kemenyffi, 1928; Steigmann, 1928; Stoeltzner, 1928; Meesemaecker, 1930; Cruz-Coke, 1930], but so far none of them has proved to be specific for vitamin D. The colour reactions of calciferol, so far described, are not sufficiently specific for its detection in the presence of other irradiation products of ergosterol.

Specificity of Ergosterol as Provitamin D.

Ergosterol is the only substance known to acquire intense antirachitic activity on irradiation. It would seem that this is an inherent property of the substance and not due to an impurity, for Windaus and Brunken [1928] found that ergosterol recovered from its inactivable peroxide still becomes highly active on irradiation.

This unique property of ergosterol appears to be due to its molecular structure, and in particular to the specific position of the three unsaturated bonds in their relation to the hydroxyl group. None of the artificially prepared isomerides, of which twelve are already known, can be activated (*iso*-ergosterol [Reindel *et al.*, 1927], *u*-ergosterol, ergosterol D [Windaus and Auhagen, 1929], ergosterol F, suprasterol I and suprasterol II [Windaus *et al.*, 1930] etc.). Other substances which gave negative results, although possessing sterol structure, are digitaligenin, choledienic and choletrienic acids, dehydro-ergosterol, dihydro-ergosterol, dihydro-cholesterol, coprosterol, sitosterol, stigmasterol, cholesterilene, and oxycholesterilene; further, all the open chain unsaturated compounds examined: squalene, nerolidol, ψ-ionone and sphingosine.

The relatively slight activity acquired by naturally occurring sterols (cholesterol, zymosterol, fungisterol, α-sitosterol, &c.) is assumed to be due to admixture of ergosterol, the evidence for its presence being based so far mainly on spectroscopic observations. The difficulty of removing the last traces of admixed ergosterol probably accounts for the results reported by various observers [Jendrassik and Kemenyffi, 1927; Bills *et al.*, 1928; Koch, Koch and Lemon, 1929; Koch and Ragins, 1929] who found irradiated purified cholesterol slightly antirachitic when administered in large doses. It has also been claimed by the last-named authors that products possessing after irradiation a low but definite antirachitic activity are obtained by heating purified cholesterol above its melting-point under conditions which avoid appreciable oxidation. These undefined substances seem to be of the

same type as those described by Bills and McDonald [1926], which are formed from cholesterol by the action of fuller's earth (floridin). These authors considered that the mode of action of their products differed from that of vitamin D, a conclusion confirmed by Kon, Daniels and Steenbock [1928].

Photochemistry of Ergosterol.

The Technique of Irradiation.

Many sources of radiation have been tried, but it seems probable that the quartz mercury lamp is the most convenient. It suffers, however, from the defect that the output of ultra-violet rays falls off with use, and for accurate work the output needs to be controlled [cf. Gillam and Morton, 1927]. Windaus and his collaborators made extensive use of the magnesium spark. The preparations obtained from ergosterol by means of either source of ultra-violet rays show no essential differences in antirachitic activity or stability.

It is difficult to define optimal conditions. Provided that measures are taken for an efficient circulation or stirring of the solution, and for exclusion of oxygen during the irradiation, preparations of high activity may easily be obtained if not less than 35 per cent., and not more than 80 per cent. of ergosterol are changed in the process. The rate of change may be ascertained by estimating, by precipitation with digitonin, the amount of unchanged ergosterol in the irradiated product.

Effect of Radiations of Different Wave-length on Ergosterol.

It would appear that only ultra-violet rays and, to a less extent, cathode rays [Knudson and Moore, 1928, 1929] are able to supply the form of energy which is suitable for the conversion of the ergosterol molecule into vitamin D. The ultra-violet radiations of relatively long wave-lengths contained in sunlight are adequate for this purpose [Rosenheim and Webster, 1927, 5], and are presumably the source of energy by means of which the synthesis of vitamin D from ergosterol is effected in nature.

It seems to be established that irradiation with wave-lengths shorter than 270 $m\mu$ ('short-wave irradiation') leads to a rapid inactivation of the vitamin which is formed, and that in 'long-wave irradiation' (with wave-lengths longer than 280 $m\mu$) both the production and the inactivation of the antirachitic substance take place at a slower rate.

X-rays do not produce any antirachitic activity, and according to Morrison, Peacock and Wright [1928] even exert an inactivating effect on ergosterol irradiated by ultra-violet rays [cf. Sumi, 1929].

Exposure to white light in the presence of sensitizers (eosin, chlorophyll, haematoporphyrin, &c.) changes ergosterol into well-defined crystalline products, which are devoid of antirachitic activity [Windaus, Borgeaud and Brunken, 1927]. Attempts to activate ergosterol by the action of heat with catalysts, or by purely chemical methods, have also been unsuccessful.

Role of Temperature.

Antirachitic activity is produced by irradiating alcoholic solutions of ergosterol over the wide temperature range of $-195°$ to $+78°$ C. [Web-

ster and Bourdillon, 1928, 1, 2]. The products obtained at −183° and −195° are definitely less active. Cholesterol containing ergosterol as an impurity can also be activated when irradiated at −183° [Bills and Brickwedde, 1928]. The results provide no grounds for departing from the assumption that the formation of vitamin D from ergosterol and its subsequent inactivation are purely photochemical processes.

Stability.

Although irradiated ergosterol has been found to be unstable, the crystalline vitamin D preparation, calciferol, is remarkably stable under ordinary laboratory conditions. On heating *in vacuo* to 180° it loses its antirachitic potency and undergoes a change (isomerization ?) which has not yet been fully investigated. A similar effect is produced by further irradiation with long- or short-wave ultraviolet rays.

From evidence collected by the Accessory Food Factors Committee it appears that solutions of irradiated ergosterol in olive oil, prepared under certain defined conditions, retain their activity unchanged for 2 years at 0°, but are liable to lose their activity slowly at room temperature. (See Appendix II.)

Whilst the stability of the irradiation mixture in oily solutions seems therefore well established [cf. Holtz and Schreiber, 1930], the experience of different observers as to its stability when kept in a dry condition is somewhat conflicting. This may partly be due to differences in preparation and to the difficulty of completely excluding traces of oxygen during the irradiation. But even when all the operations were carried out in an evacuated system constructed of quartz and glass, Windaus and Auhagen [1931] found a decrease in the optical activity and an increase in absorption of their preparations when kept in a high vacuum at ordinary temperature. The changes due to 'age', however, seem to be unconnected with the vitamin itself, for the decrease of antirachitic activity is only slight in comparison with the rapid change of the other properties. At temperatures between 100° and 200° the antirachitic activity is lost, and a stable substance with an intense absorption at 290 $m\mu$ is formed.

These facts seem to confirm the suggestion advanced by Askew *et al.* [1930, 1] that one of the first products of irradiation of ergosterol is an unstable substance of low absorption and no antirachitic activity, which is converted by heat into a substance absorbing at 290 $m\mu$. The remarkable stability of calciferol, whether prepared from the crude irradiation product by means of the dinitrobenzoate or maleic anhydride or by distillation, is probably due to the removal of this unstable substance by the processes employed.

The Absorption Spectrum of Vitamin D.

The discovery of the fact that a highly antirachitic substance is produced from ergosterol by a photochemical reaction raised the hope that a study of the changes taking place in its ultra-violet absorption spectrum during irradiation would lead rapidly to the elucidation of the mechanism of the vitamin formation. In spite of many years' intensive and laborious research, the information supplied by photo-

chemistry has proved disappointing in its results and the isolation of the crystalline substance, considered to be vitamin D, was finally effected by the methods of organic chemistry.

A detailed discussion of the extensive literature dealing with the spectrographic data, often contradictory, is outside the scope of this Report. The early investigators were misled by the disappearance of the characteristic absorption bands of ergosterol on irradiation, and the appearance of a new band at 247 $m\mu$ [Pohl, 1927; Morton, Heilbron and Kamm, 1927]. They assumed that this new band was characteristic for vitamin D, but such a view, although offering a simple explanation of what proved to be a complex reaction, became untenable. It was soon shown that the exposure of ergosterol to ultra-violet irradiation produced a mixture of substances, and that the amount of vitamin present in the mixture was independent of the period of irradiation, apparently owing to simultaneous formation and inactivation of the vitamin [Rosenheim and Webster, 1927, 5]. Moreover, animal experiments proved that the substance (or substances) showing absorption between 230–250 $m\mu$ was devoid of antirachitic activity [Webster and Bourdillon, 1928, 2; Bills, Honeywell and Cox, 1928; Bourdillon *et al.*, 1929]. For a time it was assumed that three groups of substances were formed in succession, the first of which had an absorption similar to that of ergosterol (maximum at 280 $m\mu$), but twice as intense, and was tentatively held to be vitamin D [Bourdillon *et al.*, 1929]. This view also was later abandoned [Bourdillon *et al.*, 1930], mainly on account of the results obtained with filters, which allowed a differentiation between the action of irradiation with rays of short and long wave-length respectively [Reerink and van Wijk, 1929; Windaus, 1930, 2; Askew *et al.*, 1930, 2].

The uncertainty concerning the absorption spectrum was finally settled by the isolation of the crystalline vitamin D. The absorption of calciferol was found to be between 260–270 $m\mu$ with a maximum of $\epsilon = 48{\cdot}5$ at 265 $m\mu$ which disappears rapidly on further irradiation with short or long waves. The fact, however, that the absorption between 260–270 $m\mu$ is liable to be increased by the simultaneous formation of small quantities of substances with high absorption at 280 $m\mu$ and 290 $m\mu$ makes the study of absorption of doubtful utility for the estimation of vitamin D in mixtures.

The Estimation of Vitamin D.

At the present time purely chemical or physical methods for estimating vitamin D are not available. The only methods are biological, and depend on the power of vitamin D to promote or restore normal bone formation in rats which would otherwise become rachitic when maintained on diets lacking vitamin D and containing calcium and phosphorus in an unfavourable ratio.

Standard of reference. A standard preparation of irradiated ergosterol (vitamin D) has been made available for workers in all laboratories by the National Institute of Medical Research of Great Britain [Med. Res. Council, 1930], and has been provisionally adopted by the (League of Nations) International Conference 1931 on Vitamin

Standards and Units (see Appendix II). By reference to this standard it is possible to state the antirachitic potency of any substance in terms of the unit of the standard, and the results obtained in different laboratories are directly comparable, whether the criteria employed in the tests are macroscopic or X-ray examination of the bones or estimation of the ash content. Further, the use of a stable standard enables trustworthy comparison to be made of results obtained at different times in the same laboratory, and makes it possible to obtain relative values of the antirachitic potencies of substances such as summer and winter milk which obviously cannot be compared directly.

The unit of Vitamin D adopted by the International Conference is defined as the amount of vitamin that possesses the antirachitic activity of 0·001 g. of the standard solution. This corresponds approximately to 1/10,000 mg. of the ergosterol originally used in the preparation of the standard.

Animals suitable for vitamin D tests. The young rats used in tests for vitamin D should be obtained from a healthy and vigorous colony. The stock breeding diet should be kept as nearly uniform as possible throughout the year and be so low in vitamin D content that the reserves of that factor in the young animals are not too great to permit of the development of a suitable degree of rickets on standard rachitogenic diets.

Experimental diets. A severe degree of rickets can in general be produced in young rats by feeding them for about three weeks on a diet devoid of vitamin D and having a high-calcium, low-phosphorus content. The two such diets in common use, in each of which the ratio Ca : P is about 4 : 1, are :

(*a*) McCollum's diet No. 3143 [McCollum, Simmonds and Becker, 1922] consisting of :

Ground yellow maize	33 per cent.
Whole wheat	33 ,, ,,
Wheat gluten	15 ,, ,,
Gelatin	15 ,, ,,
Sodium chloride	1 ,, ,,
Calcium carbonate	3 ,, ,,

and (*b*) Steenbock's diet No. 2965 [Steenbock and Black, 1925] consisting of :

Ground yellow maize	76 per cent.
Wheat gluten	20 ,, ,,
Sodium chloride	1 ,, ,,
Calcium carbonate	3 ,, ,,

These diets may be given dry or mixed with water to form a stiff paste. It should be noted, however, that the constituents may vary in rachitogenic properties.

At the end of three or four weeks' feeding on this diet, young rats will have noticeably swollen wrists and show other signs of rickets, provided that their initial reserves of vitamin D were not too great. Control with X-ray examinations is desirable before beginning the period of test feeding when the curative method is employed, p. 85.

Comparison of Test Substance with the Standard.

Three methods of estimation have been recommended by the Accessory Food Factors Committee, viz. (*a*) estimation of the ash con-

tent of the bones (humeri or femora) [Steenbock and Black, 1924; Chick, Korenchevsky and Roscoe, 1926; Soames and Leigh-Clare, 1928], (*b*) McCollum's 'line' test using the tibia or ulna and radius [McCollum, Simmonds, Shipley and Park, 1922; Steenbock and Black, 1925; Dyer, 1931; Key and Morgan, 1932], (*c*) X-ray examination of these bones [Poulsson and Lövenskiold, 1928; Schulz, 1929; Bourdillon *et al.*, 1931]. Whichever method is used, the rats of each litter must be divided equally between test and standard.

(*a*) *Ash Content of Bones* (*Prophylactic Test*).

Young rats of about 40 grams weight are used. The aim is to find a dose of test substance which, given daily for 3–4 weeks to young rats on one of the above diets, will cause the production of bones having the same percentage of ash as those produced when some selected daily dose of the standard is administered. A preliminary experiment may be necessary to find an approximation to these doses. This is followed by a further comparison using a large number of animals (about 10 on each dose); if the average ash contents in bones of the two groups of rats are not in reasonable agreement, a further experiment is performed with doses more nearly equal in antirachitic value.

To determine the ash content of the bones the animals are killed by coal gas, the bones of both hind legs dissected free from all flesh (the patella being also removed), weighed, dried at 105° to constant weight, extracted with alcohol and ether to remove fatty substances, weighed, ashed, and the ash is weighed. The extraction of fat in this process is important, since the fat content of the bones varies widely with differing nutritional conditions of the animals [Chick, Korenchevsky and Roscoe, 1926].

The calcium in the bones has been shown to be a constant proportion (37 per cent.) of the total ash. The degree of calcification (or degree of rickets) can, therefore, be expressed either as A, the percentage of ash contained in the dried, fat-extracted bone, or as the A / R ratio, the ratio of the amount of ash in the bone to the amount of organic material, other than fat.

A convenient dose of the standard preparation for this test is 0·1 unit daily, but 0·05 to 0·2 may also be used. These doses cover the useful range. The standard and, if necessary, the test substance, should be so diluted with an inert oil that the daily dose is contained in some amount easily and accurately administered.

(*b*) *The 'Line Test'* (*Curative*).

Rats of about 50–60 grams weight are fed on one of the rachitogenic diets for 3–4 weeks, after which period the selected doses of test material and of standard are given daily for 10–14 days. At the end of that time the rats are killed, the distal ends of the ulnae and radii are removed and immersed in 4 per cent. formaldehyde for 24 hours to clear. They are then split longitudinally, immersed in 1·5 per cent. silver nitrate for a few minutes and exposed to light. This converts the phosphates of the bones into silver phosphate, which is reduced on exposure to light, black colloidal silver being deposited in the calcified parts of the bones. The degree of healing produced by

the doses is estimated by macroscopic examination of the blackened areas of the cut surfaces.

The degree of rickets produced in control (untreated) rats is shown by the wide uncalcified cartilaginous metaphysis between the shaft and the head of the bone. In a completely healed rat, this region is completely calcified, i.e. blackened. Different degrees of healing are indicated by narrower or wider 'lines' of calcification across the metaphysis. By comparing the widths of these 'lines' the efficacy of the various doses in curing rickets may be compared. It is useful to draw up a scale of reference showing degrees of healing to which are given values 1 to n, where n is the number of different degrees which the worker finds distinguishable with his own technique. This scale can then be used to assess the result for each rat and from the values thus obtained an average can be calculated for those of a group receiving the same dose. A preliminary experiment is often necessary to determine the dose approximately equal to the selected dose of the standard. When this has been found, a larger experiment should be made with about ten animals on the dose of standard and ten on the dose of test substance that appears to be equal in activity.

A convenient dose of standard for this test is 0·5 or 1 unit.

The bones of rats used in a prophylactic test may be examined by this method also and judged according to the width of metaphysis produced.

(*c*) *X-ray Examination* (*Prophylactic or Curative*).

The bones of rats treated as described in either of the two previous methods are examined by X-ray photography and the degrees of rickets or degrees of healing respectively judged with the use of a scale of reference drawn up as described in the 'line' test.

If the curative method is used the degree of rickets can be estimated at the end of the preparatory period by an X-ray photograph of one knee-joint after lightly anaesthetizing the rat with ethyl chloride. Daily doses of the standard and of the material to be tested are then administered to rats of the same litter, which, after 14 days, are killed with coal gas, and X-ray photographs taken of the same joint as before.

(*d*) *Ash Content of the Bones and Degree of Growth of Rats on Basal Diets deprived of Vitamin D but complete in other respects* (*Prophylactic Tests*).

Methods (*a*), (*b*), and (*c*) are those commonly in use and, though of undoubted practical value, are open to criticism on theoretical grounds because the basal diets are defective not only by absence of the vitamin to be estimated, but also by the unfavourable salt mixture they contain. In method (*d*) the basal diet contains a normal salt mixture and is deprived only of fat-soluble vitamins, vitamin A being conveniently added in the form of 20 per cent. wheat embryo [Soames and Leigh-Clare, 1928].

The period of experimental feeding lasts 50 to 60 days, after which the rats are killed and the leg bones removed and analysed as in method (*a*). The increase in body-weight during the test period may also be used as a measure of the efficacy of the vitamin D dose which has been received [Steenbock, Nelson and Black, 1924–25].

In this method the period of experimental feeding is much longer than in the other methods, but on the other hand, the experimental diet contains optimal proportions of all constituents other than vitamin D; the general health of the rats is more satisfactory and intercurrent illness is avoided. At the present time, however, there is but limited experience of this method and it has not been employed in routine tests.

(*e*) *Estimation of Vitamin D by the change produced in Faecal pH.*

Curative tests. This method elaborated by Jephcott and Bacharach [1926; 1928] is based on the change in reaction of the faeces from slight alkalinity to slight acidity observed when healing takes place in rachitic rats [Zucker and Matzner, 1923–24]. The authors obtained quantitative results when certain precautions were observed, and adopted, as unit for measurement of vitamin D, 1/10 of the amount required to change the faecal pH from 7·3 to 6·7. At present there is, however, little evidence of the applicability of this method in practice [see Redman, 1928; 1929].

Comparison of Methods.

In comparing the above methods, it is evident that X-ray examination has the advantage that the condition of the joints prior to the curative test can be controlled. The procedure is rapid and a permanent photographic record for future reference is provided. In the 'line' test photographs can also be taken of the macroscopic appearance of the bones. Both these methods are extensively used, although involving an error depending on the judgment of the observer in matching the scale values. Estimation of the ash content of the bones has the advantage that the criteria adopted are entirely objective; the tests, on the other hand, are more laborious and time-consuming than in the other methods.

Distribution of Vitamin D in Foodstuffs.[1]

So far as it is possible to compare the distribution of the different vitamins, it would appear that vitamin D occurs more sparsely distributed in natural foodstuffs than the other vitamins. This is at least partially compensated for by the direct influence of sunlight on the skins of animals, for it has been shown experimentally that vitamin D from irradiated impure cholesterol can be absorbed through the skin of rats and rabbits when it has been rubbed on the surface of the skin after shaving off the hair [Hume, Lucas and Smith, 1927]. There is also experimental evidence that some birds derive vitamin D from the oil of their sebaceous 'preen' gland, which is expressed from the gland by pressure of the beak, and the ergosterol of which becomes activated by exposure to sunshine on the surface of the feathers when the birds preen themselves [Hou, 1928, 1929].

The chief sources of vitamin D in the ordinary food supplies of human beings are milk, butter, eggs, and, to a very small extent, fresh green salads and vegetables. For therapeutic purposes cod-liver oil and certain other oils are valuable, and in some cases proprietary preparations of irradiated ergosterol of stated potency are useful.

[1] See Table XXI, Appendix I.

Milk, Butter, and Eggs.

Crawford, Golding, Perry and Zilva [1930] have shown that vitamins D and A are associated wholly with the fat of milk. Thus the potency of the butter may be taken as a measure of the antirachitic activity of the milk from which it was made.

The antirachitic potencies of milk, butter, and eggs vary according to the diet on which the cows and hens respectively have been fed. With ordinary methods of feeding, summer milk is more potent than winter milk, and eggs laid in summer are richer than those laid in winter. Winter milk may, however, be made as rich in vitamin D as summer milk [Golding and Zilva, 1928] and winter eggs as rich as summer eggs [Bethke, Kennard and Sassaman, 1927] by giving the cows and hens cod-liver oil daily in amounts which need not be great enough to flavour the milk, butter, or eggs. The vitamin D content of the milk of cows has also been raised by the daily addition of 200 g. of irradiated yeast to the cows' diet [Wachtel, 1929; Hart, Steenbock, Kline and Humphrey, 1930; Steenbock, Hart, Hanning and Humphrey, 1930]. Direct sunlight incident on the animal also influences the potency of the milk of the cow according to Chick and Roscoe [1926, 2] and of the goat according to Steenbock, Hart *et al.* [1925], but the latter workers were unable to increase the vitamin D content of milk by irradiation of the cow from a quartz mercury lamp [Hart, Steenbock, Scott and Humphrey, 1927; Steenbock, Hart, Riising, Hoppert *et al.*, 1930]. The milk from the same cow was, however, increased about fifteen times in potency by direct irradiation from the same lamp.

Green Vegetables.

The cow, when on pasture, probably obtains some vitamin D from grass, which appears to contain a small amount of this factor when directly under the influence of sunlight, though the vitamin so formed seems to be destroyed when the green leaf is cut and stored for a day or two either in the light or in darkness [Coward, unpublished results on artificial irradiation of growing plants]. Thus it is probable that green salads and vegetables contain appreciable amounts of vitamin D immediately after cutting, if previously exposed to direct sunlight, but that by the time they are purchased from retail shops, they probably contain almost none. Slight amounts of vitamin D have been detected in summer spinach, but none in winter spinach [Chick and Roscoe, 1926, 1; Roscoe, 1927, 1; Boas, 1926]. None was found in the green marine diatom (*Nitzschia closterium*) grown under laboratory conditions, when exposed to sunshine through filters permeable to the ultra-violet portion of the sun's spectrum [Leigh-Clare, 1927, 1].

The vitamin D content of eggs has been increased appreciably by the irradiation of hens kept in pens on a winter feed [Hart, Steenbock, Lepkovsky, Kletzien and Johnson, 1925], but when the hens are kept out of doors in the sunlight and given an adequate diet, irradiation cannot bring about any further improvement.

Mellanby, Surie and Harrison [1929] found that mushrooms bought in the open market in winter were inactive. Vitamin D has, however, been found in ergot of rye (from which ergosterol was first isolated by Tanret in 1889), and also in the embryo of rye by

Mellanby, Surie and Harrison [1929]. Scheunert and Rescke [1931] have shown that the fungi 'Pfifferlinge' and 'Steinpilze', freshly collected or preserved, also contain vitamin D. Preserved 'Morchel' also contained this factor; but 'Champignons' grown in the dark and tested when fresh or preserved were inactive. The daily rat dose necessary to indicate the presence of vitamin D in these plants was about 2 g. per day.

Liver Oils.

Fish-liver oils constitute the richest known source of vitamin D in nature. A valuable quantitative survey of liver oils from different species of fish is given by Bills [1927] who found the antirachitic value of the liver oil from the puffer fish to be fifteen times that of the cod and shark, which were roughly equal to one another in potency [see also Hess and Weinstock, 1925–26]. In comparison with these, negligible amounts only would seem to be present in the liver and body fats of mammals, such as whale, seal, sheep and ox [Rosenheim and Webster, 1927, 6]. According to Poulsson [1929] and S. and S. Schmidt-Nielsen [1930] the liver oils of cartilaginous fishes (dogfish, etc.) contain less vitamin D than cod-liver oil, and this is also true of the liver oils of Cyclostomata (lampern and sea lamprey) [Callow and Fischmann, 1931].

The stomach oil of the fulmar petrel (*Fulmarus glacialis*) [Rosenheim and Webster, 1927, 1] and of the Australasian petrel (*Aestralata lessoni*) [Leigh-Clare, 1927, 2] have been found rich in vitamin D, though inferior to a good sample of cod-liver oil.

Potency of liver oils compared with that of other sources of vitamin D. By the use of the standard of reference described in the section on the estimation of vitamin D (p. 83) the relative antirachitic potencies of samples of milk, butter, cod-liver oil, and other oils can be expressed in terms of the unit there defined.

Of thirteen samples of milk thus tested at different times of the year at the Pharmaceutical Society's Laboratory in London, using the 'line' test, the two most potent contained only 0·2 vitamin D unit per c.c., or 112 units per pint. These samples were produced in the months of August and October respectively. Using a similar method of estimation, the vitamin D value of twelve samples of butter bought in the open market varied from 0·1 to 1·5 units per g., the average being about 1·0 unit per g. The two most potent samples were obtained in May and June and contained 1·4 and 1·5 units per g. respectively.

Thirty-one samples of cod-liver oil similarly examined had potencies ranging from 25 to 200 units per g. Even higher potencies are given for some cod-liver oils by Drummond and Hilditch [1930]. Thus cod-liver oil may possess from 20–400 times the potency of an equal weight of fresh butter in summer and 1 g. of cod-liver oil may possess the vitamin D value of a pint of fresh milk.

It must be remembered, however, that in milk the rich supply of calcium salts and phosphates enhances the efficiency of the relatively small amount of vitamin D which it contains. The value of milk as an antirachitic agent in diet would be therefore much underestimated if gauged only by its content of vitamin D (see also p. 217).

Origin of vitamin D in cod-liver oil. There is, as yet, no satisfactory explanation of the rich deposits of this vitamin which are present in the liver oils of many fishes. It is difficult to suppose that a deep-sea fish, such as the cod, is exposed to a sufficient intensity of sunshine to activate a precursor in its superficial tissues and it has been assumed that the antirachitic vitamin in its liver is derived from the food it consumes. The ultimate source of the fat-soluble vitamins has been traced by Drummond, Zilva and Coward [1922] and others, through a series of intermediaries which include the smaller fish on which the cod feeds, and the copepods and mollusca in the plankton which forms the diet of the former, to unicellular marine organisms. The liver oil of the caplin, *Mallotus villosus*, which forms the chief food of the Newfoundland cod in midsummer when its liver is richest in oil and in vitamin D, is, however, relatively poor in this vitamin [Bills, 1927] and although a marine diatom *Nitzschia closterium* was found able to synthesize large amounts of fat-soluble vitamin A [Jameson, Drummond and Coward, 1922], no traces of vitamin D have been detected in it [Leigh-Clare, 1927, 1]. It must be remembered, however, that although the amount of vitamin in the daily food of the fish may be small, it may be retained in the liver so effectively that in the mature fish the accumulation will be considerable.

The suggestion that the rich depots of vitamin D in fish-liver oils may be formed by some unknown method of direct synthesis has been made by Bills [1927] on the basis of some interesting experiments with young catfish (*Ictalurus punctatus*) under laboratory conditions.

CHAPTER IV

VITAMINS AND DENTAL TISSUES.

The importance of vitamins in relation to teeth and associated periodontal tissues has been revealed by experimental investigation on animals. In some cases the results have been afterwards subjected to clinical trial on human beings and the facts obtained by animal investigation have been shown to be of significance in human dental problems. Clinical trials on man are obviously necessary before any appraisement can be made of the relative importance of the experimental results obtained with animals, because of the variation among different species in their reaction to deficiencies of the various vitamins.

Two types of dental defect are commonly found in civilized man: (1) dental caries, and (2) periodontal disease, including pyorrhoea alveolaris. The first of these concerns the tooth itself, the second involves both the soft structures adjacent to the tooth and the hard alveolar bone. The aetiology of these diseases seems to be closely bound up with the dietetic factors which control the structure of the hard and soft dental tissues respectively. Various vitamins seem to control the structure of these tissues, although other factors of the diet appear also to be of importance. Thus vitamin D is concerned with the structure of the teeth and alveolar bone, while vitamin A plays a similar part with regard to the structure of the marginal epithelium of the gums. Experimental work on guinea-pigs has demonstrated that a deficiency of vitamin C in the diet also produces pathological changes in the teeth and periodontal tissues.

Vitamin D in Relation to the Teeth.

The investigations carried out by M. Mellanby (1918–30) have included animal experiments and clinical investigations on man. The former have demonstrated the importance of specific dietetic factors in controlling the development of the teeth, and in determining their reaction to harmful stimuli. The earlier experiments proved that the production of normal or perfectly calcified teeth in young dogs could be ensured by an adequate supply during the period of growth of a fat-soluble vitamin, later identified as vitamin D, thus showing a complete analogy with the production of well-calcified bones and protection from rickets [M. Mellanby, 1918; 1920; 1923, 2; 1929].

Young puppies fed on diets deficient in vitamin D exhibit obvious defects in the development of their teeth and jaws, the most important of which are the following:

1. Delay in the eruption of the permanent teeth, affecting particularly those teeth which normally erupt at a late period.
2. Thickening of the jaw bones and irregularity in the arrangement of the teeth, most noticeable in the lower incisors.
3. Irregularly formed and poorly calcified enamel, the surface of which is pitted and grooved and frequently pigmented.

(*a*)

FIG. 7.

(*a*)

FIG. 8.

(*a*)

FIG. 9.

Photographs (*a*) of the lower jaws and photomicrographs (*b*) of ground sections of the carnassial teeth of three puppies of the same family all of which were given the same basal diet consisting of bread, separated milk powder, lean meat, orange juice, yeast and salt. The diets were begun and the animals all killed at the same time.

FIG. 7. Basal diet plus olive oil. (*a*) The surface enamel of the teeth is rough and pigmented. Eruption delayed. (*b*) The enamel and dentine are thin and imperfectly formed, and many 'interglobular' spaces (uncalcified areas) are present in the dentine.

FIG. 8. Basal diet plus olive oil, but for the last month of life cod-liver oil was given. (*a*) The enamel surface is rough, pigmented and thin, having been formed prior to the improvement in diet. (*b*) The earlier formed dentine is similar to that seen in FIG. 7*b*, but has had much well formed tissue added since the change of diet from one containing little vitamin D to one containing an abundance of this substance.

FIG. 9. Basal diet plus cod-liver oil. (*a*) The teeth are smooth, white and shiny. (*b*) The enamel and dentine are thick and well calcified, and there are no 'interglobular spaces' in the dentine.

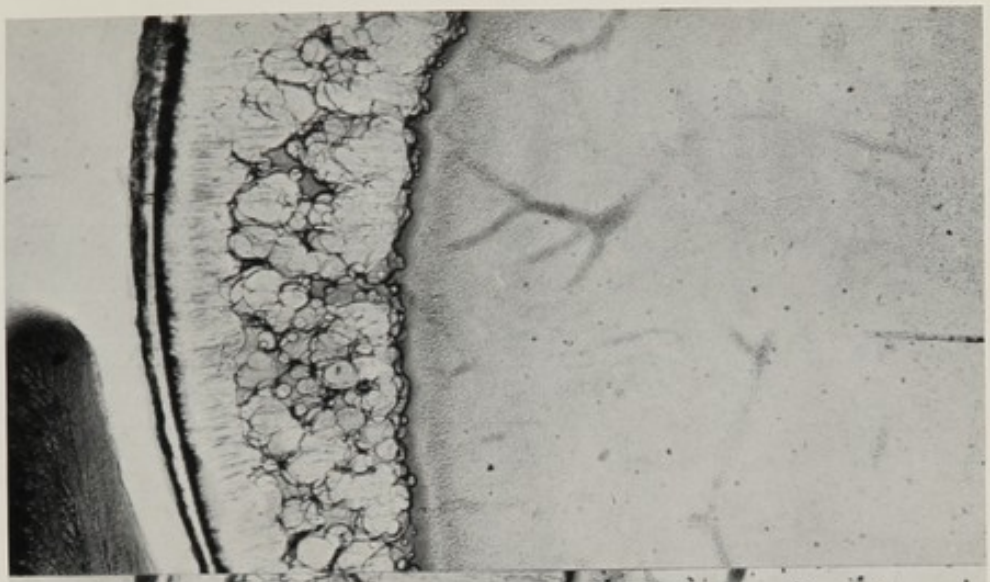


FIG. 7 (*b*)

FIG. 8 (*b*)

FIG. 9 (*b*)

4. Defective development and irregular calcification of the dentine, which can be satisfactorily studied only by microscopic examination of ground sections of the teeth.

5. Abnormal development of the calcified tissues at the gingival margin and hence irregularity of the periodontal membrane.

Macroscopical and microscopical appearances of the structure of well and badly formed teeth are represented in Figs. 7–17. It will be seen that when olive oil, which contains no vitamin D, was given as a supplement to a rachitogenic diet, the tooth surface in puppies was rough and pigmented, only a thin layer of enamel was formed and the subjacent dentine was not only defective in amount, but was also irregularly laid down, and contained numerous dark areas of defective calcification (the so-called interglobular spaces) interspersed between the spheroidal masses of calcium deposit. When an adequate supply of cod-liver oil, which contains abundance of vitamin D, was given in place of olive oil, the surface was smooth, white, and shiny, and much thicker layers both of enamel and of dentine were produced; the dentine exhibited a perfectly uniform structure with a complete absence of interglobular spaces. It was easily possible to devise diets intermediate in their calcifying activity between these two examples.

M. Mellanby urges that the term hypoplasia should not be limited to gross naked-eye defects, but should be used for any defect of tooth structure. This point is of considerable importance in connexion with the relation of dental structure to dental disease.

Reference to Figs. 7*b*, 8*b*, 9*b* makes it clear that during the period of growth the quantity and character of the dentine laid down at any given time may depend on the vitamin D content of the diet at that time. The puppy whose tooth is figured in Fig. 8*b* received no vitamin D for 5 months, during which time only a small amount of badly calcified dentine was laid down. For the next month it received cod-liver oil, and during this period the dentine formed was quite regular in structure and was almost equal in amount to that formed during the previous five months. The change from a diet with poor to one with good calcifying qualities was found to be reflected very quickly in an improvement in the structure of the developing teeth, whereas the reverse change from a good diet to a poor one was followed by defective calcification of the teeth only after a long and variable period. This delay is interpreted as indicating a utilization of vitamin that has previously been stored.

Effect of Maternal Feeding.

The amount of vitamin D consumed by a bitch during pregnancy and lactation was found to influence the calcification of the deciduous teeth of her offspring [M. Mellanby, 1921; 1928; 1929]. These teeth erupted at an earlier age and were better calcified when the mother received liberal supplies of vitamin D. Of great practical importance was the observation that the feeding of the mother during pregnancy and lactation might have an even greater effect on the development and structure of the permanent, than of the deciduous, teeth of the offspring. When the puppies themselves received a liberal supply of vitamin D after

weaning, the character of the previous maternal feeding had only a comparatively slight influence on the development of the permanent teeth. But when the puppies were fed after weaning on diets deficient in vitamin D the previous maternal feeding had great influence on the state of calcification of these teeth. When the mother's diet had possessed poor calcifying properties the teeth of her offspring erupted at a later date, and their structure was much more defective than when her diet had contained liberal supplies of vitamin D. Even after a puppy had been fed for a period on a good diet the character of the dental tissues laid down subsequently during a spell of bad feeding was influenced by the previous maternal feeding. The results demonstrated that the principles of maternal feeding which have been found to operate in the case of experimental rickets operate also in determining the structure of the teeth.

Factors other than Vitamin D which may influence the Structure of the Teeth of Puppies.

Variations in the amount of protein or pure carbohydrate in the diet of puppies were found to have no definite effect on the structure of the developing teeth, whether the basal diet contained much or little vitamin D. Similarly the quantity of fat in the diet had little influence, although the quality, that is to say its content of vitamin D, was of paramount importance. The water-soluble vitamins, including the B complex and C, had little or no effect. The principal food factors, other than vitamin D, which were found capable of influencing the calcification of the teeth, were the type and quantity of cereal and the amount of calcium [M. Mellanby, 1929].

Effect of Cereals on the Structure of the Teeth.

When the diet of a puppy contained an abundant supply of vitamin D, the developing teeth exhibited a perfect structure whatever the quantity or type of cereal. But when there was little or no vitamin D in the diet, both the quantity and quality of the cereal might exert an influence on the type of calcification of the teeth. The observation that the greater the amount of carbohydrate eaten, either in the form of cereal or of starch or sugar, the more defective is the development of the teeth, may possibly be explained in part by the suggestion that the more rapid increase in weight which occurs with an increase in the energy-producing portion of the diet demands an increased supply of vitamin D, and if this is not forthcoming the relatively greater deficiency of the vitamin will be reflected in more defective calcification of the teeth. But that this is not the whole explanation is clear from the results obtained with different cereals. Of those tested, white flour and rice were associated with the production of the least badly calcified teeth and oatmeal of the worst. Rye, barley, and maize were intermediate in their effects. The anticalcifying action of the cereal in the puppy's diet was observed only in the absence of an adequate supply of vitamin D, and could be completely controlled by liberal quantities of this factor, no matter what type of cereal was included in the diet [M. Mellanby, 1923, 2; 1929].

Fig. 10.

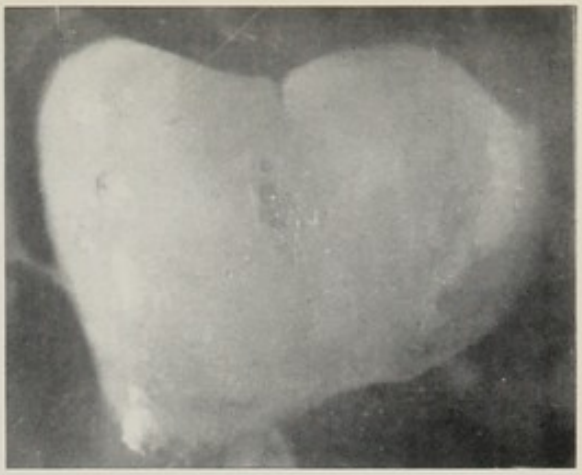


Fig. 1

Fig. 11.

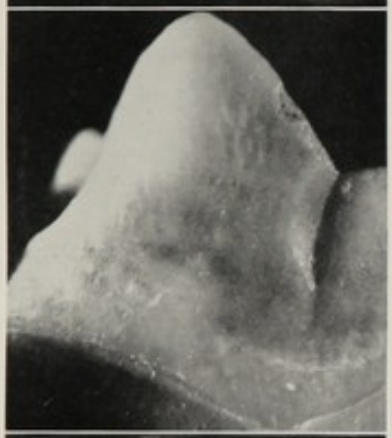


Fig. 1

Fig. 12.

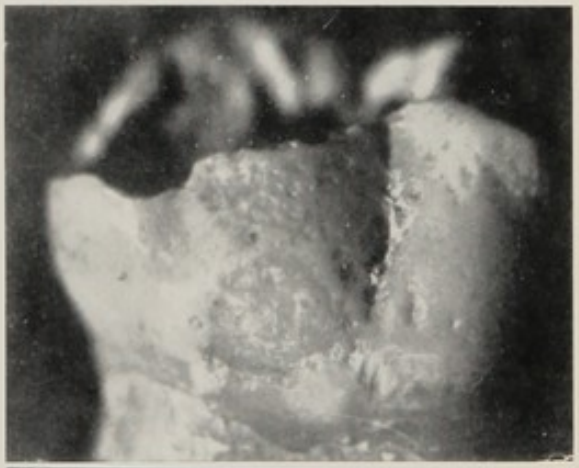


Fig. 1

Fig. 13.

Fig. 1

Figs. 10–13. Photographs (enlarged) of the surface enamel of the molar teeth of experimental dogs 10, abundance of fat-soluble vitamins; 11, some fat-soluble vitamins; 12, little supply of fat-solubl vitamins; 13, very little supply of fat-soluble vitamins. Figs. 14–17. Photographs (enlarged) of th surface enamel of human molars.

Figs. 10 and 14. Normal—smooth and shiny.

Figs. 11 and 15. Hypoplasia—some roughness.

—rough.

—comparatively large areas of deficient enamel.

Effect of the Mineral Supply on the Structure of the Teeth.

The absolute or relative amounts of calcium and phosphorus in an experimental diet were found to influence the degree and character of the calcification of the teeth. When there was little or no vitamin D in the diet extra supplies of calcium in the form of carbonate resulted in a slight but distinct improvement in the structure of the teeth. The effect of adding phosphates under these conditions was variable, but the beneficial effect sometimes observed was only slight. The addition of calcium salts, the carbonate, phosphate, or hydrogen phosphate, to diets containing butter resulted usually in great improvement in the calcification of the teeth. When liberal quantities of vitamin D were given, the addition of calcium salts to a diet otherwise containing very little of this element sometimes resulted in the production of an increased amount of dentine, though the structure was always perfect or nearly perfect even when the diet contained only small amounts of calcium. In the presence of sufficient quantities of vitamin D the ratio of calcium to phosphorus could also be varied within considerable limits without producing any effect on the development of the teeth. It therefore appears safe to conclude that an adequate supply of vitamin D is of greater importance in determining the perfect development of the teeth than an abundant or carefully balanced supply of calcium and phosphorus [M. Mellanby, 1923, 2 ; 1929].

Effect of Ultra-violet Irradiation on the Structure of the Teeth.

Exposure of puppies fed on diets deficient in vitamin D to the rays of a mercury vapour lamp for half an hour three times a week resulted in a distinct improvement in the calcification of their teeth [M. Mellanby, 1924 ; 1929]. When the diets had a potent ant-calcifying action, as for example when they contained large amounts of oatmeal, the effect of irradiation was comparatively slight ; in no instance did the irradiation of puppies under these conditions bring about the same improvement in the structure of the teeth as was observed when a potent source of vitamin D was included in the diet.

The irradiation of food destined for the feeding of animals that received no other source of vitamin D was followed by a great improvement in the calcification of their teeth. Fats such as olive oil which previously had no stimulating effect were found after irradiation to be capable of promoting the formation of teeth with an almost perfect structure. Oatmeal, after exposure to the mercury vapour lamp, not only ceased to have a deleterious effect on calcification, but actually acquired definite calcifying properties, which were due in all probability to the activation of the parent substance of vitamin D (ergosterol) originally present in the cereal. Precisely similar results were obtained by irradiating the germ of wheat and maize. The administration of small quantities of irradiated ergosterol to puppies resulted in the production of teeth of good structure.

The series of experimental results just outlined makes it quite clear that a plentiful supply of vitamin D during the period of growth is essential for the production of normal or perfect teeth in the dog. M. Mellanby has carried out similar experiments on rabbits and rats

[1930, 2], which indicate that this principle holds good for these species also. Toverud [1926, 1931] studied the calcium content of the teeth of rats fed on complete and deficient diets. His analysis showed the calcium and phosphorus of the ash of the teeth to be reduced and the magnesium increased, especially with diets poor in calcium and fat-soluble vitamins, while with diets poor in calcium and rich in vitamin almost normal values were obtained. Toverud also concluded from his experiments that the chemical composition of the molars is influenced if the mothers have been fed during pregnancy on diets deficient in calcium and fat-soluble vitamins. Orban [1927], also working with rats on defective diets, found big differences in the rate of growth of the incisors as well as in the structure of the developing dental tissues. He concluded that milk, cod-liver oil, and calcium salts greatly improve calcification.

Applicability of Animal Results to Man.

The same factor, therefore, which influences the calcification of the long bones in the dog, the rabbit and the rat, determines the calcification of the teeth in these species. Since it has been abundantly proved that this factor is just as effective in promoting good calcification of the bones in the child as in experimental animals, it may reasonably be expected that the calcification of the teeth of the child will also be influenced by the available supply of this factor during the period of growth. Although it has not yet been possible to test this assumption on any large scale, limited trials have shown [M. Mellanby, 1928] that the addition of a daily dose of cod-liver oil to children's diets results in the production of good teeth which appear sound to naked-eye examination and show a normal structure microscopically.

Vitamin D however is usually deficient in the ordinary diet, especially of the poorer classes, and on the assumption that this factor is of primary importance in the development of normal deciduous teeth in man, as well as in dogs and other animals, it might be expected that the teeth of the average child would be defective in structure. This, indeed, has been shown to be the case [M. Mellanby, 1923, 1; 1927, 1]. Microscopic examination of a large and representative collection of human teeth showed that the majority were defective in structure, contrary to the view generally expressed that only a negligible proportion of children's deciduous teeth in England are defective, or hypoplastic. The sections examined varied considerably in structure, from perfect teeth, as seen in dogs fed on diets containing liberal amounts of vitamin D, to very defective teeth resembling those seen in dogs on diets very deficient in this vitamin. It soon became evident that the macroscopical structure of the majority of these teeth was also defective or hypoplastic, as revealed by a careful examination of the enamel surface for discoloration and obvious pits and grooves, and more especially for the general 'feel' as gauged by rubbing with a blunt probe. The macroscopic appearance is, in fact, related to the histological structure of the enamel and dentine, teeth with varying grades of roughness usually showing corresponding histological defects (see Figs. 10-17). With practice it is generally possible to predict the internal structure of a tooth from a superficial examination.

In Tables I and II are classified according to their microscopical

structure over 1,000 shed and extracted deciduous teeth of British children. These teeth were obtained partly from private sources and partly from children attending public elementary schools.

TABLE I.

Teeth obtained from private sources.

	Number examined.	*Normal Per cent.*	*Slight hypoplasia Per cent.*	*Hypoplasia Per cent.*
Incisors . . .	113	53	43	4
Canines . . .	54	11	57	32
1st molars . .	55	14	31	55
2nd molars . .	40	0	15	85
Total	262	28·3	39·3	32·4

TABLE II.

Teeth obtained from children attending public elementary schools.

	Numter examined	*Normal Per cent.*	*Slight hypoplasia Per cent.*	*Hypoplasia Per cent.*
Incisors . . .	123	45	31	24
Canines . . .	102	6	27	67
1st molars . .	194	5	16	79
2nd molars . .	355	1	6	93
Total	774	9·7	15·4	74·9

These results show that varying degrees of abnormality were found in 72 per cent. of the former and in over 90 per cent. of the latter, indicating that the majority of children's deciduous teeth are defective in structure, that the old criteria of dental structure are not justified, and that the teeth of poorer children are more defective than those of the child in better circumstances.

RELATION OF STRUCTURE OF TEETH TO DENTAL DISEASE.

To demonstrate the possibility of promoting the development of good teeth in man would be in itself a noteworthy achievement. From the point of view of preventive medicine, however, it is still more desirable to be able to lessen the incidence of dental caries. It has not been easy to study in detail the influence of food factors on the production of dental caries, for the reason that it is difficult to produce this condition in experimental animals. McCollum, Simmonds, Kinney and Grieves [1922] and Marshall [1922], Bunting [1925] and others have described caries-like lesions in rats fed on diets deficient in fat-soluble vitamins and calcium. M. Mellanby [1930, 2] found it almost impossible to produce dental caries in dogs, even when their diets were thoroughly bad in the sense both of producing defective calcification and of encouraging fermentation in the mouth. Nevertheless, she had previously found it possible to study the effect of variations in the diet on the response of dog's teeth to harmful stimuli other than microbic invasion [M. Mellanby, 1923, 3; 1930, 2]. When the teeth of puppies or grown dogs are filed at regular intervals it is found that the dentine in such teeth exhibits a reaction to the injury. Changes can be seen in the already existing primary dentine, and over a localized

area, corresponding with the site of injury, a new formation of secondary dentine occurs adjacent to the pulp. The amount and the quality of this secondary dentine were found to vary in the dog according to the calcifying properties of the diet on which the animal was fed during the experiment. With a liberal supply of vitamin D in the diet, abundant well-formed secondary dentine was usually produced, while, with an insufficient supply of the vitamin, secondary dentine was either not produced at all or was obviously defective in character. The animal experiments therefore demonstrated that the calcifying properties of a diet influenced the character of the reaction of teeth to injury as well as their original structure.

Dental Caries.

Relation between Structure and Presence of Caries in Human Teeth.

M. Mellanby [1923, 1 ; 1927, 2] concluded from a study of a large number of human deciduous teeth that there was a close relation between structure, or calcification, and liability to caries, as indicated in Table III. Out of a total of 1,036 teeth examined superficially and microscopically, 925 were found to support the general principle that caries is liable to occur in teeth that are imperfectly calcified, while well-calcified or properly formed teeth are not subject to caries.

Table III.

	Normal 149.		*Slight hypoplasia* 223.		*Hypoplasia* 256.		*Severe hypoplasia* 408.	
	No.	*Per cent.*	*No.*	*Per cent.*	*No.*	*Per cent.*	*No.*	*Per cent.*
No caries . .	89	60·0	104	46·6	*11*	4·3	*26*	6·3
Slight caries.	31	20·6	46	20·6	39	15·1	*22*	5·4
Caries . . .	*6*	4·0	50	22·4	86	33·9	59	14·5
Severe caries	*23*	15·4	*23*	10·4	120	46·7	301	73·8

It is obvious that in a rough estimate of caries and structure in teeth, some of which were shed, some extracted and some obtained post-mortem, it was impossible to regard as exceptions teeth showing no caries although slightly hypoplastic. It was therefore decided to regard as exceptions to the main rule only those teeth in Table III, the figures for which are printed in italic type, and which number 111.

An explanation of most of these discrepancies was forthcoming when the character of the secondary dentine was examined. Of 59 teeth in which the primary structure of the tooth was defective and there was no caries, 33 had well calcified secondary dentine. Similarly, of 52 teeth with carious cavities and yet well calcified primary dentine, 47 either had no secondary dentine, or it was badly calcified. It would therefore appear likely that in 80 of the 111 exceptions to the general principle, the resistance to caries had been raised or lowered after eruption by the same factor which had been shown in experimental animals to determine the character of the response of their teeth to harmful stimuli, namely the calcifying potency of the diet at the time of application of the stimulus.

These facts suggest strongly that one very important factor determining the incidence of dental caries is the initial structure of the teeth, which in turn depends on the specific calcifying properties of the diet taken during the period of their development, and especially on the presence of sufficient vitamin D. They also suggest the possibility that even when the initial structure of the teeth is defective the resistance to dental caries may be raised by increasing the calcifying potency of the diet, which ensures the formation of well calcified secondary dentine at any spot where bacterial invasion may be threatened or may actually have begun. This second suggestion has been put to the test of direct experiment.

The Influence of Dietetic Factors on the Initiation, Spread and Healing of Dental Caries in Children.

A series of four investigations to test the effects of dietetic variation on the spread of dental caries in children was made by M. Mellanby and Pattison in a Sheffield Hospital for surgical tuberculosis. In the first of these [M. Mellanby, Pattison and Proud, 1924] the diets of different groups were varied so as to differ as far as possible in calcifying properties. Thirty-two children aged about 7 years were divided into three groups; group 1 were given a diet which was designed to promote perfect calcification of the teeth; group 3 received a diet which had poor calcifying properties, including little vitamin D and no extra sources of calcium, but much cereal; group 2 were kept on the ordinary hospital diet. The teeth were carefully examined at the beginning of the investigation, records being made of the position, extent, and degree of hardness of each carious point. After a lapse of seven and a half to eight months the teeth were again examined. The results are given in Table IV.

TABLE IV.

Sheffield Investigation I.

Diet.	*Average no. of teeth per child showing initiation or increase of caries.*	*Average amount of 'arresting' of caries per child.*
1. Abundant fat-soluble vitamins (no oatmeal).	1·4	1·5
2. Intermediate amount of fat-soluble vitamins.	2·9	1·0
3. Comparatively little fat-soluble vitamins (some oatmeal).	5·1	0·7

In the second investigation [M. Mellanby and Pattison, 1926] 71 children of ages varying from 6 to 12 were also arranged in three groups. The diets given to the members of the three groups were similar to those used in the first experiment, but were so adjusted that the total quantities of protein, carbohydrate, and fat were approximately the same. The energy values of the diets, the calcium and phosphorus intake and ratios, and the acid-base ratios were also fairly uniform. Every child used a toothbrush twice a day. At the beginning of the investigation the teeth of the children about to receive the best calcifying diet were, according to careful naked-eye examination, rather worse calcified and were more carious than those of the other children. At the end of the period of special feeding ($6\frac{1}{2}$ months) examination of the teeth gave the results shown in Table V.

TABLE V.

Sheffield Investigations II, III, and IV.

	Diet.	*Average no. of teeth per child showing initiation or increase of caries.*	*Average 'degree' of increase of caries per child.*	*Average amount of 'arresting' (hardening) of caries per child.*
II.	4. Abundant fat-soluble vitamins (no oatmeal)	1·8	2·0	2·0
	5. Intermediate amt. of fat-soluble vitamins (no oatmeal).	3·0	4·0	1·2
	6. Comparatively little fat-soluble vitamins (some oatmeal).	5·8	6·7	0·04
III.	7. Much vitamin D added (Irradiated ergosterol).	1·0	1·1	3·9
IV.	8. Cereal-free; much vitamin D.	0·37	0·32	4·7

The striking correspondence between the results of these two investigations leaves little room for doubt that the initiation, spread, and arrest of dental caries in children can be controlled to a very considerable extent by including in their diets an abundance of those food factors which had previously been shown in animal experiments to exert a favourable influence on the calcification of the teeth.

In the third investigation undertaken by M. Mellanby and Pattison [1928] the effect on the spread of caries of giving a liberal supply of vitamin D in the form of irradiated ergosterol only without other alteration in the hospital diet was tested on 21 children. After these children had received the vitamin D preparation for 6½ months it was found that the average number of teeth per child in which caries had spread was 1·0, the average 'degree' of increase 1·1 and of hardening 3·9 (see Table V, Investigation III, diet 7). In many instances the soft and active caries observed at the beginning of the test was found to be undergoing arrest or actually to have become arrested when the final examination of the teeth was made. This experiment makes it clear that it is the fat-soluble vitamin D that is responsible for raising the resistance of the teeth to caries and not the fat-soluble vitamin A, which in the previous investigations had been supplied to the children together with vitamin D.

Since 1928 an extension of the Sheffield investigations has been in progress in three 'Cottage Homes' in Birmingham, under the auspices of the Dental Disease Committee of the Medical Research Council. Although in this case the basal diets were not standardized as in the Sheffield work and the vitamin additions were less, this scheme had the advantage of extending over a longer period and of involving larger numbers of children (332). The results corroborate those obtained in Sheffield, as will be seen from Table VI, p. 99. In this Table the Sheffield results are given in a form comparable with the Birmingham figures. The progress of caries was significantly reduced in the group receiving additional fat-soluble vitamins as cod-liver oil and in the group having additional vitamin D in the form of irradiated ergosterol.

By analogy with the animal experiments, showing that cereals, and especially oatmeal, interfere with calcification, it seemed probable

that extra cereals in the form of oatmeal might increase the spread of caries when the supply of vitamin D was limited. Extra oatmeal was given to a group of children in the Sheffield investigations, and the spread of caries in this group was found to be greater than in any other. The effect of the omission of cereals was not tested in the investigations described above. Independently, Boyd and Drain [1928; Boyd, Drain and Nelson, 1929] observed during the routine examination of the teeth of children attending a hospital department that in twenty-eight instances arrest of previously extensive and active caries was taking place. It was then found that without exception these children were receiving treatment for diabetes and were being strictly dieted, their food consisting chiefly of milk, cream, butter, eggs, cod-liver oil, meat, bulky vegetables, and fruit. Two most noticeable features of such diets are their richness in fat-soluble vitamins and mineral salts and their poverty in cereals, features which had characterized the diets used by M. Mellanby and her co-workers to raise the resistance of children's teeth to caries.

TABLE VI.

	Percentage of teeth carious.			*Percentage increase in 'degree' or extent of caries.*
	First examination.	*Final examination.*	*Increase.*	
Sheffield:				
Olive oil group. (Diet 6)	25·96	35·96	10·00	62·13
Cod-liver oil group. (Diet 4)	42·86	44·41	1·55	10·28
Vitamin D group. (Diet 8)	45·13	46·14	1·01	6·57
Birmingham:				
Olive oil group.	15·59	23·22	7·63	45·85
Cod-liver oil group.	19·26	22·23	2·97	9·81
Vitamin D group.	22·18	24·27	2·09	10·12

Further observations on the effect of giving similar diets to thirteen children not suffering from diabetes were carried out by Boyd and Drain, who report that in each instance pre-existing caries was completely arrested in ten weeks or less. It was found that considerable quantities of carbohydrate could be taken by the children without hindering the progress of the arrest of their caries, providing that dairy produce, cod-liver oil, vegetables, and fruit were consumed in sufficient quantities at the same time. Incidentally it was remarked that oral hygiene did not seem to play any part in the arrest of caries, for this was noted in several instances in which the children did not use a toothbrush.

Believing that the results obtained by Boyd and Drain were due to the fact that vitamin D was given in abundance while cereals were absent from the diet (although these authors did not themselves make any statement to this effect) M. Mellanby and Pattison [1932] have carried out a similar investigation to test this supposition. The results (see p. 98, Table V, Investigation IV, diet 8) indicate that the total omission of cereals from the diet, together with the addition of vitamin D, hastens the arrest of existing caries and prevents the initiation of new carious points to greater extent than the addition of vitamin D to a diet containing cereals.

Bunting and his collaborators [1930] have compared the active caries in children in different institutions in which the dietary was controlled to some extent. In one of these each child had one pint of milk, some fruit and green vegetables daily ; the amount of sugar was reduced and a mouth wash was used. At the end of one year, the teeth of 80 per cent. of the children were found to be free from active decay as compared with 24 per cent. in a second institution in which the mouth wash alone was used, and 18 per cent. in the control institution where no additions to the diet were made and no mouth wash used. They therefore conclude that caries is a specific infective process the activity of which is dependent on certain metabolic changes, and state that, of the methods they employed for combating dental caries, dietary measures appeared to be the most important.

There is, therefore, a considerable body of evidence pointing to the fact that dietetic factors, and especially vitamin D, determine to a large extent the resistance of children's teeth to dental caries. For practical purposes the ideal to aim at is by correct feeding during prenatal life, infancy, and childhood, to encourage the production of normal or perfect teeth in the adult. This, with the continuance of a sound dietetic régime throughout life, should result in a very greatly diminished incidence of dental caries in the community.

Periodontal Disease.

Dogs, especially pet dogs, are, like man, very susceptible to periodontal disease. These animals were therefore chosen by M. Mellanby for an extensive study of the aetiology of this disease of the tissues surrounding the teeth [1920 ; 1929 ; 1930, 1, 2]. It was found that dogs could be kept under laboratory conditions for periods up to 8 years and, so long as the diet was complete from birth onwards, they remained free from periodontal disease. If, however, the diet was deficient in vitamins A and D and rich in cereals, especially during the period of growth in the early months of life, this disease ultimately developed. These dietetic defects acted in two ways, (1) by allowing the abnormal development of periodontal tissues, and (2) by allowing these abnormally formed tissues to change their anatomical relations to the teeth and become subject to invasion by micro-organisms.

Vitamins A and D in relation to the Structure of Periodontal Tissues.

Experiments on puppies brought up on diets rich and poor respectively in both vitamins A and D, or deficient in either vitamin A or D only, showed that vitamin A primarily controls the normal development of the soft periodontal tissues, including the sub-gingival epithelium and the underlying connective tissues, while, as might be expected, vitamin D is largely responsible for the normal calcification of the alveolar bone and the other hard tissues.

Thus, if the diet is deficient in both fat-soluble vitamins A and D, as for instance when olive oil replaces cod-liver oil, the following abnormalities develop :

(1) Thickening of the gingival region (see Figs. 18, 20 ; cp. Fig. 19).

FIG. 18. FIG. 19. FIG. 20.

The comparative effects of a deficiency of vitamins A and D together and of vitamin A alone on the development of the gingival tissues.

Photomicrographs (X 40) of the gingival regions of the carnassials of three dogs. *Note*: The alveolar bone and periodontal membrane are not seen here. (Sections decalcified and cut in celloidin; enamel therefore lost.)

FIG. 18. Diet poor in vitamins A and D. *Note*: Gingival regions hypertrophied; sub-gingival epithelium (S. Ep.) much hypertrophied and irregular with small processes; some connective tissue reaction or cell infiltration (C.I.) in corium.

FIG. 19. Liberal supply of vitamins A and D in diet. *Note*: Gingival region thin; sub-gingival epithelium (S. Ep.) thin and regular; no connective tissue reaction or cell infiltration in corium.

FIG. 20. Much vitamin D but very little vitamin A in diet. *Note*: Gingival region hypertrophied; sub-gingival epithelium (S. Ep.) hypertrophied and irregular with some processes; some connective tissue reaction or cell infiltration (C.I.) in corium.

(2) Imperfect development of the alveolar bone, particularly of the alveolar crest, and of the laminae durae, with the inclusion of osteoid tissue and poorly calcified bone.

(3) Imperfection of the epithelial attachment to the hypoplastic enamel.

(4) Poor calcification of the laminae durae.

(5) Hypertrophy of the sub-gingival epithelium, with the development of finger-like processes (see Figs. 18, 20, cp. Fig. 19).

(6) Abnormal arrangement of the connective tissue of the corium, with connective tissue reaction and cell infiltration (see Figs. 18, 20, cp. Fig. 19).

(7) Irregular thickness of the periodontal membrane.

Irregular alignment of some of the teeth and tilting of the teeth in relation to the jaw are also sometimes found when the diets are deficient in vitamins A and D.

Vitamins A and D in relation to Periodontal Disease.

The term 'periodontal disease', which is susceptible of various meanings, is here used in its widest sense, that is to say, to denote any morbid condition affecting the tissues surrounding the teeth, ranging from simple gingivitis to 'pyorrhoea alveolaris' with extensive absorption of bone.

McCollum, Simmonds, Kinney and Grieves [1922] found what they term 'attaching-tissue' lesions after death in some rats fed on experimental diets, especially when these were low in calcium, phosphorus and fat-soluble vitamins.

In M. Mellanby's experiments [1930, 1, 2] the majority of the dogs used were about 2 months old when the experiments were begun, and in some cases were born in the laboratory from mothers whose diets were controlled throughout pregnancy and lactation. In a few experiments in which curative rather than prophylactic measures were tested, the animals were several years old when brought to the laboratory suffering from periodontal disease.

The periodontal tissues of dogs kept in kennels in a laboratory and fed from the time of weaning for 8 years on a diet containing among other ingredients white bread and a liberal supply of vitamins A and D are rendered very resistant to periodontal disease. On the other hand, the periodontal tissues of animals kept under similar conditions of diet and environment, but receiving comparatively little vitamins A and D for the first $6\frac{1}{2}$ years, are very susceptible to the disease.

These experiments stress the fundamental importance of including vitamins A and D in the diet during the early developmental period if the periodontal tissues are to resist disease. A large supply of these vitamins given later, even from the fifth month onwards, does not entirely compensate for a deficiency during the early period of life, although vitamin A given in large doses may then prevent the spread of the pathological condition. Conversely, the addition of a liberal supply of vitamins A and D to a diet composed largely of white bread, for nearly a year after weaning (i.e. for the full developmental period), renders the periodontal tissues resistant to disease in later life, even when the diet of the adult animal is deficient in these vitamins for long periods.

It will be noted that hyperplasia of the squamous epithelium adjoining the teeth produced by vitamin A deficiency in the developing period of growth is a special instance of the general effect of this deficiency (see p. 33). The susceptibility to bacterial infection of this hyperplastic epithelium of the gums or gingival margin is a condition which would also be expected from other known instances, in which infective foci and epithelial overgrowth are commonly associated with a deficient intake of this vitamin. Just as the administration of vitamin A in such conditions tends to correct the pathological changes of the epithelium and to heal the septic foci, so also in pyorrhoea alveolaris in dogs massive vitamin A therapy seems to increase the resistance of the gum tissues to micro-organisms to the extent that the exudate of pus is greatly diminished and may even be suppressed [M. Mellanby, 1930, 2].

Vitamin C and Dental Tissues.

One of the earliest investigations on the influence of the diet on the teeth was that of Zilva and Wells [1919], who fed guinea-pigs on a scorbutic diet of oats, bran, and autoclaved milk. Ground and decalcified sections of the teeth showed defects in the pulp, odontoblasts and dentine. In the pulp various degrees of degeneration of a type designated as 'fibroid' were found, and in the dentine an irregular 'osteoid' condition. Changes in the teeth were observed even in the mildest degree of scurvy, but never when a sufficiency of vitamin C was added to the basal diet (see Figs. 21–24).

Howe [1919; 1921; 1923] has also carried out much experimental work on guinea-pigs and monkeys, primarily, however, with a view to establishing the relation of diet, and in particular of vitamin C, to caries. He concludes that a deficiency of this vitamin leads to decalcification of the teeth. Toverud [1923; 1926] made a histological and chemical examination of some of the teeth of Howe's guinea-pigs and found the defects described by Zilva and Wells. Robb and collaborators [1921] found similar changes in the teeth of guinea-pigs on a scorbutic diet.

Extensive investigations by Höjer [1924; 1926; Höjer and Westin 1925] and others have also emphasized the importance of vitamin C in determining the structure of the teeth in guinea-pigs. They summarize the defects that follow vitamin C deficiency as follows:

(1) The gradual change and disappearance of the odontoblastic layer.

(2) The amorphous calcification of the 'predentine' and the absence of canals in this layer.

(3) The previously calcified dentine becomes porous.

(4) The dilatation of the vessels in the pulp.

(5) The production of nerve lesions and oedema of the pulp tissues.

As a result of these investigations a method was devised for the estimation of the antiscorbutic value of foods (p. 204).

The results experimentally obtained on guinea-pigs have not been tested on man and it is difficult to appraise their value in terms of human beings. In the case of the dog, M. Mellanby [1929] was unable to show that vitamin C played any significant part in the

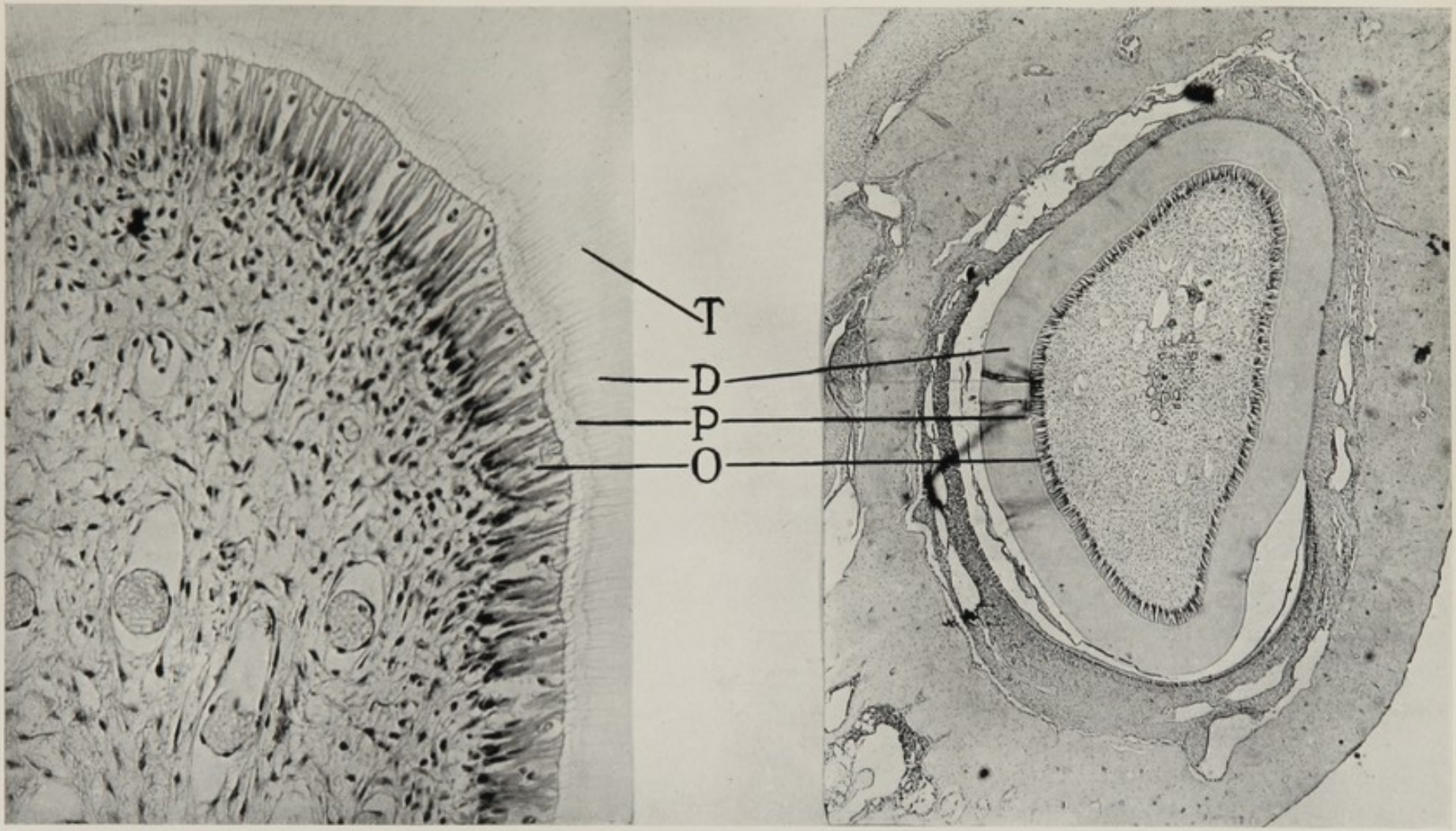


FIG. 21. (×270)

FIG. 22. (×40)

Effect of vitamin C deficiency on the teeth of guinea-pigs. Photomicrographs of transverse section of the decalcified incisor root, stained with haematoxylin-eosin.

FIGS. 21 and 22. Guinea-pig protected from scurvy (10 c.c. of decitrated lemon juice per diem).

Tomes canals (T) well marked in the uniformly stained broad layer of dentine (D), 'predentine' (P) lightly stained, odontoblasts (O) long and parallel.

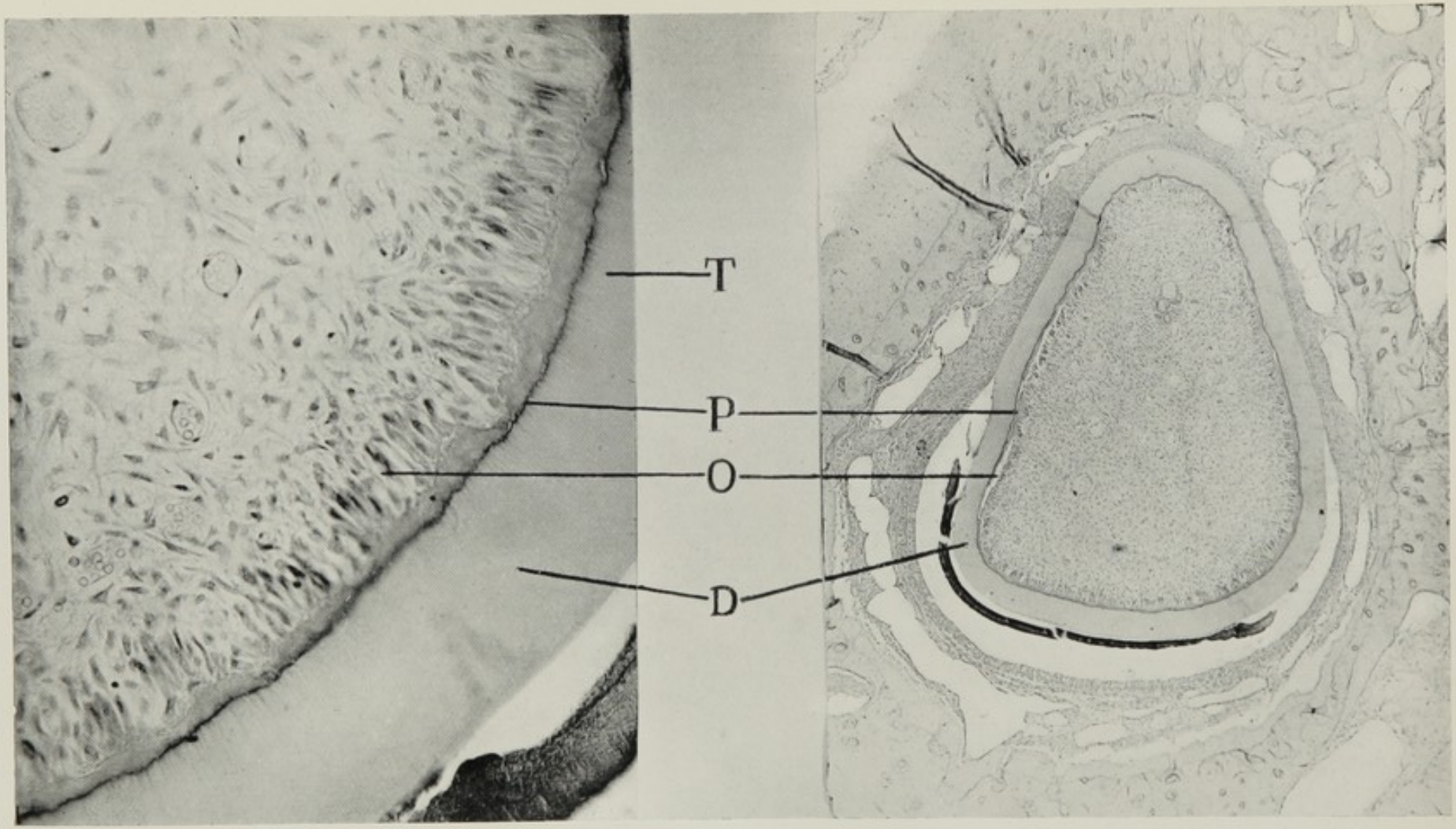


FIG. 23. (×270)

FIG. 24. (×40)

Effect of vitamin C deficiency on the teeth of guinea-pigs. Photomicrographs of transverse section of the decalcified incisor root, stained with haematoxylin-eosin.

FIGS. 23 and 24. Guinea-pig partially protected from scurvy after 14 days on experimental diet.

Tomes canals (T) in dentine (D) poorly marked, and absent in the 'predentine' (P) which is darkly stained, odontoblasts (O) short, disorganized and separated from the dentine (D).

calcification and structure of dental tissues, but this may be explained by the relative insusceptibility of the dog to the absence of this vitamin from the food. Rats also appear to exist in health on diets lacking this vitamin.

Florid scurvy in man is now relatively rare except in persons living under special conditions. When it occurs, periodontal changes similar to those described by Zilva and Wells in scorbutic guinea-pigs may develop. Since periodontal disease in man is pathologically similar to that observed in guinea-pig scurvy, it has been unduly assumed that it is related to a vitamin C deficiency. In dogs it has been shown experimentally that this is not the case [M. Mellanby, 1930, 2], but that periodontal disease is related to vitamin A deficiency. As regards man, Hanke [1929 ; 1930] claims to have produced evidence that dental caries and pyorrhoea are sometimes associated with a deficiency of vitamin C alone. If vitamin C deficiency plays a part in the aetiology of human periodontal disease, it seems clear that the deficiency is only partial and is not accompanied by other well-recognized scorbutic changes. The work of Zilva and Wells has clearly shown that dental tissues in guinea-pigs are peculiarly susceptible to vitamin C deficiency and are among the earliest to suffer from this dietetic defect. It may be, therefore, as they suggest [1919], that this also applies to man and that the so-called 'latent scurvy' may play a part in human dental disease. Whether this is the case or not remains to be tested by further work.

CHAPTER V

VITAMIN E.

Historical.

It is only within the last ten years that the existence of a specific vitamin essential for reproduction (at any rate of the rat and mouse) has been established. The view held previously was that a diet which would satisfy the animal's needs for growth and well-being would also be adequate for reproduction. The first indication that this was not necessarily the case was given by Mattill and Conklin [1920], who found that rats reared on whole milk, though growing well and appearing to be in good health, were usually sterile. They therefore suggested that milk was deficient in some dietary factor essential for reproduction. Evans and Scott [1922], during a study of the effect of nutrition on the oestrous cycle, found that rats reared on a diet containing adequate amounts of protein, of salts and of all the known vitamins were partially sterile in the first generation and wholly so in the second. This sterility was corrected by an unknown substance X, present in certain natural foodstuffs, e.g. fresh lettuce leaves, wheat embryo, and dried alfalfa. In subsequent investigations Evans and Bishop [1923, 1, 2, 3] showed that X was fat-soluble, but was not identical with any of the known vitamins. In the female, sterility was a disturbance of uterine function leading to foetal death and resorption. Evans's standard sterility diet contained caseinogen 18, starch 54, lard 15, butter fat 9 and salts 4 per cent., the rats receiving in addition a daily ration of dried whole yeast. If the butter fat was replaced by 2 per cent. of cod-liver oil the results were unchanged.

Sure [1923, 1, 2], during an investigation of the influence of diet on reproduction, had independently come to the same conclusion as Evans and Mattill and their co-workers. He found that sterility resulted when rats were maintained on diets containing whole or skim milk as the source of protein, with cod-liver oil and an alcoholic extract of wheat embryo to supply vitamins A, D, and B respectively. This sterility was neither corrected by increasing or improving the supply of protein, nor by increasing the dose of water-soluble vitamins. It disappeared, however, when an unknown substance, apparently identical with Evans's factor X, present in Georgia velvet bean pod meal, polished rice, yellow corn, rolled oats, and lettuce leaves, was added to the diet. Sure suggested that the new factor should be called vitamin E and this suggestion was universally adopted in 1925.

Mattill and his co-workers [Mattill and Carman, 1922-23; Mattill and Stone, 1923; Mattill and Congdon, 1924; Mattill, Carman and Clayton, 1924], in the course of further work on milk diets, became satisfied that vitamin E corresponded with the essential factor which they had considered to be lacking in whole milk. They

described degeneration of the testes resulting from deprivation of this dietary factor.

The chief opposition to the acceptance of the existence of vitamin E as an essential anti-sterility dietary factor came from the work of Nelson. Mattill, Evans, Sure and Daniels and Hutton [1925–26] had all reported failure of reproduction when rats were fed on diets containing milk as the only possible source of vitamin E, but Nelson and his co-workers [Nelson, Heller and Fulmer, 1923; Anderegg, 1924] and also Mazé [1925], Woods [1925], and Palmer and Kennedy [1922–23] obtained good reproductive records when rats were reared on diets of whole milk powder alone. When, however, a certain amount of lard was added to such a diet, sterility occurred. Nelson suggested that the sterility-producing power of Evans's diet was due to its high lard content. The resulting high calorie value of the diet would occasion a low intake and hence a possible deficiency of some of the known dietary essentials. Though at one time more inclined to accept the existence of vitamin E [Anderegg and Nelson, 1925], Nelson and his co-workers were still unconvinced in 1926 [Nelson *et al.*, 1926; Guest, Nelson, Parks and Fulmer, 1926], and reported that whereas sterility resulted from the ingestion of a skim-milk diet, supplemented by cod-liver oil and dried yeast, in the form of a dry powder, good reproduction occurred when the same diet was fed as a liquid paste [Anderegg and Nelson, 1926]. Sure was, however, unable to confirm this [1926, 3; 1927, 1].

The arguments of Nelson and his co-workers against the existence of vitamin E have been satisfactorily met [Mattill, Carman and Clayton, 1924; Mattill and Clayton, 1925, 1926; Evans, 1924, 1; Evans and Burr, 1927, 1]. These workers agree that lard has the power of increasing the sterility-inducing properties of a diet, but claim that this does not invalidate the proof of the need of the rat for vitamin E. That a high content of fat is not in itself sterility-producing was shown by the fertility associated with diets containing a high content of butter fat.

It was afterwards shown that lard neutralizes the effect of vitamin E when the latter is mixed with the diet and not given as a separate ration [Evans and Burr, 1927, 2, 3]. This fact accounts for many discrepancies in the literature of the subject. Butter fat constituting 5 per cent. of the diet appeared to be an inadequate source of vitamin E when the diet also contained 15 per cent. of lard, but proved an effective source when mixed in a diet, low in fat, such as was used by Nelson and his co-workers.

Sure's later investigations of vitamin E [Sure, 1924–25; 1925; 1926, 1, 2, 3; 1927, 1, 2, 3, 4; 1928, 1; 1929–30] have been collected in a single pamphlet [1930]. The researches of Evans and his co-workers [Evans, 1924, 1, 2; 1925; Evans and Bishop, 1924; Evans and Burr, 1924; 1925, 1, 2] were summarized in a monograph in 1927 which contains most of the established facts concerning vitamin E [Evans and Burr, 1927, 1]. When not otherwise stated the information given below is derived from this monograph.

The Results of Deprivation of Vitamin E.

Female Sterility.

In female rats rendered sterile by deprivation of vitamin E, the ovaries are undamaged, ovulation and oestrus occur as usual, and the proportion of successful matings is within normal limits. Implantation of the fertilized ovum is proved by the appearance of red blood-cells in the vaginal smear—the 'placental sign' of Evans and Burr—at the normal time, about the fifteenth day of pregnancy. Instead, however, of the birth of a litter at the end of term, the foetuses die in the uterus and are resorbed. The weight curve of the pregnant rat follows at first the curve of normal pregnancy, but soon falls more and more below it: after the twentieth day there is a gradual loss of weight. The foetus shows the first signs of retarded development at the eighth day of gestation. By the twelfth day defects in the blood system are shown by the solid appearance of the liver and by the deficiency of blood islands in the yolk sac and of erythroblasts in the heart and blood-vessels. Death of the foetus occurs about the thirteenth day, the allantois at this stage showing complete atrophy. Resorption of the foetus is accomplished by the twentieth day. The placenta, which is unaffected, persists and grows after the death of the foetus until the twentieth day; after this it also is rapidly resorbed. Foetal death appears to be due to starvation and asphyxia resulting from defects in the allantois and yolk sac.

The sterility in the female is not incurable as is the case in the male. The ingestion of vitamin E in adequate amounts will always permit a successful gestation, however long has been the previous period of deprivation. The minimum daily dose need not be given until the fifth day after mating, but administration must be continued until the nineteenth day. Parenteral injection has been found to be as effective as oral administration.

Male Sterility.

In male rats and mice [Beard, 1925–26, 1, 2] lack of vitamin E causes a gradual loss of reproductive power leading to complete and incurable sterility within 85 to 150 days [Evans and Burr, 1927, 1; Mason, 1925; 1926]. These observers agree that the susceptibility of different rats to deprivation of vitamin E shows a wide range of variation. Mason found that young rats placed at weaning on a diet devoid of vitamin E showed the first signs of sterility within 50 to 65 days, while if the rats were sexually mature before they were deprived of this factor, no ill effects were seen for 75 to 100 days. Evans and Burr showed that rats born of mothers deprived of vitamin E, except for minimal amounts during gestation, if reared without addition of vitamin E had no period at all of fertility.

Evans and Burr divide the onset of sterility in the male into six stages. In the first stage, in spite of a sufficiency of apparently normal spermatozoa, there is a loss of reproductive potency; in the second the spermatozoa lose their motility. In the third stage groups of spermatozoa fused together are found in the vaginal

plug, which is, in the succeeding stage, devoid of spermatozoa. The penultimate stage is characterized by loss of power to form the plug, although some degree of sex interest is retained. This disappears in the final stage.

Mason [1926] in a careful study of the histology of the testis found a gradual degeneration and removal of the germinal epithelium completed in 35 to 50 days. The germ cells degenerate in an order which is the reverse of that of their formation, the mature spermatozoa being the first to disappear.

The histological studies of Evans and Burr [1927, 1], Juhasz-Schäffer [1931], and Mattill, Carman and Clayton [1924] are in accord with those of Mason, except for the fact that Mattill and his co-workers found a great proliferation of the interstitial tissue in the degenerated testis. Evans and Burr showed in addition that a considerable degree of sterility, as shown by mating tests, precedes any abnormality of the testis which can be detected by histological examination. Both sets of observers found great variations in the susceptibility to lack of vitamin E of the different tubules of the same testis. This variation explains the fact that a partial success sometimes appears to attend curative efforts. Once degeneration has set in, in any given tubule, the process in that tubule cannot be arrested by the administration of vitamin E. If, however, when the attempted cure is begun the testis still contains a few normal tubules, these can be preserved and thus a certain measure of reproductive power retained.

Mason [1929] found a normal erythrocyte and haemoglobin content in the blood of sterile male rats suffering from deprivation of vitamin E. No histopathological changes were shown by the adrenals, spleen, liver, pancreas, thymus, thyroids, or lymph glands.

Verzár *et a* . [1931] noted a lowered basal metabolism in male rats reared on a diet devoid of vitamin E.

Vitamin E and Lactation.

Sure [1925; 1926, 3; 1927, 1, 3] brought forward evidence tending to indicate that vitamin E consisted of two parts, one thermostable and promoting reproduction, and the other thermolabile and promoting lactation. He [1928; 1930] stated later, however, that he could not find sufficient satisfactory evidence in favour of this theory, and has since concluded that vitamin E is not needed in lactation.

Evans and Burr [1928, 2] reported the development of paralysis about the time of weaning in suckling young of mothers deprived of vitamin E. Approximately 35 per cent. of the animals thus affected died, about 17 per cent. recovered, and the remaining 48 per cent. remained paralysed but appeared otherwise healthy. Prophylaxis with vitamin E administered either to the mother or directly to the young was successful, but curative measures failed. Sure [1930] has not observed this paralysis.

Decreased Growth and Vigour.

The earlier work on vitamin E was generally taken to show that rats receiving a diet deficient in vitamin E, but complete in all

other respects, exhibit normal growth. Evans and Burr [1927, 1] noticed, however, when attempting the cure of male sterility, that although the cures were not successful, yet the administration of vitamin E was marked by an increase in the rate of growth. Evans [1928, 2] subsequently investigated this point more fully, and found that rats of both sexes reared on a diet deficient in vitamin E showed, after the eighth month of life, but not before, a consistently poorer rate of growth than did rats when receiving the same diet supplemented daily by a few drops of wheat germ oil. The same effect was obtained by using the unsaponifiable fraction in place of the crude oil, showing that it must be attributed to the vitamin E content of the oil rather than to any other constituent. As castrated rats behaved in the same way, it appeared that the growth and well-being of the rat did not depend on the condition of the testis. Mason [1929] recorded that in groups of male rats receiving a sterility-inducing diet, the ingestion of fresh or dried lettuce leaves or dried alfalfa markedly improved the rate of growth. Since this effect was apparent in young rats from the beginning of the experiment, it does not appear that Mason was dealing with the same condition as Evans. The possibility that the differences in the stock diets used by different observers may cause variations in the amount of vitamin E present in the bodies of rats bred on these diets must not be disregarded. A careful consideration of Mason's work, however, suggests that the increased growth in his experiments might have been due to the vitamin B_2 content of the leaves rather than to their content of vitamin E.

Juhasz-Schäffer [1931] observed a 150 per cent. increase in the rate of growth of embryonic chicken tissues, cultured in adult chicken's plasma, as the result of the addition of wheat germ oil. If the oil was autoclaved, no such effect was observed (see p. 110).

Uterine Deciduomata.

Evans [1928, 1] stated that when pseudopregnancy was induced in rats by mating with vasectomized males, a large proportion (50 per cent.) developed uterine deciduomata. When, however, such females received vitamin E in abundance, the proportion developing the tumours was only 3–7 per cent. No explanation of this phenomenon is yet available.

Function of Vitamin E in the Body.

The part played by vitamin E in the reproductive processes is far from clear. In the pregnant female, lack of this vitamin appears to cause intra-uterine death by preventing the normal development of the yolk sac and allantois. The maternal parts of the placenta remain unaffected and the germ cells suffer no damage. In the male, on the other hand, permanent degeneration of the germinal epithelium occurs. The fact that vitamin E plays such different parts in male and female reproductive organs led Mason [1929] to suggest that vitamin E might be composed of two factors, one influencing the testis and the other concerned with uterine function. He found evidence for this in the observation that dried lettuce was less

effective than fresh in preventing male sterility, whereas Evans had reported that desiccation of lettuce caused no decrease in its power to cure female sterility. Mason, however, himself concluded that there was insufficient evidence for this theory of a dual nature of vitamin E.

Vitamin E disappears from the body of the female rat during normal metabolism at a rate which is unaffected by the occurrence of gestation. It would appear, therefore, that it either fulfils some definite function even when pregnancy does not occur, or is excreted unused.

Verzár [1931] found that the intraperitoneal injection of vitamin E concentrates from wheat germ caused a genital hypertrophy in young female rats similar to that following the injection of anterior pituitary hormone. He also noted [Verzár and Kokas, 1931, Verzár *et al.*, 1931] that male rats, reared on a diet devoid of vitamin E, had a silky infantile coat like that of castrated rats, and that if, during the period of deprivation, anterior pituitary hormone was injected daily, the rats developed, instead, the bristly coat characteristic of the normal male. He suggested, therefore, that vitamin E is necessary for the synthesis of the hormone of the anterior lobe of the pituitary.

Storage of Vitamin E.

Rats reared from weaning on a standard sterility-inducing diet show an extent of initial fertility which indicates that the animal has a considerable power to store vitamin E. Once the body's reserve has been exhausted there is no return of fertility, and the second generation is consistently sterile. Vitamin E has been found present in normal rat muscle and absent from the tissues of rats deprived of this factor. Further proof that storage takes place is given by the fact that a single large dose of a curative substance given either orally or parenterally about the day of mating, produces an effect equal to that of the same total amount administered in small daily doses during gestation. It has been observed that a sterile female, enabled by the ingestion of a diet rich in vitamin E during pregnancy to give birth to a litter, will sometimes store sufficient of this factor to carry through one or more successful gestations after a return to the sterility-inducing diet. The extent of this after-fertility will depend upon the amount of the vitamin administered.

Vitamin E content of faeces. The amount of vitamin E present in the faeces depends on the amount ingested by the rat [Juhasz-Schäffer, 1931]. There is no evidence that the factor is synthesized in the alimentary canal.

Chemical Investigation of Vitamin E.

Solubility. Vitamin E is fat-soluble and can be extracted by fat solvents; light petroleum, ether, acetone, benzene, absolute alcohol, and 76 per cent. alcohol have all provided successful extracts. Extraction with alcohol is not so complete as with ether; in the latter case the extracted residue was found to have lost all power to prevent or cure sterility. Vitamin E is very slightly soluble in water

and highly soluble in methyl alcohol, but the solubility falls away rapidly on dilution with water. It is more soluble in pentane than is sitosterol, a fact which is of use in preparing a concentrate of the active fraction.

Stability. Saturation with bromine in glacial acetic acid, treatment with acetic anhydride, and exposure to ashing temperatures are all destructive of vitamin E. It is also partially destroyed by exposure to ultra-violet radiation for 45 minutes. It is undamaged by hydrogenation, by distillation *in vacuo* at 250° C., by exposure in air for two hours to a temperature of 170° C., or by autoclaving at 20 pounds pressure for two hours [Sure, 1926, 3]. If any destruction occurs when a current of air is passed through it at a temperature of 97° C. it only does so at a very slow rate. Neither strong acids (e.g. 20 per cent. HCl) nor the enzymes naturally occurring in wheat germ destroy it. Vitamin E is not destroyed by saponification, and is found present in the unsaponifiable fraction.

Vitamin E, therefore, appears to be remarkably stable to heat, resembling in this respect vitamin A. The evidence was, however, all obtained by Evans and Burr in experiments with wheat germ oil, and it does not necessarily follow that the vitamin will prove to be equally stable when present in other foodstuffs. Evans and Burr found that the vitamin E content of many animal and vegetable tissues was unaffected by cooking or by rapid drying, as judged by the power to cure female sterility. Mason [1929] recorded, however, that when lettuce was dried most of the power to prevent male sterility was lost, but he used a lower temperature than did Evans and Burr and in consequence the period of desiccation was more prolonged. Evans and Burr were unable to obtain a potent unsaponifiable fraction from ox muscle, but Sure [1927, 1] was successful in doing so from cotton-seed oil.

Under certain conditions vitamin E is susceptible to oxidation. Supplee and Dow [1925] observed that whole-milk powder packed in air lost its content of this vitamin after two years, whereas the same sample of powder kept in the presence of de-oxygenated air for the same length of time had retained all its original potency. Mattill [1927] stated that vitamin E was destroyed by the oxidative changes which accompany the development of rancidity in fats and oils. This process was accelerated by the presence of ferrous sulphate and by some other conditions, but was delayed by the presence of substances containing hydroxy-groups, and the stability of vitamin E in wheat germ oil was probably due to the abundance of these groups in the oil. Butter fat when protected from oxidative changes was found to contain a considerable amount of vitamin E.

This connexion between rancidity and the destruction of vitamin E was further elucidated by Evans and Burr [1927, 2, 3]. This work was undertaken to investigate the fact, repeatedly observed, that a high lard content increased the sterility-inducing power of a diet. On a diet containing 9 to 15 per cent. of lard there was a certain amount of initial fertility, which, when the lard was increased to 22 per cent., disappeared. At the same time a daily dose of wheat germ oil was still effective in inducing fertility, showing that the lard was not in itself responsible for the sterility. Evans and Burr found further

that, if the wheat germ oil given as a daily dose was replaced by wheat germ mixed with the other constituents of the diet, there was a definite ratio between the amount of lard in the diet and the amount of wheat germ which must be included to ensure fertility. (This observation was not confirmed by Mattill and Clayton [1926]). The same relation held true with wheat germ and hardened cotton-seed oil or oleic acid, but not with butter or stearic acid. It appeared that lard, hardened cotton-seed oil, and oleic acid in some way neutralized or antagonized the vitamin E in the wheat germ. This antagonism was found to increase with the degree of rancidity of the fats, but it was not proportional to the acid number. The unsaponifiable fraction of commercial oleic acid was found to contain most of the antagonistic or destructive body [Evans and Burr, 1927, 3].

Waddell and Steenbock [1928; 1931] demonstrated an apparent destruction of vitamin E in their stock diet and in wheat germ when an ethereal solution of ferric chloride was poured over the diet, the ether afterwards being allowed to evaporate. Both male and female sterility occurred. If the ferric chloride was dissolved in water, no such effect was observed. Although female rats might have large reserves of vitamin E they were apparently unable to use them while consuming the iron-treated ration. These reserves became effective when the diet was replaced by one devoid of the vitamin.

Cummings and Mattill [1931] suggested that the destructive action of certain fats on vitamin E was due, as suggested by Fridericia [1924] in the case of vitamin A, to the action of peroxides produced during autoxidation. They showed that the fats, such as cod-liver oil and lard, which most readily destroy vitamin E, are those which have the highest autoxidizable value. These fats contain powerful pro-oxidants while anti-oxygenic capacity is shown by wheat germ oil, cotton-seed oil, and other substances.

Olcovich and Mattill [1931; see also Olcott and Mattill, 1931, 1, 2] obtained from the unsaponifiable material of green lettuce leaves a non-crystallizable fraction containing both vitamin E and the anti-oxidant. Separation of the two was effected by solution in light petroleum and subsequent treatment with 92 per cent. methyl alcohol. Vitamin E remained in the petroleum and the anti-oxidant passed into the alcohol. Fractional distillation of the latter *in vacuo* yielded a fraction distilling at 160–180° C. which, on cooling, gave monoclinic crystals with marked anti-oxidant capacity. The formula of this substance appeared to be $C_{13}H_{14}O_5$ and one hydroxyl group of a phenolic character was present.

Concentration of Vitamin E.

Evans and Burr obtained from wheat germ oil a concentrate of the active principle which represented 0·25 per cent. by weight of the original oil, and of which 5 mg. given as a single dose to the female rat on the day of mating supplied sufficient vitamin E to permit of the birth of a litter. The concentrate was prepared as follows. The unsaponifiable fraction was treated with cold pentane and the precipitate removed, after which hot methyl alcohol was added to the solution and another precipitate discarded; after standing, the

additional precipitate formed on cooling was removed and the portion soluble in light petroleum was treated with digitonin to remove sterols. The remaining solution was again saponified and the sterols in the unsaponifiable fraction removed by precipitation with digitonin. After extraction with methyl alcohol, the active fraction was distilled *in vacuo* at 200–233° C.

The refractive index of the concentrate was about 1·5018, the iodine number 220, and the molecular weight 400. Analysis gave a composition of 81·7 per cent. C, 12·2 per cent. H, 6·1 per cent. O. This work emphasizes further the strong similarity between vitamin E and vitamin A (see p. 37).

Methods employed in Testing for the Presence of Vitamin E.

Rats reared on a diet deficient in vitamin E show wide variations in the length of the period of initial fertility. Some are able to give birth to two or even three litters in succession before sterility occurs. This is partly due to the reserves of vitamin E stored in the body at birth and partly to the presence in the basal diet of small amounts of the vitamin. For this reason the most satisfactory method of testing a foodstuff for the presence of vitamin E is to investigate its power to restore fertility to a female rat which has exhibited one or more typical resorptions as the result of receiving a sterility-inducing diet. A typical resorption cannot be presumed in the absence of any one of the following conditions:

(1) a normal oestral cycle and ovulation, proved by taking vaginal smears;

(2) insemination at the time of oestrus, proved by the presence in the vagina of the vaginal plug containing spermatozoa;

(3) implantation of the fertilized ovum, proved by the placental sign;

(4) foetal death and resorption, proved by the characteristic weight curve.

The normality of the reproductive power of the rat must be subsequently established by the successful birth of a litter after vitamin E has been administered.

It has been generally accepted that resorptions of the foetus as described above are characteristic only of the deprivation of vitamin E, but Sure [1930] has claimed to have observed resorptions of apparently identical character in rats deprived of vitamin A but receiving abundance of vitamin E. In view of the cessation of ovulation in rats deprived of vitamin A, these observations are hard to interpret.

The other method of testing for vitamin E is to add the substance under investigation to a diet deficient in vitamin E but complete in all other respects, and to observe the reproductive behaviour of young rats reared from weaning on this diet. This method is inconclusive unless observation is continued for a very long period. A report of sterility in female rats mated with males bred on the same diet is obviously inconclusive of deprivation of vitamin E: resorptions must be observed after mating with normal males.

Distribution of Vitamin E in Nature.

The suggestion of Cummings and Mattill [1931], that vitamin E is destroyed during the autoxidation of fats, offers an explanation for the conflicting nature of some of the evidence regarding its distribution in nature. If the test substance is incorporated in a diet containing lard or some other highly autoxidizable fat, the estimated vitamin E content will be much lower than would have been the case had the lard been replaced by butter or cotton-seed oil, or the test substance been offered as a separate ration. The efficiency of any natural substance as a source of vitamin E will depend not only on the actual amount of the factor contained in it, but also on the balance of anti- and pro-oxidants associated with it. The reserves possessed by the experimental animals will also markedly influence the results obtained.

Evans is the only observer who has consistently employed the curative test, which is far more satisfactory than any other method, owing to the wide variations existing in initial fertility (see p. 112).

The richest natural sources of vitamin E appear to be green leaves and the embryos of seeds. From wheat germ an oil can be extracted that is effective in the cure of female sterility in the rat in daily doses of 15 to 20 mg. Vitamin E is absent from the endosperm of cereals. Vegetable oils, as a rule, contain definite but small amounts of this vitamin.

Vitamin E is present in small amounts in animal tissues, the richest sources being the muscle and subcutaneous fat. Ox and cow tissues appear to be uniformly richer than the corresponding tissues of the pig or rat. Although vitamin E is present in fresh pig fat, lard owing to high autoxidizability is almost entirely devoid of it. Simmonds, Becker and McCollum [1928], however, reported the presence of vitamin E in samples of lard.

Vitamin E is present in milk and butter, the varying results obtained by different observers being partly due to differences in technique and partly to variations in the samples used. Mattill [1927] showed that much of the vitamin E in the original milk-fat was lost in the usual processes of butter-making.

Owing, presumably, to its high autoxidizable index, cod-liver oil is as a rule a poor source of vitamin E [Evans and Bishop, 1923, 1; Sure, 1927, 2; 1930; Mattill, 1922; Hogan and Harshaw, 1926], but evidence has been produced that some samples of the oil may contain it [Simmonds, Becker and McCollum, 1928; Nelson *et al.*, 1927].

Evans and Hoagland [1927] carried out some experiments which indicated a synthesis of vitamin E by green and etiolated Canadian field pea seedlings grown in nutrient solution. The possibility of contamination of the solution by bacteria was not, however, ruled out.

The accompanying Table VII gives an approximate estimate of the distribution of vitamin E in those substances which have been investigated. When the dose is expressed in g. it indicates that this amount was given as a separate daily dose; when expressed as per cent., that the substance was included in this proportion in the dry diet.

Table VII.

Distribution of Vitamin E.

o indicates that the vitamin was not detected ; + that the material contains the vitamin ; + + that it is a good source of it ; + + + that it is a rich source of it.

Foodstuff.	*Anti-Sterility Value.*	*Curative Dose.*	*Prophylactic Dose.*	*Observer.*
Fats and Oils.				
Almond oil	o	...	...	Sure [1926, 1]
Butter fat	+ +	...	5–10 %	,, [1927, 4]; Nelson *et al.* [1926]
,, ,, pasture	+	24 %	24 %	Evans and Burr [1927, 1] (high lard diet)
,, ,, dry feed	o	...	...	,, (,,)
Coco-nut oil	+	...	15 %	,, (,,)
Cod-liver oil	o to +	<12 %	...	,, (high lard diet); Sure [1927, 2]
	+ +	...	4–5 %	Simmonds *et al.* [1928]; Nelson *et al* [1927]
Cotton-seed oil	+	...	3–15 %	Evans and Burr [1927, 1]; Sure [1924]
,, ,, ,, hydrogenated	+	...	15 %	Kennedy and Palmer [1926]
Hemp-seed oil	+ +	...	5 %	Sure [1924–25]
Lard	o	...	...	Evans and Burr [1927, 1]
	+	...	15 %	Simmonds *et al.* [1928]
Linseed oil	o	...	...	Sure [1926, 1]
Maize oil	+ + +	...	3 %	,,
Mustard-seed oil	o	...	...	,,
Mutton fat	+	5.0 g.	...	Evans and Burr [1927, 1]
Oat oil	+ + +	...	5 %	Kennedy [1926]
Olive oil	+	...	5–15 %	Evans and Burr [1927, 1]; Sure [1926, 1]
Palm oil	+ +	...	5 %	Sure [1926, 1]
Palm-kernel oil	o	...	...	,,
Peach-kernel oil	+ +	...	5 %	,,
Peanut oil	+	...	<5 %	,,
Pig fat	+	5.0 g.	...	Evans and Burr [1927, 1]
Sesame oil	o	...	...	Sure [1926, 1]
Walnut oil	+	22 %	...	Evans and Burr [1927, 1]
Wheat germ oil	+ + +	0.025 g.	...	,,
Yeast fat	o	...	...	,,

Cereals.				
Flour, white wheaten	o	...	...	Evans and Burr [1927, 1]
Maize, whole grain, yellow	+	...	60-62 %	Sure [1923, 2]; Miller and Yates [1924]
„ embryo	+++	0.5 g.	5 %	Evans and Burr [1927, 1] (lard diet) Miller and Yates [1924]
Rice, polished	o to +	<76 %	62 %	„ „ (lard diet) Sure [1923, 2]
„ polishings, ether extract	+++	<0.1 g		„ „ „
Wheat, whole grain	+	5.0 g	50 %	„ „ „
„ embryo	+++	0.12 g.	33 %	„ „ „
Oats, whole grain	+	33 %	...	„ „ „
„ rolled	...	...	62 %	Sure [1923, 2]
Legumes.				
Georgia velvet bean pod meal	++	...	20 %	„ [1923, 2]
Soya bean	+	...	50 %	„ [1926, 1]
Other Seeds				
Alfalfa seed	+++	0.5 g.	...	Evans and Burr [1927, 1]
Lettuce „	+++	0.5 g.	...	„ „
„ „ ether extract	+++	0.046 g.	...	„ „
Nuts.				
Coco-nut press cake	+	...	16 %	„ „ (lard diet)
Peanuts	+	<1.0	...	„ „
Walnuts	+	22 %	...	„ „ (lard diet)
Vegetables.				
Alfalfa, fresh	+++	...	...	„ „
„ dried	+++	0.6 g.	...	„ „
Begonia leaf, fresh	+++	2.5 g.	...	„ „
Kale, green fresh	++		20 g.	Miller and Yates [1924]
Lettuce, fresh	+++	2.5 g.	5.0 g.	
		(0.25 gm dry wt.)	(male fertility)	Evans and Burr [1927, 1]; Mason [1929]
„ dried	+++	0.25 g.	...	„ „
„ „	++	...	1.6 g.	Mason [1929]
			(male fertility)	Evans and Burr [1927, 1]
„ ether extract	+++	...	...	„ „
Pea seedlings, green, fresh	+++	4.0	...	„ „
„ „ etiolated, fresh	+++	4.0	...	„ „
Tea leaf, dried	++	<1.0 g.	...	„ „

TABLE VII (*continued*).

Foodstuff.	*Anti-Sterility Value.*	*Curative Dose.*	*Prophylactic Dose.*	*Observer.*
Fruits.				
Banana, fresh	++	27 g.	...	Evans and Burr [1927, 1]
Orange juice	+	4–16 c.c.	...	,, ,,
Meat.				
Ox, hypophysis	++	4·0	...	,, ,,
,, liver	++	5·0	5·0, <20 %	,, ,, ; Clayton [1929]
,, muscle	++	5·0	1·0, 20 %	,, ,, ; ,, ,,
Pig, heart	++	<5·0	...	,, ,,
,, liver	++	<5·0	...	,, ,,
,, muscle	++	<5·0	...	,, ,,
,, pancreas	++	5·0	...	,, ,,
,, spleen	++	5·0	...	,, ,,
Placenta, human	++	<5·0	...	,, ,,
Rat, heart	++	<1·25	...	,, ,,
,, kidney	++	<2 kidneys	...	,, ,,
,, liver	++	9·0	...	,, ,,
,, muscle	++	8·0	...	,, ,,
,, testes	++	<2 testes	...	,, ,,
Milk and Milk Products.				
Caseinogen	+	...	18 %	,, ,, (lard diet)
Milk, cow's, fresh	+	...	100 %	,, ,,
,, ,, dried	+	...	No fertility with 25 %	,, ,, (lard diet).
			50 %	Anderegg and Nelson [1925] (no lard)
,, ,, skimmed, dried	o	...	...	Evans and Burr [1927, 1]
Miscellaneous.				
Cholesterol	+	...	...	Knudson and Randles [1925]
Egg-yolk, raw	++	$\frac{1}{3}$ of one yolk	...	Evans and Burr [1927, 1]
Yeast	o to +	...	5 %	,, ,, ; Mattill and Stone [1923]

Practical Applications of the Work on Vitamin E.

The discovery of vitamin E is of practical importance to those engaged in the breeding of rats for experimental purposes. In many cases it is desirable to plan stock diets, which, while securing adequate growth and health, do not produce young rats with large stores of the other fat-soluble vitamins. A knowledge of the distribution of vitamin E will prevent the occurrence of sterility as the result of the use of such diets.

Some work of Vogt-Møller and Bay [1931] indicates that vitamin E may be of importance to the farmer and stock-breeder. They investigated the effect of intramuscular injection of single doses of wheat germ oil on 11 healthy but sterile cows; in 9 cases pregnancy ensued.

Card *et al.* [1929; 1930] found that if hens were deprived of vitamin E for a sufficient length of time, their eggs failed to hatch, though development started normally.

A number of workers are at present investigating the part played by vitamin E in the problems of human fertility. The only results as yet published are those of Vogt-Møller [1931]. Two women who had experienced 4 and 5 previous miscarriages were treated with wheat germ oil. In both cases a successful pregnancy occurred.

CHAPTER VI

THE VITAMIN B COMPLEX.

Multiple Nature of the Vitamin B Complex.[1]

In 1915 McCollum and Davis [1915, 1, 2] reported that young rats, fed on a purified synthetic diet containing butter fat to supply the fat-soluble vitamin already discovered by them [1913], required for normal growth an additional food factor, which was named 'water-soluble vitamin B' in the following year [McCollum and Kennedy, 1916]. 'Water-soluble vitamin B' was considered by McCollum and his co-workers to be identical with the antineuritic vitamin discovered by Eijkman [1897, 1], and found by him and many others to be preventive and curative of polyneuritis developing in birds on a diet of polished rice. It had already been shown by Eijkman and numerous clinical investigators, that the antineuritic vitamin was protective against human beri-beri, and avian polyneuritis and beri-beri were and are generally taken to be analogous.

The fact that the distribution and properties of the water-soluble B factor, as ascertained by the use of rats, closely agreed with those of the antineuritic factor established by experiments on pigeons, coupled with the similarity of the symptoms exhibited by rats and pigeons suffering from deficiencies of these factors, led to the supposition that these two factors were identical [see Drummond, 1917, where the evidence is summarized]. This supposition was provisionally accepted by most workers, the evidence to the contrary [see Mitchell, 1919, where this evidence is critically reviewed] being regarded as insufficient to decide the question [see, for example, Emmett and Luros, 1920].

More recent work, however, on the rat, has shown that this animal requires for normal growth at least two water-soluble vitamins, here called vitamin B_1 and vitamin B_2 (see note below), both of which are

[1] *Note on the Nomenclature of the Vitamins of the B Complex.*

In England the Biochemical Society has adopted the plan of indicating the various vitamins of this complex by subscribed numbers, e.g., B_1, B_2, B_3, &c., a descriptive adjective or phrase being added when necessary.

The following terms are at present in use:

Vitamin B_1 for the more heat-labile, antineuritic (antiberi-beri) vitamin, discovered by Eijkman [1897, 1].

Vitamin B_2 for the more heat-stable, 'P-P' (pellagra-preventive) factor discovered by Goldberger and others in 1926, found necessary for prevention of dermatitis in rats.

Vitamin B_3 for a heat-labile factor of this group, described by Williams and Waterman [1927–28], present in yeast and whole wheat and necessary for full normal nutrition in the pigeon.

Vitamin B_4 for the heat- and alkali-labile factor described by Reader [1929, 2], present in yeast and necessary for the rat; this factor has been known previously as 'vitamin B_3'.

Vitamin B_5 for the factor described by Carter, Kinnersley and Peters [1930, 1, 2], which is necessary for weight maintenance of pigeons.

Factor Y for the heat- and alkali-stable factor described by Chick and Copping [1930, 2], present in yeast and many other foodstuffs, deficient in egg-white.

All the above are found in fresh yeast.

In America some workers use the term vitamin B for vitamin B_1, as defined above

contained in water-soluble vitamin B. It can now be seen that the assumption that 'water-soluble vitamin B' as originally defined by McCollum and his colleagues was a single entity and identical with the antineuritic vitamin was due to several reasons. Since the antineuritic vitamin—vitamin B_1—necessary for life, is also necessary for growth, it forms an important part of the vitamin B complex; to find some similarity between its distribution and properties and those of 'water-soluble vitamin B' was natural, since in experiments with rat growth no substance could be found rich in 'water-soluble vitamin B' unless it contained a due proportion of the antineuritic factor. The abundant presence of both factors in yeast, a substance much used in nutritional researches, assisted in the confusion. Again, an observation of Chick and Roscoe [1928], that caseinogen, the usual source of protein in vitamin experiments with rats, is only with great difficulty completely freed from vitamin B_2, suggests that the study of 'water-soluble vitamin B' may sometimes have approximated to that of vitamin B_1 alone, owing to the presence of some vitamin B_2 in the basal diet.

Evidence for the Multiple Nature of Vitamin B.

Vitamins B_1 and B_2. For some years before vitamin B was shown clearly to be separable into two or more components, suggestions of its multiplicity are to be found in the literature. Schaumann [1911], Cooper [1912] and Chick and Hume [1917–19, 1] demonstrated that some materials, potent in the prevention or cure of polyneuritis, were incapable of maintaining the weight of birds on a diet of polished rice. Cooper [1912; 1914, 1], working with pigeons, observed that the weight-maintaining and antineuritic factors are differently distributed in natural foodstuffs. The factors which avert loss of weight in rice-fed birds are, however, not identical with vitamin B_2, and are apparently not all essential for the growth of young mammals. R. R. Williams and Waterman [1927-28, 1928] and Eddy, Gurin and Keresztesy [1930] have found that one 'pigeon' factor, now termed vitamin B_3, is thermolabile and present in substances which are relatively lacking in the more heat-stable vitamin B_2.

Smith and Hendrick [1926] recorded an observation of importance in the history of vitamin B research. In attempting the chemical identification of vitamin B, Seidell and his co-workers [Seidell, 1921, 1, 2; 1922, 1, 2; 1924, 1; 1925; R. R. Williams and Seidell, 1916; Bertrand and Seidell, 1923] had learned that the vitamin, after adsorption on fuller's earth, could be extracted therefrom by sodium hydroxide or baryta solution and converted into a crude picrate, which given in small doses was found to protect rice-fed pigeons from polyneuritis. Smith and Hendrick discovered that a daily dose of 1 or 2 mg. of Seidell's vitamin picrate could not induce growth in rats when given as the sole source of vitamin B in an otherwise complete diet. When autoclaved yeast was added to the ration good growth occurred, though loss of weight and death were the result when autoclaved yeast was

and vitamin G for vitamin B_2, whilst others term the antineuritic factor vitamin F (Sherman). Funk gave the name of vitamin D (now reserved for the antirachitic vitamin) to 'Bios'.

given without the picrate. Since the antineuritic vitamin is known to be rapidly destroyed by temperatures much over 100°C., it followed that the growth-stimulating factor in autoclaved yeast could not be that vitamin.

Shortly afterwards Goldberger, Wheeler, Lillie and Rogers [1926] put forward some parallel results. During the previous ten years Goldberger and his colleagues in the U. S. Public Health Service had been engaged in a clinical investigation of pellagra, the various steps of which will be recounted later (p. 177), and it was with direct reference to their clinical researches that the laboratory study of vitamin B in rats was undertaken. Goldberger and his co-workers observed that autoclaved yeast could not sustain growth in young rats when given as the sole source of vitamin B in amounts equal to 30 or 40 per cent. of the basal diet. 40 per cent. of an 85 per cent. alcoholic extract of whole maize, capable of curing polyneuritis in the rat (and, therefore, potent in vitamin B_1), was likewise ineffective for growth. But when small amounts of both were given together (10 per cent. autoclaved yeast : 5 per cent. maize extract), the rats grew normally. It was thus clearly shown, in this and the preceding experiment, that the antineuritic vitamin will not of itself promote growth in rats fed on diets totally lacking in vitamin B, unless supplemented by a second factor present in autoclaved yeast.

In the same year Goldberger and Lillie [1926] produced by means of diet a 'pellagra-like' condition in the albino rat. It was found that when 6 per cent. of the alcoholic maize extract, rich in the antineuritic vitamin B_1, was fed to rats on a vitamin B-free diet, the animals failed to grow and a certain number developed 'a dermatitis at one or other of the following sites: ears, front of neck and upper part of chest, forearms, backs of forepaws, shins, and the backs of the hind paws. This dermatitis, particularly as it affected the paws, was sharply defined and bilaterally symmetrical.' The dermatitis rapidly disappeared and growth was resumed when 9 per cent. of autoclaved yeast or 6 per cent. of Seidell's [1922, 1] 'activated solid', a preparation containing both factors in the vitamin B complex, was included in the diet. Goldberger and Lillie tentatively suggested that the experimental rat disease, which developed on diets deficient in the B-vitamin contained in autoclaved yeast, was the analogue of pellagra in man. They termed this vitamin the P-P, or pellagra-preventive factor. It is now known in this country as vitamin B_2 (antidermatitis) and is termed vitamin G by some American authorities.

Confirmation of Goldberger's results, and those of Smith and Hendrick, came from Chick and Roscoe [1927]. These workers fed to young rats as the sole source of vitamin B an alcoholic extract made from material adsorbed on charcoal in Peters's [1924] process for purification of the antineuritic vitamin contained in brewer's yeast. This extract, known to be highly effective in curing polyneuritis in pigeons, was found incapable of sustaining growth in rats unless supplemented by autoclaved yeast. Animals fed on the extract alone remained stationary in weight, and in some cases, after six to eight weeks, developed symptoms roughly similar to those described by Goldberger—loss of hair, blood-stained urine, conjunctivitis, and red,

inflamed patches of skin on the nose and the backs of the forepaws, which later became oedematous.

Almost simultaneously with the publication of the experiments of Goldberger and his colleagues, and those of Chick and Roscoe, a number of investigators put forward further evidence indicating that vitamin B is separable into two components. Randoin and Lecoq [1926, 1, 2; see also Scotti-Foglieni, 1926, 2] showed that certain yeasts of different origin, possessing equal power of maintaining growth, varied in their antineuritic potencies. Hauge and Carrick [1926, 1] discovered that a certain brand of yeast would produce growth in chicks and yet not prevent the development of polyneuritis, while whole maize possessed converse properties. It was observed by Salmon [1927] that the seeds of the velvet bean were more effective than the leaves in preventing polyneuritis in pigeons, while the leaves were superior for the maintenance of growth in young rats.

R. R. Williams and Waterman [1927–28] found that when an aqueous yeast extract was passed through fuller's earth, a substance was adsorbed (vitamin B_1), which prevented polyneuritis in pigeons on a vitamin B-free diet, yet did not promote growth in rats unless supplemented by autoclaved yeast. Sherman and Axtmayer [1927] recorded a supplementary relationship between whole wheat and autoclaved yeast and between whole wheat and dried skim milk, when these were used to supply vitamin B in an otherwise complete diet.

Among others who contributed data bearing indirectly on the question of the complex nature of vitamin B may be mentioned Hassan and Drummond [1927], who observed that a yeast extract autoclaved in alkaline solution at 120° C. for one hour could accelerate growth in rats fed on diets excessively high in protein and low in vitamin B; Palmer and Kennedy [1927, 1, 2], who found that when wheat embryo, an excellent source of vitamin B_1, was given as the sole source of vitamin B, better growth could be obtained by the addition of autoclaved yeast; and Evans and Burr [1928, 3], who recorded that 'tikitiki', a dilute alcoholic extract of rice polishings used with good effect in infantile beri-beri, is deficient in the second component of the vitamin B complex. Other results obtained by these last two groups of investigators, and by Chick and Roscoe [1928], suggested that commercial caseinogen is liable to contain some vitamin B_2. Careful purification is thus necessary if caseinogen is to be used for experimental work on this vitamin.

To sum up, it may be said that differences in heat-stability, distribution in nature, and solubility in alcohol made possible the identification and separation of the components of the vitamin B complex now known as vitamins B_1 and B_2.

Further Components of the Vitamin B Complex.

Vitamins B_3 and B_5. It has been found that pigeons require for their nutrition not only vitamin B_1 but a second factor, vitamin B_3 [R. R. Williams and Waterman, 1927–28], which occurs in wheat but is only present in traces in marmite, and without which the birds fail to increase in weight. These two factors, however, do not suffice for the full normal nutrition of the pigeon, a third, vitamin B_5, being also

required [Kinnersley and Peters, 1925; Carter, Kinnersley and Peters, 1930, 1, 2] (see p. 165).

Vitamin B_4. Reader [1929, 2; 1930, 1, 2] has demonstrated that rats require for their growth, in addition to vitamins B_1 and B_2, a third factor (vitamin B_4) which is thermolabile and differs from the vitamin B_3 required by the pigeon. Its presence is necessary for the growth of the rat and its deficiency leads to characteristic symptoms (p. 167).

Factor Y. In addition to the foregoing it has been shown [Chick and Copping, 1930, 2] that yeast and watery yeast extracts contain a factor (factor Y) differing from all the preceding by not being inactivated by prolonged heating in alkaline solution. It is necessary for the continual normal growth of the rat and although plentiful in yeast is entirely absent from egg-white, which is rich in vitamin B_2 (p. 167).

The rat thus requires for satisfactory nutrition four vitamins of this group, B_1, B_2, B_4, and factor Y; the pigeon at least three vitamins, B_1, B_3, and B_5.

Our present knowledge of the various components of the vitamin B complex is rendered imperfect and unsatisfactory by the conflicting statements of different workers on such properties as solubility and stability to the action of heat and alkali. These discrepancies are especially marked in case of the antidermatitis vitamin B_2, and the facts suggest that these characteristics are greatly affected by the source from which the vitamin is derived and the medium in which the test is carried out.

Properties such as the above are largely used as criteria in the experimental work directed to separation of the different members of the group from one another and in the evidence produced for their separate identity. The study of the more recently described members of the group, vitamins B_3, B_4, B_5, and 'factor Y' is thereby greatly complicated.

Vitamin B_1 (Antineuritic Vitamin).

Beri-beri.

There is now a general consensus of opinion that beri-beri is a disease of dietetic origin. It occurs principally among the rice-eating populations of Japan, India, the Malay Peninsula, Dutch Indies, and Philippine Islands, but is also endemic among the inhabitants of Newfoundland, Labrador, etc., who subsist largely on a diet of white wheaten flour. In Europe under normal peace conditions beri-beri is rare owing to the great variety of foods consumed, but in war time when the diet becomes considerably restricted there is a liability to outbreaks of the disease.

Beri-beri may appear in two well-marked forms. In one ('dry' type) there is great muscular wasting, anaesthesia of the skin, and finally paralysis of the legs, and in some cases of the arms, diaphragm and intercostal muscles. There is an associated degeneration of the peripheral nerves involving both motor and sensory fibres. In the 'wet' type of the disease the most marked symptom is oedema involving the limbs and trunk and effusion of fluid into serous cavities. There is marked dilatation of the heart, particularly of the right side,

together with congestion of the liver and abdominal viscera. There is usually little or no congestion of the pulmonary circulation. Heart failure, frequently sudden in onset, is common and the mortality is high.

As regards the aetiology of beri-beri the earlier workers [Eijkman, 1897, 1, 2, 3; Grijns, 1901; Braddon, 1907; Fraser and Stanton, 1909; Strong and Crowell, 1912; and many others] have laid stress on the association of the disease with the eating of highly milled rice from which the germ and pericarp have been removed, and its cure or prevention when these milling products are restored to the diet (see Fig. 25).

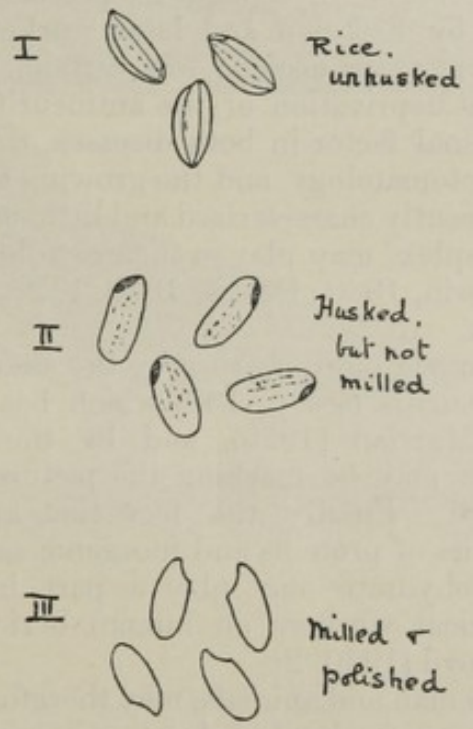


FIG. 25. Showing the various stages in milling of the rice grain. I. Rice grain in the natural condition, retaining the husk or enclosing glumes. II. After removal of the husk, but retaining the pericarp or 'silver-skin', and the embryo, which is shaded. III. After milling and polishing; both 'silver-skin' and embryo are removed and the grains are then 'polished' by rubbing with talc between sheepskins.

As early as the year 1885 Tahaki [1885; see also 1887; 1906] had found that beri-beri in the Japanese navy was abolished by changes in the dietary, the chief of which consisted in the partial replacement by barley of the polished rice which had been the staple article of diet.

In 1886 a commission was appointed by the Dutch Government to investigate the cause and nature of beri-beri. After the departure of the Commission from Java, Eijkman, who was one of the assistants to the Commissioners, Pekelharing and Winkler, continued the investigation in a laboratory attached to the military hospital of Batavia (Java) and observed a sudden outbreak among the laboratory experimental fowls of a disease strongly reminiscent of human beri-beri, the birds developing marked polyneuritic symptoms. The epizootic lasted only about five months and was traced to the use by the laboratory assistant, on economical grounds, of boiled rice from the hospital kitchen for feeding his fowls. The disease disappeared when a new chef in the hospital kitchen refused to supply 'military' rice

for fowls which were under the 'civil' administration of the laboratory [Eijkman, 1890, 1896, 1929].

Following this pioneer observation the development of polyneuritis in animals, and in particular in fowls and pigeons, has been the subject of intensive study on the part of Grijns, Schaumann, Vedder, Chamberlain, Funk, and McCarrison among others. [For bibliographies upon the subject the following should be consulted: Braddon, 1907; Schaumann, 1910; McCarrison, 1921; Funk, 1922; Kruse and McCollum, 1929; Peters, 1930, 1, 2; Sherman and Smith, 1931.]

The discovery of avian polyneuritis was an important step towards the elucidation of beri-beri, though its precise relation to human beri-beri, accepted by Eijkman and later workers as an analogous disease, has of recent years become less certain. While there is no room for doubt that deprivation of the antineuritic vitamin must be regarded as a cardinal factor in both diseases, the diversity of their pathology and symptomatology, and the growing uncertainty as to the rôle which other recently characterized and little-understood factors of the 'vitamin B complex' may play in their aetiology [R. R. Williams, Waterman and Gurin, 1930; Peters, 1930, 1, 2] render the problem extremely complex.

The question is made more obscure by the association of inanition with avian polyneuritis (see p. 130) which has been stressed by Drummond and Marrian [1926], and by the possibility that a coexistent inanition may be masking the picture of pure deficiency [McCarrison, 1928]. Finally the fact that an ill-balanced diet involving deficiencies of proteins and inorganic salts and an excessive allowance of carbohydrate may play a part has been repeatedly mentioned by clinical workers on human beri-beri, and has been discussed by Aykroyd [1930, 2].

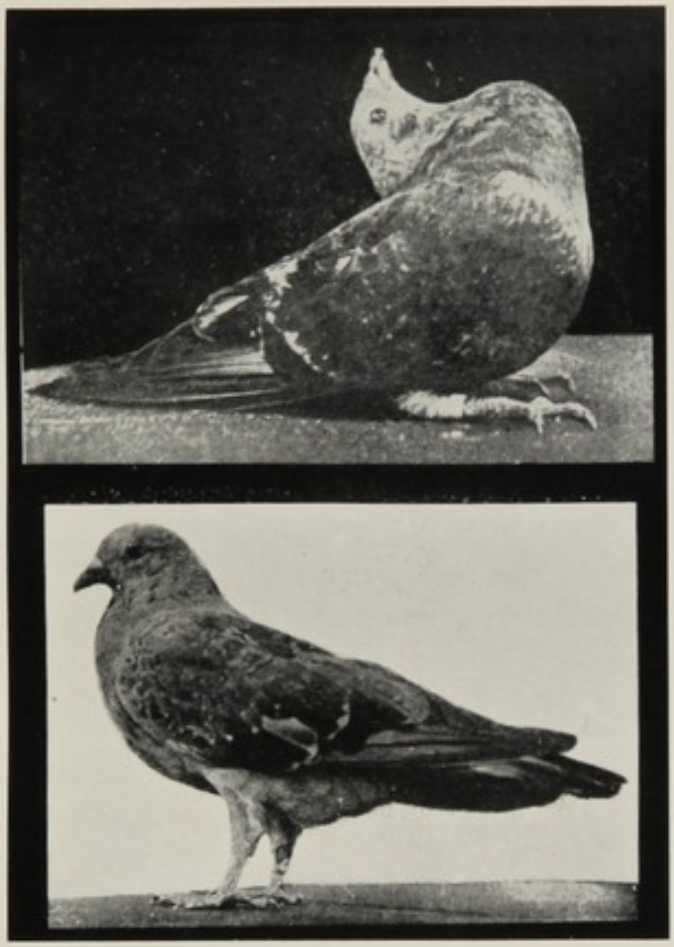
Beri-beri, both in man and animals, may therefore prove to be a syndrome rather than a clinical entity due to a vitamin deficiency.

In an extensive study of avian polyneuritis McCarrison [1928] has distinguished two types of the disease, which he terms polyneuritis columbarum, analogous to the condition described by Eijkman and others, and beri-beri columbarum.

Polyneuritis columbarum is characterized by loss of appetite, asthenia, intestinal fluxes, and symptoms attributable to derangements of the nervous system (opisthotonos, emprosthotonos, peripheral neuritis) (see Figs. 26, 27, 28). Pathologically the condition shows:

(1) Degeneration of a variable number of cells of the central nervous system.
(2) Diminution in the size of the heart.
(3) Adrenal enlargement.
(4) Atrophy of all other organs (the pituitary in males excepted).
(5) Degenerative changes of the alimentary canal.
(6) Diminution of blood volume, anaemia, acidosis, and in some cases, oedema.

Beri-beri columbarum is indistinguishable clinically from polyneuritis columbarum. The pathological features of avian beri-beri which serve to distinguish it from polyneuritis proper are the hypertrophy and dilatation of the heart, particularly of the right side, associated with fatty degeneration of the cardiac muscle and sub-



Figs. 26 and 27.

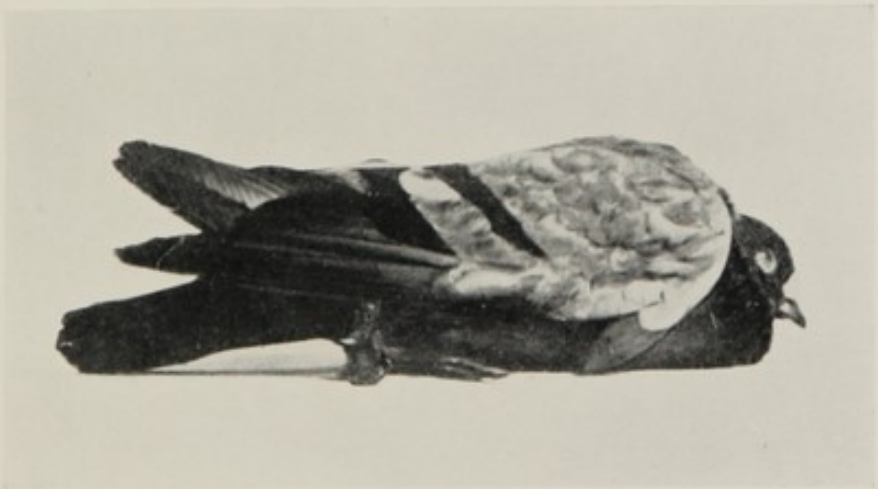


Fig. 28.

Fig. 26. Pigeon suffering from polyneuritis and showing head retraction (opisthotonos) as the result of a diet of polished rice.

Fig. 27. The same pigeon the day after having received a curative dose of yeast preparation.

Fig. 28. Pigeon suffering from polyneuritis, showing emprosthotonos.

(Reproduced from Schaumann (1910). *Arch. Schiffs. u. Tropenhyg.* 14, Beiheft. 3.)

To face p. 124]

serous ecchymoses; the chronic passive congestion of the abdominal viscera; the more marked degenerative changes of the central nervous system and skeletal muscles; and the high proportion of cases manifesting oedema, hydropericardium, hydrothorax, and ascites.

The cardiac enlargement found in human beri-beri has been ascribed by Aalsmeer and Wenckebach [1929] to oedema of the cardiac muscle which results in an interference with its contractile power without disturbance of its excitability. Newcomb [1930], on the other hand, was unable to detect any significant difference in the water content of the heart-muscle from cases of beri-beri and polyneuritis in pigeons, and concluded that the cardiac enlargement in the former case was a true hypertrophy of the ventricular muscle-fibres.

McCarrison regards polyneuritis columbarum as a true form of deficiency disease, whereas beri-beri columbarum, in its fully developed form, is believed to be something more than the manifestation of a specific dietary deficiency. He considers that in the latter type of the disease there is superimposed on a pure vitamin deficiency a toxic factor which is related rather to a relative deficiency of the antineuritic factor than to an absolute lack of it in the diet. This can be seen by reference to Table VIII. Where the intake of the antineuritic vitamin was withheld completely, uncomplicated polyneuritis resulted.

TABLE VIII.

Vitamin intake expressed as percentage of normal requirement.	*Percentage of cases developing beri-beri columbarum.*
20	3
50	23
64	40

[After McCarrison, 1928.]

McCarrison found no evidence to suggest that the toxic factor was produced from rice as a result of bacterial action, and he regarded it as being a product of disordered metabolism, as are the ketone bodies in diabetes mellitus. Thus he says: 'the diversity of symptoms and pathological changes resulting from vitamin deficiency will be determined by the varying biochemical response of different individuals to this influence, and by the extent to which the specific excitant of true beri-beri is produced within their bodies.' The conception that beri-beri is in part at least a toxaemia is no new one. Eijkman [1897, 3] first suggested that a substance present in the cortical layers of rice was required to neutralize the deleterious effects of a diet over-rich in starch. A toxic factor was stressed as a causative agent in the writings of Mott and Halliburton [1899], of Stanley [1903], of Hamilton Wright [1905], of Braddon [1905; 1907] and notably of Walshe [1917–18; 1920] among others.

McCollum, Simmonds and Pitz [1916] believed that wheat embryo contained a toxic factor which could be extracted by ether. R. R. Williams [1927] has described a toxic condition in pigeons, characterized by loss of weight and appetite, mild diarrhoea, nerve degeneration sometimes associated with paralysis, and head retraction, when the birds were fed on exclusive diets of rice-bran or rice polishings, both potent sources of the antineuritic vitamin. No such symptoms were observed on a diet equivalent to whole rice. Williams

suggests that toxaemias may play a role in determining the symptoms of deficiency diseases. The foodstuffs productive of these diseases are not necessarily toxic in the ordinary sense, though they may become so when other dietary defects remain uncorrected.

A beri-beri-like condition has been described in swine restricted to diets of corn and wheat grain or the outer coats of rice [Moore, 1914; Hart and McCollum, 1914; and Hart, Miller and McCollum, 1916]. These diets were rich in the antineuritic vitamin though deficient in proteins and inorganic salts.

It is important to remember that the diets in countries where beri-beri is prevalent are invariably ill balanced, and that there are other deficiencies in the diet which may modify the character of beri-beri.

With regard to deficiencies of proteins or salts there is comparatively little evidence, although Kohman [1920] produced oedema in rats on a diet deficient in protein.

Finally, the possibility that vitamin deficiencies other than that of the antineuritic vitamin B_1 may be involved in the aetiology of beri-beri must be considered. A number of little understood but suggestive facts indicate that the clinical and pathological picture characteristic of a particular deficiency disease may be profoundly modified when there is a coexistent deficiency of other dietary factors. Thus a rat fed on a diet involving a total deficiency of the vitamin B complex rarely develops either paralysis or dermatitis, but rapid death usually ensues without symptoms of either.

Rosedale [1927] has claimed that both rats and pigeons need a factor other than vitamin B_1, in the absence of which beri-beri-like symptoms develop, characterized by oedema and intestinal stasis. A similar view was expressed by Vedder and Williams [1913]. R. R. Williams, Waterman and Gurin [1930], and Peters and his colleagues [Peters, 1930, 2] have confirmed the finding of Jansen and Donath [1927], that highly purified preparations of the antineuritic vitamin do not protect pigeons from loss of weight in the absence of other factors, and these authors have concluded that avian beri-beri is a disease involving multiple deficiencies, which may account for its varied symptomatology.

Peters [1930, 1, 2] obtained evidence that protection against avian polyneuritis after the 30th day of polished-rice feeding is not afforded by doses of vitamin B_1 that are entirely effective in this respect before this period. The inference drawn from this observation is that in such cases the traces of vitamin B_1 contained in the diet and in the animals' own reserves are sufficient to prolong life until the development of secondary deficiencies of other vitamin factors, the store of which in the body is depleted more slowly than the antineuritic factor.

The study of polyneuritis in the rat has yielded results substantially similar to those observed in pigeons. Schaumann [1910] observed paralysis in the hind limbs of rats fed on a diet of horse flesh previously autoclaved at 120° C. Drummond [1918, 1] reported that only a small proportion of animals deprived of 'water soluble B' developed signs of paralysis. A detailed study of this condition was made by Hofmeister [1922], who found that it could best be studied in a more chronic form where rapid decline was prevented by employing a diet only partially deprived of the vitamin. In a few cases a spastic

condition of the limbs occurred, accompanied by tremors and convulsions, but in most of the animals ataxia was followed in a few days by paralysis. No degenerative changes were observed in the peripheral nerves, but slight swelling of the myelin sheath and an increased number of nuclei in the sheath of Schwann were observed. In the brain and cord Hofmeister found small capillary haemorrhages and chromatolytic changes of the nerve-cells.

Woollard [1927] and Stern and Findlay [1929] have confirmed the histological findings reported by Hofmeister. The former observed that similar, though less pronounced, changes could be detected in rats receiving daily doses of marmite (a commercial yeast extract) but otherwise starved. The latter workers noted slight chromatolytic changes in the ganglion cells of the cord following uncomplicated vitamin B_1 deficiency, while degeneration in the myelin of the peripheral nerves was only seen in cases of long-standing paralysis. These inconspicuous differences are in marked contrast to the striking changes found in birds.

The researches of recent years have not shaken the established fact that human beri-beri is primarily due to deficiency in the diet of the antineuritic vitamin. On the other hand, it is probably unwise to regard the beri-beri problem in the tropics as being solely a question of polished or unpolished rice. The work of McCarrison [1928] suggests that human beri-beri may occur in spite of the presence of a certain amount of vitamin B_1 in the diet. From this it follows that relatively undermilled rice may sometimes be associated with the disease, particularly when the diet is deficient in supplementary foods. Human beings rarely live upon rice alone, and the usual consumption of a certain amount of other foodstuffs prevents an absolute correlation between the incidence of beri-beri and the vitamin B_1 content of the rice consumed. McCarrison's observation [1924] that the vitamin B_1 content of unpolished rice may, by washing, be reduced to that of milled rice, may help to explain certain puzzling features, described by the same author, in the epidemiology of beri-beri in India. Undermilled rice of poor commercial quality may require vigorous washing before it is fit for human consumption.

Clear and convincing evidence has nevertheless accumulated from many lands that the substitution of undermilled for milled rice will cause beri-beri to disappear. Even in India, where, as McCarrison has shown, some facts seem at first sight to clash with the vitamin B_1 deficiency hypothesis, the association of beri-beri with milled rice, and its absence when undermilled rice is eaten, is as clear as elsewhere. Infantile beri-beri responds at once to treatment with extracts of rice polishings [Wells, 1921] (see p. 155), but with regard to the effect on adult beri-beri of treatment by vitamin B_1 the evidence is not so abundant. Various observers have, however, attested the value in the adult disease of rice polishings, or extracts of rice polishings [Bréaudat and Denier, 1911; R. R. Williams and Saleeby, 1915; Jansen and Donath, 1926, 1].

The number of clear-cut clinical experiments on record, in which adult beri-beri was treated by vitamin B_1 alone without other dietary alteration, is not large. Failure to secure obvious benefit by means of vitamin B_1 extracts may be due to too small a dosage. Moreover,

when nerve degeneration is excessive, improvement must necessarily be slow.

The diets on which beri-beri develops are usually deficient in other factors besides vitamin B_1, and some of the symptoms grouped under the heading 'beri-beri' may well be due to other deficiencies. The oedema of wet beri-beri has been ascribed to shortage of protein. In treatment, while stress should be laid on vitamin B_1 as the most important curative agent, it is reasonable to give in addition a good all-round diet containing abundant protein, vitamins, and mineral salts.

Physiological Significance of the Antineuritic Vitamin B_1.

Growth and Maintenance.

As a result of the work which proved the multiple nature of the vitamin B complex, it may be regarded as firmly established that vitamin B_1, in addition to affording protection against polyneuritis, is an essential factor for the normal growth of young rats, and for the maintenance of normal weight and health in adults. Deficiency of this vitamin, as of most other vitamins, is a limiting factor for normal growth and rapidly makes itself felt.

After a period of obscurity, a similar view of the relation of vitamin B_1 to weight maintenance in the pigeon has now become possible. As early as 1911 Schaumann and Chamberlain had noted that, whereas rice-bran or yeast prevented polyneuritis and maintained the weight of birds, the extracts of these substances retained their antineuritic potency but failed to prevent loss of weight. Cooper [1912, 1914, 1, 2], in his investigation of the vitamin B_1 content of foodstuffs, found that the amount needed to restore the weight of rice-fed birds did not correspond to the amount needed to cure polyneuritis, and it was also observed that activated fuller's earth, after treatment with alkali, lost its weight-maintaining properties without sensible deterioration of its antineuritic activity [R. R. Williams and Seidell, 1916]. Funk and Macallum [1916] concluded that the antineuritic and weight-maintenance factors were identical, but they suggested that a larger amount of the vitamin was required for the latter than for the former function. Emmet and McKim [1917] also observed that activated fuller's earth was potent in curing avian polyneuritis, but was much less effective in restoring weight than unmilled rice and other cereals. They recognized the necessity for postulating the existence of two factors, thus confirming the suggestion made originally by Chamberlain and Vedder. This view was not accepted by Seidell [1922, 1] who held that the curative and weight-maintaining properties of his preparations were due to the activity of one substance. The experience of later workers, however, has confirmed the belief that the antineuritic factor does not suffice for the maintenance of body-weight in pigeons when added to a basal diet of polished rice [Kinnersley and Peters, 1925; Jansen and Donath, 1926, 1; R. R. Williams and Waterman, 1928; R. R. Williams, Waterman and Gurin, 1930; Carter, Kinnersley and Peters, 1930, 1]. In the nutrition of the pigeon, as in that of the rat, the antineuritic vitamin is to be regarded as one of several water-soluble accessory factors required for maintenance of normal weight and health (see p. 121). Lack of this factor, both in the rat and in

the pigeon, and presumably in other species, results in decline in weight, as well as in the ultimate development of polyneuritis.

Vitamin B has been found necessary for the growth and maintenance not only of mammals and birds, but also of the frog [Emmett and Allen, 1919; Harden and Zilva, 1920, 2; Billard, 1922], and of insects, *Drosophila* [Northrop, 1917; Bacot and Harden, 1922], and the flour beetle, *Tribolium confusum* Duval [Sweetman and Palmer, 1928].

Storage and Utilization of Vitamin B_1.

Animals possess a definite but limited store of vitamin B_1, which in the case of the rat or the pigeon is exhausted within three or four weeks, as judged by the onset of polyneuritis after this time [Vedder and Clark, 1912; Funk and Macallum, 1915].

The amount of growth which occurs in young animals placed on a vitamin B-deficient diet seems to bear some relation to the vitamin content of the previous diet [Steenbock, Sell and Jones, 1923]. Similarly the administration of an excess of the vitamin B complex to pigeons for some days before placing them on a deficient diet delays the subsequent loss of weight for a time which varies with individual birds [Pilcher and Sollman, 1925]. It has in fact been experimentally shown that the livers of normal rats contain vitamin B, which to a large extent disappears when they are fed on a diet deficient in this vitamin [Osborne and Mendel, 1923]. In man the latent period of beri-beri appears to be not less than 90 days [Fraser and Stanton, 1911] (see p. 250).

Drummond [1918, 1] observed that the length of time over which a rat is able to maintain itself on a diet deficient in vitamin B without serious loss of body-weight is directly proportional to the age at which the restriction is imposed. Adult animals were found to be capable of surviving long periods of deprivation, during which they suffered slow decline of body-weight, though no such feature of the growth curve was seen for young animals. Ultimately both young and adult animals exhibited a rapid decline in weight, followed by death. This led to the belief that the needs of the body for vitamin B are greater during growth than during maturity. As pointed out, however, by Osborne and Mendel [1922, 1], the *rate* of decline in animals possessing undoubtedly different nutritive reserves does not necessarily indicate a difference in the requirement of the vitamin for young and adult animals. They [1922, 1; 1925] found that whereas a daily supplement of vitamin B in the form of 200 mg. of dried yeast gave normal growth in rats, at whatever age the experimental diet was imposed, with doses of 50 mg. of yeast daily the rate of growth was retarded from the beginning of the experiment. With still smaller doses of yeast the larger animals failed in every case even to maintain weight, while the smaller animals failed to give continued growth for any length of time. They concluded that the vitamin B requirement under conditions of growth or maintenance on a diet of constant composition bears some quantitative relationship to the mass of active tissue.

There is some evidence for the view that there is a definite daily utilization of the vitamin [Chick and Hume, 1917–19, 1; Kinnersley, Peters and Reader, 1928]. Not only do symptoms of polyneuritis

tend to appear in any given pigeon at a fairly constant date after the commencement of a polished-rice diet, but the duration of the cure following the administration of a given dose of vitamin B_1 is related to the size of the dose. Moreover, the rate of growth of young animals is roughly proportional to the vitamin B content of the diet [Osborne and Mendel, 1917, 2; McCollum and Simmonds 1917; Drummond, 1918, 1].

Loss of Appetite and Inanition.

In his well-known experiments with young rats fed on deficient diets, Hopkins [1912] noted that the food intake became much reduced in the later stages of avitaminosis. He made the important observation that growth is inhibited even when the food intake is more than quantitatively sufficient in calorie value to maintain normal growth. The food, although absorbed normally, is not utilized. Hopkins concluded that the influence of accessory food factors on appetite is secondary to their more direct effect on the growth processes. Diminished food consumption during vitamin B deficiency has been noted in pigeons [Chamberlain and Vedder, 1911, 1, 2; McCarrison, 1921; Kon and Drummond, 1927]. in dogs [Karr, 1920; Cowgill, Deuel and Smith, 1925], and in rats [Funk and Macallum, 1915; Osborne and Mendel, 1917, 2]. Drummond [1918, 1] regarded this as an adjustment in response to the lowered calorific requirement of the animal, a view supported by the fact that polyneuritis develops earlier in animals forcibly fed than in those allowed to feed naturally [Braddon and Cooper, 1914; Marrian, Baker, Drummond and Woollard, 1927]. The significance of loss of appetite in avitaminosis has been discussed by McCarrison [1921] and by Peters [1930, 1, 2]. They take the view that it is protective in its effect on the pigeon, whether by allowing the liberation of essential factors during tissue wasting or in some other manner not at present understood. Kon and Drummond [1927] have suggested that such an explanation may underlie spontaneous cures from head retraction observed in pigeons fed on polished rice.

The inanition resulting from lowered food intake is believed by Drummond and his associates to be responsible for many of the features observed in avitaminosis. Thus fall of body-weight, ruffled feathers, fall of body temperature, and hyperglycaemia are regarded as being due to partial starvation rather than as specific manifestations of lack of vitamin B_1. Pigeons receiving water and 1 g. of marmite daily, but otherwise starved, presented a picture closely resembling that seen in avitaminosis. A number of other observers, whose work is referred to below, have called attention to the fact that many of the features of vitamin B deficiency are closely bound up with the condition of inanition which always accompanies it. It has, indeed, proved extremely difficult, if not impossible, to disentangle the effects of the one from the other [see for example, Simonnet, 1920; 1921]. Nevertheless, avitaminosis is not to be explained merely in terms of inanition. Much evidence has accumulated to show that the vitamin exerts some favourable influence on the metabolism of the animal which is reflected in its continued growth and well-being.

The immediate effect on the appetite of removing either vitamin B_1 or B_2 was well shown in the work of Boas-Fixsen [1931] with adult rats. A decline was noticed within 48 hours, even when the previous diet was entirely adequate, and was greater when vitamin B_1 was removed. These facts argue a very limited capacity for storage of the vitamin, which appears to be excreted day by day chiefly in the faeces.

Relation of Vitamin B_1 to Metabolism.

Much of the literature bearing on the relation of vitamin B_1 to metabolism is conflicting. Attention has been divided between the exact significance of vitamin B_1 for metabolic processes as a whole and its relation to one or other of the main sources of food material. It is important to bear in mind that many of the findings refer to the significance of vitamin B as a whole and require reinterpretation in the light of the multiple nature of this complex.

Many observers have recorded the fall of body temperature in monkeys, rats, and birds during avitaminosis, particularly in the terminal stages, in which there appears to be complete loss of temperature regulation [Eijkman, 1897, 3; Drummond, 1918, 1; Novaro, 1920; Abderhalden, 1920, 2; Cramer, Drew and Mottram, 1921; McCarrison, 1921; Farmer and Redenbaugh, 1925–26, 1; Drummond and Marrian, 1926; Kon, 1927, 1]. In such cases administration of the antineuritic vitamin is followed by a recovery of body temperature within 24 hours.

An indirect relation between vitamin B_1 deficiency and failure in certain oxidase systems was suggested by two studies. Dutcher [1918, 1] observed a diminution in the catalase content of certain organs, while G. M. Findlay [1921, 1] noted that the glyoxalase content of the liver was decreased in the absence of vitamin B, but increased again when it was administered.

Opinion is, however, sharply divided as to whether there is a specific diminution in the oxidative capacity of the tissues due to vitamin B deficiency. In the living animal, associated with the fall in temperature, there occur a lowered basal metabolism and alterations in respiratory exchange, first described by Ramoino [1916] and confirmed by others, but especially stressed by Abderhalden [numerous papers, including some with Schmidt and Wertheimer, 1920–22]. Their view that the changes were specific to vitamin B_1 deficiency was successfully challenged by Drummond and Marrian [1926], whose contention that they were due to the accompanying inanition receives support from the work of Anderson and Kulp [1922] and Gulick [1922; 1924] on poultry and rats respectively [see also Farmer and Redenbaugh, 1925–26, 1; Galvao and Cardoso, 1931]. In comparative experiments with the oxidase systems in isolated tissues from normal and avitaminous animals, it is necessary to distinguish between experiments in which (*a*) actual oxygen uptake or (*b*) hydrogen transfer in the presence of dyes is measured. These processes can be carried out either in presence or absence of various substances added as substrates, and have been studied upon muscle, liver and brain tissue, usually obtained from pigeons. Drummond and Marrian [1926] working with liver and muscle did not confirm

the claims of Abderhalden *et al.* [1920-22], or of W. R. Hess [1921] and Hess and Messerle [1921] that there was a specific change either of actual oxygen uptake or of dye reduction in presence and absence of succinate; a similar view was reached by Roche [1925]. This failure to demonstrate changes has not, however, deterred further investigation. Later, Kollath [1929], from a comparison of the effects of vital staining by methylene blue in normal, starving and polyneuritic pigeons, concluded that in polyneuritis a definite change in the oxidation-reduction potential of certain organs could be demonstrated, associated with changes in cell permeability. This was succeeded by a reiteration by Abderhalden and Vlassipoulos [1930] of the claim that differences in oxygen uptake of pigeon's tissues can be demonstrated in the absence of added substrate. As compared with the normal, an average of 12 observations showed a fall for the liver of avitaminous pigeons of 18 per cent. (not shown by starving birds), and for the brain falls of 12 per cent. for starving and 23 per cent. for avitaminous birds. Using similar methods, Gavrilescu and Peters [1931; Gavrilescu, Meiklejohn, Passmore and Peters, 1932, unpublished results] find a slight, statistically insignificant, reduction in oxygen uptake by pigeon's brain without added substrates for avitaminous as compared with normal birds. With added glucose, or more readily with lactate, decreases of 20 per cent. and 40 per cent. respectively were found, more marked in the optic lobes. On the other hand, tissue from avitaminous birds behaved normally in the presence of succinate as substrate. Vitamin B_1 concentrates added in amounts of 1 γ (0·001 mg.) or less per 100 mg. tissue largely restored the oxygen uptake in presence of glucose and lactate, but had little influence on normal brain with added lactate or on avitaminous brain with added succinate. They inferred that the active agent in the concentrates was vitamin B_1. At least these results exclude the possibility that the observed diminution in oxygen uptake was due solely to a diminished amount of tissue substrates. The present position, therefore, is that the deprivation of vitamin B_1 superimposes specific biochemical lesions in the glucose-lactate system upon any changes that may be due to the accompanying inanition. There appears to be some correlation between these specific changes and the opisthotonus symptoms.

The problem of the relation of vitamin B to the quantity and type of foodstuff metabolized has been the subject of a number of investigations.

Calorie intake. As regards the total calorie intake, it has been claimed that the vitamin B requirement of an animal is roughly proportional to the total calorie intake × weight $^{5/3}$ [Cowgill, Smith and Beard, 1925; Beard, 1925], whereas Plimmer, Rosedale and Raymond [1927] consider that it is simply proportional to the calorie intake; these relations, however, have been disproved by Roscoe [1931, 1].

Protein intake. Although Funk observed that increase in the ration of protein did not affect the rapidity of onset of polyneuritis, some relation between vitamin B and protein metabolism has been established. Thus the amount of vitamin B required by the rat in order to ensure proper lactation has been found to be proportional to the protein content of the diet [Hartwell, 1922], and in order to

obtain normal growth there must also be a definite relation between the vitamin B content of the diet and the protein intake [Drummond, Crowden and Hill, 1922; Reader and Drummond, 1926]. Later, Hassan and Drummond [1927] showed that the vitamin related to protein metabolism is stable to treatment with hot alkali, indicating that vitamin B_1 is not the accessory factor concerned, although vitamin B_2 may be.

Tscherkes [1926] claimed that vitamin B bore a relation both to protein and to carbohydrate metabolism.

Carbohydrate intake. It was first observed by Funk [1914, 1, 2] that polyneuritis in pigeons was the more rapidly produced the greater the amount of carbohydrate ingested by the birds. The difference in the time taken to develop polyneuritis by different individuals was regarded as being determined, in part at least, by the amount of vitamin stored in the body as a consequence of the previous diet, and by the amount and nature of the food eaten during the avitaminous period. Funk's observation was confirmed by Braddon and Cooper [1914], though they did not accept the suggestion that the protection afforded against polyneuritis during starvation was due to the liberation of a supply of the antineuritic substance in the process of tissue wasting. Abderhalden and Lampe [1913] observed that pigeons starved to death did not develop polyneuritis, though this may have been due to the fact that in most cases death from starvation falls within the period required for the development of the disease [Chamberlain and Vedder, 1911, 1, 2]. In a few cases symptoms resembling those of polyneuritis have been observed in starving birds before they have succumbed [Chamberlain, Bloomberg and Kilbourne, 1911; Marrian, Baker, Drummond and Woollard, 1927].

Collazo [1923] found that, after the introduction of considerable amounts of carbohydrate into the crops of normal and avitaminous animals, death invariably occurred in the latter though not in the normal controls. Monosaccharides produced the most toxic, starch the least toxic, effect, while disaccharides occupied an intermediate position. He concluded that carbohydrate metabolism is deranged as a result of the deprivation of vitamin B_1 with the production of toxic substances, and showed [Rubino and Collazo, 1923] that the carbohydrate thus introduced was actually absorbed, but that the liver was unable to store it, the toxic effect not being due to physical reasons as suggested later by Kon [1927, 2].

It has also been claimed that the vitamin B requirement is lower on a high protein than on a high carbohydrate diet [Funk and Collazo, 1925; Randoin and Simmonet, 1924] and can be diminished by substituting part of the carbohydrate of the diet by fat [Evans and Lepkovsky, 1929; 1931].

Carbohydrate metabolism. Disturbance of carbohydrate metabolism when the vitamin B complex is absent has been noted by Randoin and Simonnet [1924] and confirmed by Roche [1931], who found an increase in the ratio C/N of the deproteinized blood filtrates partly caused by hyperglycaemia and partly by the accumulation of intermediate products of carbohydrate metabolism [cf. Kon, 1929; 1931].

Kinnersley and Peters [1929; 1930, 1] have found that the brains of pigeons in the terminal stages of deficiency of vitamin B_1 have an

increased lactic acid content. The increased lactic acid appears to be localized in the region of the hind brain, and in this respect differs from the condition after exercise when the raised lactic acid content is general. Fisher [1931], using monoiodoacetic acid [Lundsgaard, 1930] as fixative agent for lactic acid, has shown that the lactic acid content is also raised in the heart, liver, and muscles of the polyneuritic pigeon, returning more slowly after exercise to the normal. Birds cured with marmite behave normally.

Inawashiro and Hayasaka [1928; Hayasaka and Inawashiro 1929–30], in an exhaustive investigation of the metabolism of beri-beri in man, have found that the lactic acid content of the blood is higher than normal and takes longer than usual to return to the initial level after exercise. They think that the resynthesis of lactic acid in the muscles is retarded in beri-beri.

These observations justify the belief that a deficiency of vitamin B_1 induces a fault in the metabolism of lactic acid and agree with the results of the brain studies of Kinnersley and Peters.

Hyperglycaemia. Studies of the blood-sugar level and the respiratory quotient during avitaminosis have contributed further evidence bearing on the theory relating the antineuritic vitamin to carbohydrate metabolism.

Funk and Schönborn [1914] first recorded the fact that pigeons fed on a diet of polished rice developed hyperglycaemia with a corresponding diminution in the glycogen content of the liver, and this has been confirmed as regards the hyperglycaemia by many other workers for the pigeon, and for the rat by Lepkovsky, Wood and Evans [1930] and by Eggleton and Gross [1925]. Since a similar condition was observed in pigeons during starvation [Marrian, Baker, Drummond and Woollard, 1927], it was attributed to inanition.

During avitaminosis B_1 no change occurs in the concentration of non-glucose reducing substances in the blood [Gulland and Peters, 1930] or in the rate of absorption of glucose [Eggleton and Gross, 1925].

While there can be little doubt that inanition is an important contributory factor in the development of hyperglycaemia as well as of certain other features of avitaminosis, it is difficult to accept the view stated by Drummond and his associates, that no relation exists between vitamin B_1 and carbohydrate metabolism, in the light of the evidence of Funk, Braddon, Cooper and later workers.

Relation of Vitamin B_1 to Secretion of Glands.

A considerable literature has accumulated bearing on the relation of the antineuritic vitamin to glandular function. Much of this is conflicting, owing to differences of technique, not infrequently faulty, and to the variety of experimental animals employed. There appears to be no justification for the view that this vitamin is specifically related to the activity of any particular organ of the body. Disorders of function of glands, as of other organs, undoubtedly occur as the result of deprivation of vitamin B_1, but it is probably safer to regard these as local manifestations of a general 'tissue anarchy'.

Nevertheless, a marked feature of vitamin B_1 deficiency is the atrophy and cellular degeneration produced in many organs (intestinal

and gastric mucosa, pancreas, salivary glands, and liver), a special feature being the disappearance of lymphoid tissue [see Funk and Douglas, 1914; McCarrison, 1921; Cramer, Drew and Mottram, 1921; G. M. Findlay, 1928].

Digestion. The suggestion that vitamin B might be identical with secretin [Voegtlin and Myers, 1919] has been negatived by the demonstration that extracts containing vitamin B failed to evoke any secretions from the pancreas, liver, or salivary glands [Cowgill and Mendel, 1921; Anrep and Drummond, 1920–21]. On the other hand there is evidence that the flow of gastric juice in dogs deprived of vitamin B_1 is greatly diminished or entirely inhibited [Miyadera, 1921] and that in pigeons the lessened amount of gastric juice is almost free from pepsin [Danysz-Michel and Koskowski, 1922]. Karr, on the other hand [1920], claimed that lack of vitamin B in the dog did not involve disturbances in the digestion and absorption of protein.

The inhibition of the flow of gastric juice in dogs is possibly due to the lack, not of vitamin B_1, but of some stimulating substance which is absent from vitamin-free diets; thus administration of alcohol produces an immediate flow of gastric juice. Drummond [1918, 1] found that flavouring agents increased the food intake without influencing growth, confirming the original observation of Hopkins [1912] that vitamins stimulated appetite in a manner quite different from the action of flavouring agents.

As regards the effect of vitamin B_1 deficiency on the secretion of specific enzymes, Braddon and Cooper [1914] found that 90–98 per cent. of the starch ingested by polyneuritic pigeons, even on a high carbohydrate diet, was normally digested and absorbed; no change has been observed by a number of workers in the activity of the amylolytic enzymes in polyneuritic pigeons [Tiger and Simonnet, 1921; Rothlin, 1922; Kon and Drummond, 1927; Farmer and Redenbaugh, 1925–26, 2]. Braddon and Cooper also observed a diminution of proteolytic activity in polyneuritic pigeons and were confirmed in this by the experiments of Rosedale and Oliveiro [1928], who examined the enzyme content of the crop, proventriculus, pancreas, and intestine of normal and polyneuritic pigeons and found absence of trypsin and lipase from the latter. Similarly the secretion of pancreatic juice in dogs fed on polished rice was found by Tsukiye and Okada [1922] to be diminished, but was restored on giving vitamin B.

Endocrine glands. The effect of a deficiency of vitamin B on the ductless glands was first examined in pigeons which had died of beriberi by Funk and Douglas [1914], who found a great diminution in size in every case, accompanied by degenerative changes of the cells. R. R. Williams and Crowell [1915] noted great atrophy of the thymus gland in polyneuritic pigeons and to a lesser extent in chickens, but were unable to find any specific relationship between the antineuritic vitamin and the function of the gland.

Adrenal glands. McCarrison [1919], who made a systematic study of the subject, found that the organs of pigeons on a diet deficient in vitamin B lost weight much as in inanition, with the exception of the pituitary and adrenal glands. The adrenals increased to two or three times the weight of those of control birds. The adrenaline content of the enlarged glands was found to be increased proportionately

to the size of the organ, while the cortex showed an almost complete absence of lipoids. McCarrison noticed a relationship between this adrenal hypertrophy and the occurrence of hydropericardium in his experimental birds. He regarded the excess of fluid as analogous to 'wet' beri-beri in man, and developed a theory of the cause of oedema in this disease on the basis of excessive adrenaline secretion. The enlargement of the adrenal gland and its increased adrenaline content were confirmed by Kellaway [1921] and by Korenchevsky [1923].

There has been much uncertainty as to the relative parts played by vitamin B deficiency and the associated inanition in the hypertrophy of the adrenal glands. The weight of opinion is in favour of the view that in pigeons the hypertrophy due to avitaminosis B is confined to the cortex [G.M. Findlay, 1921, 1; Stern and Findlay, 1929; Beznák, 1923; Marrian, Baker, Drummond and Woollard, 1927; Marrian, 1928]. The adrenal hypertrophy was found to be greater in cases of uncomplicated vitamin B_1 deficiency than in cases where the bird was deprived of both vitamins B_1 and B_2 [Marrian *et al.*, 1927; Marrian, 1928], thus confirming the finding of G. M. Findlay [1928] with regard to rats. Gross [1923] working with rats found neither hypertrophy of the gland nor increase of adrenaline content as compared with normal animals.

Sexual glands. Deficiency of vitamin B also exerts a powerful influence on the sexual glands. In male animals (monkeys, pigeons, fowls) atrophy of the testis occurs [Funk and Douglas, 1914; McCarrison, 1919; Dutcher and Wilkins, 1921], accompanied by absence of spermatozoa and spermatocytes. Similar changes were observed in the rat by Allen [1919] and Parkes and Drummond [1925], but there is some doubt whether this was due to deficiency of vitamin B or of vitamin E [Mattill, 1926–27]. The testicular degeneration in birds seems to be a true deficiency effect and not due to inanition. The effect of vitamin B_1 deficiency is much more marked than that of vitamin B_2 [Marrian and Parkes, 1928].

In female mice on a diet deficient in vitamin B anoestrus commences abruptly, the normal oestrous cycle ceasing just before decline in weight sets in. Anoestrus is due to the failure to produce oestrin and not to an inability to respond to it on the part of animals suffering from vitamin B deficiency [Parkes, 1928].

Sure [1927, 3; 1928, 2, 3], who has been ineffectively criticized by H. G. Miller [1926–27], finds that the requirement of the female rat for vitamin B_1 and B_2 is three to five times greater for lactation than for normal growth. This increase in the requirement of B vitamins appears to be due to inefficient transfer of vitamin to the milk by the mammary gland. Thus a daily dose of 500 mg. of a yeast concentrate was required by the mother for the normal suckling of a litter of six, whereas a dose of 180 mg. sufficed for successful rearing when 30 mg. was supplied to the mother and the remaining 150 mg. was given to the young directly.

On the other hand Evans and Burr [1928, 1] consider that 'lactation delinquency' is related particularly to the antineuritic vitamin and is in no way due to an inadequate supply of vitamin B_2, the requirement of the lactating animal for vitamin B_1 being five times that for normal growth of the young non-lactating animal.

Relation of Vitamin B_1 to Gastro-intestinal Movements.

Many observers have noted that intestinal atony or stasis results from prolonged vitamin B deficiency in pigeons [Vedder and Clark, 1912; McCarrison, 1921; Anderson and Kulp, 1922; Plimmer and Rosedale, 1926; Rosedale, 1929], in rats [Gross, 1924], in monkeys [McCarrison, 1921], and in dogs [Cowgill *et al.*, 1925; 1926; 1930]. The last-named authors have specially studied the gastric movements and consider that the defective motility of the stomach is a secondary effect due to the condition of anhydraemia which accompanies the vitamin deficiency. On the other hand, Kon and Drummond [1927] found no delay in the passage of a barium meal through the intestinal canal of polyneuritic pigeons.

Bradycardia.

Carter and Drury [1929] showed that pigeons fed upon a diet of polished rice develop bradycardia and heart block. This condition is abolished by vagal section or atropine, and can be relieved by giving whole wheat or yeast. Deprivation of the vitamin B complex in young rats leads to a severe bradycardia which is different from that seen in the pigeon in that it is of 'sinus' origin and is not due to vagal influences [Drury, Harris and Maudsley, 1930]. These authors found that the lowered heart rate could be restored to normal within a few hours after administration of materials rich in vitamin B_1 and the response was found to be roughly graded to the size of the dose given. It is, however, premature as yet to ascribe these effects to lack of any particular member of the vitamin B complex.

Relation of Vitamin B to Bacterial Infection.

Rats and rabbits fed on diets deficient in vitamin B show no loss of ability to form agglutinins, precipitins, haemolysins, and bacteriolysins [Zilva, 1919, 2; Werkman, 1923] and the opsonic index of the serum of such rats were found to be normal [G. M. Findlay and Mackenzie, 1922]. On the other hand a lowered bactericidal action of such rat serum has sometimes been observed [Smith and Wason, 1923; G. M. Findlay and Maclean, 1925]. Rice-fed pigeons have a lowered resistance to bacterial infection [G. M. Findlay, 1923, 1] and may develop septicaemia [Barlow, 1930].

Blood Changes in Relation to Vitamin B_1 Deficiency.

Anaemia has been observed in polyneuritic pigeons [Abderhalden, 1921, 1; Weill, Arloing and Dufourt, 1922; Palmer and Hoffman, 1922–23; Barlow, 1930], and in young rats deprived of vitamin B_1 [Sure, 1929; Sure and Smith, 1929; Sure, Kik and Walker, 1929]. This is sometimes complicated, especially in the later stages of the deficiency, by loss of water from the blood (anhydraemia), a condition which is also seen in inanition due to restricted diet [see Rose, Stucky and Mendel, 1929–30; Stucky and Rose, 1929].

A marked fall in the white-cell count, due to a reduction in the number of lymphocytes, has been observed [Cramer, Drew and Mottram, 1921], whilst a large proportion of aged or moribund polymorphs are found in the blood-stream [Happ, 1922].

Excretion of Vitamin B.

Much discussion has centred round the question as to whether vitamin B is excreted unchanged [see Van der Walle, 1922, where the literature is quoted]. Positive results were obtained by Cooper [1914, 1] with an alcoholic extract of pigeons' faeces, and by Muckenfuss [1918, 1919] and Gaglio [1919] with urine. Van der Walle has carefully examined the question, and has shown that the undoubted curative effect of normal urine on polyneuritic pigeons is due to the presence of a small amount of the antineuritic vitamin. This possesses the characteristic properties of the vitamin, and disappears from the urine when the diet is deficient in vitamin B_1.

The excretion of the B-vitamins in the faeces would explain the rapid depletion of reserves and the habit of coprophagy which are commonly observed in rats on diets deprived of these vitamins. To diminish access to faeces it is usual, in the study of these vitamins, to maintain the experimental animals in cages provided with bases of wide-meshed wire. Steenbock, Sell and Nelson [1923] found that rats allowed to consume their own faeces might derive as much as half their necessary intake of vitamin B from this source. When coprophagy was prevented it was found necessary to double the vitamin B content of the diet in order to ensure an adequate supply. Although these findings were at first disputed by McCollum, Simmonds and Becker [1925], they have been accepted by later workers [Salmon, 1925; Smith, Cowgill and Croll, 1925]. Heller, McElroy and Garlock [1925] considered that the B-vitamin present in rat faeces was formed by spore-bearing organisms in the intestinal tract, which were capable of synthesizing the vitamin; these organisms when grown on a synthetic medium, stated to be free of B-vitamins, caused a marked response in growth when fed to rats. Kennedy and Palmer [1928] found a substance present in rat faeces which improved a diet 'complete' in all respects including the antineuritic vitamin; this substance was soluble in strong alcohol and only moderately heat-stable, which properties resemble those of vitamin B_1 and do not coincide with those of vitamin B_2. The work of Roscoe [1931, 3], however, suggests that both B-vitamins are to some extent present in rat faeces, although much better growth was obtained when the faeces were used to supplement diets already containing either vitamin B_1 or B_2. At present it must be regarded as uncertain whether the B-vitamins present in the faeces are derived from the animal's reserves or from synthesis by micro-organisms in the intestine (see p. 174).

Attempts to Isolate Vitamin B_1.

The numerous early claims recording the isolation of the antineuritic vitamin have proved, in the light of later work, to have been premature. Even now the unequivocal isolation of this factor has not been realized, though the preparations of a number of workers [Kinnersley and Peters, 1925, 1927, 1928; Jansen and Donath, 1926, 1, 2; 1927; Levene, 1928; Guha and Drummond, 1929; Windaus, Tschesche, *et al.* 1931] have reached a high degree of activity, and represent a considerable advance upon the work of former investigators. The earlier literature, therefore, need only be briefly reviewed.

Cooper and Funk [1911] found that the curative substance present in an alcoholic extract of rice polishings was precipitated by phosphotungstic acid. The active fraction thus obtained was stated to be free from proteins, carbohydrates, and salts, and to resemble an organic base. Employing the method usually adopted for separating certain organic nitrogenous bases Funk [1911] obtained from the silver nitrate-baryta fraction a crystalline substance melting at 233° C., and having the formula $C_{17}H_{18}O_7N_2$. This substance was curative for pigeons in doses of 20–40 mg. The same process was applied to yeast, milk, and ox brain and led to the isolation of the same substance [Funk, 1912–13, 1]. Funk considered that the chemical properties of the curative substance placed it in the class of pyrimidine bases. Nicotinic acid, which was isolated during the fractionation of yeast and rice polishings, was believed to be a decomposition product of the vitamin [Funk, 1913, 1]. The substances isolated by Funk, however, proved not to be the active vitamin in pure form, but nitrogenous bases contaminated with traces of the vitamin [Barger, 1914; Drummond and Funk, 1914].

Vedder and Williams [1913], using a hydrolysed alcoholic extract of rice polishings, confirmed Funk's observation that the curative activity for avian polyneuritis was precipitated in the pyrimidine fraction, and they concluded that the antineuritic factor is present as a constituent of certain nucleic acids present in rice polishings and yeast. The precipitation of the active substance by silver nitrate and baryta has been confirmed by many workers [Cooper, 1913; R. R. Williams and Saleeby, 1915; Myers and Voegtlin, 1920; Seidell, 1921, 1; Tsukiye, 1922; Jansen and Donath, 1927; Kinnersley and Peters, 1925, 1928; Guha and Drummond, 1929].

Further attempts to isolate the factor were made by Suzuki, Shimamura and Odake [1912], who separated a crystalline picrate which they termed oryzanin; and by Edie *et al.* [1912], who obtained a crystalline basic substance, torulin, belonging to the pyrimidine group. Abderhalden and Schaumann [1918] attempted to isolate the antineuritic vitamin from yeast, and by precipitation of an alcoholic concentrate with acetone they obtained a crystalline product which they named 'eutonine'. This substance was curative for polyneuritic pigeons in doses of 5 mg., but did not maintain body-weight.

Hofmeister [1920] attempted unsuccessfully to purify the curative substance present in rice-meal by fractional precipitation of an alcoholic extract with potassium bismuth iodide.

Seidell [1916; 1917; 1921, 1; 1922, 1] employed fuller's earth to adsorb the vitamin from autolysed yeast, or from an aqueous yeast extract. The fuller's earth was washed and dried after adsorption, constituting his 'activated solid', which protected pigeons from polyneuritis in doses of 0·1–0·2 g. daily. Baryta extracts the vitamin from the activated solid, and on fractionation of the resulting solution by silver precipitation the vitamin is found to follow the histidine fraction. The final preparation protected pigeons from loss of weight in doses of less than 1 mg. on alternate days. Later Seidell [1924, 1, 2] prepared a crude picrate from an extract of his activated solid. By fractional crystallization from 95 per cent. acetone he obtained a crystalline picrate of an active product having an empirical formula

$C_6H_{18}O_2N_3.C_6H_2(OH)(NO_2)_3$. This product protected pigeons from loss of weight in doses of 2 mg. daily.

A concentrated preparation of the vitamin B complex which has been much used in feeding experiments and as a starting-point for further purification may be made by the method of Osborne and Wakeman [1919]. An acidulated aqueous extract of yeast was submitted to fractional precipitation by alcohol, the fraction thrown out by 80 per cent. alcohol containing the bulk of the activity. After washing and drying, a preparation was obtained which had, weight for weight, sixteen times the activity of the original yeast as tested by growth experiments on rats. A method of concentrating the Osborne-Wakeman fraction has been described by Levene and van der Hoeven [1924; 1925; 1926], which involves a preliminary de-amination with nitrous acid followed by repeated treatment of the extract with small quantities of silica gel at pH 3·0. It is claimed that under these conditions vitamin B_1 is adsorbed by the silica gel almost entirely free from vitamin B_2. The activated gel is extracted with lithium hydroxide at pH 9·8, and the extract fractionated in alcohol and finally precipitated by acetone. The preparation thus obtained, when examined by growth tests on rats, was active for vitamin B_1 in daily doses of 0·07–0·1 mg.

Kinnersley and Peters [1925; 1927; 1928; 1930, 2] employ an aqueous extract of baker's yeast which is treated successively with normal lead acetate, baryta to remove yeast gum, sulphuric acid to remove barium, and mercuric sulphate at pH 2·0 to 3·0. The acid filtrate, from which many impurities have been removed, is brought to pH 7·0 and treated with purified norite charcoal which adsorbs the active factor. The vitamin B_1 can be recovered from the charcoal either by boiling with 50 per cent. alcohol (by volume) acidified with HCl or by boiling successively first with *N*/10 HCl followed by the acid alcohol. In the latter case two different types of extract are obtained of which the first has a curative activity of approximately 0·4 mg. per pigeon day-dose, and the latter 2·0 mg. per day-dose. The extracts so obtained can be freed from metals in aqueous solution with H_2S at pH 4·0, concentrated at pH 3·0, and stored as stable vitamin B_1 solutions after addition of alcohol to 15 per cent. Alternatively they can be used for the preparation of purer concentrates. The methods which have been found most successful have been alcohol fractionation and fractionation with sodium phosphotungstate at varying acidity. Preparations have been obtained with a pigeon day-dose of 0·012 mg. and the purer specimens have been found free from vitamin B_4.

In 1926 Jansen and Donath [1926, 1, 2], working in the laboratory in Batavia in which Eijkman made his fundamental discovery, announced the isolation of the antineuritic vitamin in crystalline form. In their method the vitamin present in an extract of rice polishings is adsorbed on acid clay at pH 5·0, and extracted therefrom with baryta by Seidell's method. The baryta extract, freed from barium with H_2SO_4, is submitted to fractionation with silver nitrate and baryta which precipitates more than 50 per cent. of the vitamin at pH 6·5. The decomposed precipitate is decolorized by norite, and the clear filtrate treated with phosphotungstic acid. The filtrate obtained after decomposition of the precipitated phosphotungstates is evaporated to

dryness and extracted with absolute alcohol. Addition of platinic chloride precipitates nearly all the vitamin. The platinum precipitate is decomposed and the filtrate fractionated with alcohol and acetone. The acetone-insoluble fraction is further purified, and the vitamin obtained as a crystalline hydrochloride. It consists of rosettes of small bars, melting at 250° C., the composition $C_6H_{10}ON_2$ being assigned to the free base. Jansen and Donath found that daily doses of 0·002 mg. of the crystals protected rice birds (*Munia maia*) from polyneuritis for three weeks.

Eijkman [1927], and R. R. Williams, Waterman and Gurin [1930], have confirmed the potency of these crystals, and a careful test [Jansen, Kinnersley, Peters and Reader, 1930] has yielded the following results for curative activity—pigeon day-dose, by mouth, 0·009 mg.; by injection, 0·007 mg.; rat day-dose, 0·005 mg.

The application of the methods of Jansen and Donath and others to yeast has led to the isolation of a highly active crystalline compound by Windaus, Tscheche, *et al.* [1931]. The analysis of a crystalline picrolonate (M.P. 229° C.) showed that the substance contained sulphur and led to the tentative formula $C_{12}H_{17}ON_3S$ for the free base. The conversion of the picrolonate into the hydrochloride, melting at 245° C. (Jansen and Donath's hydrochloride melts at 250° C.) entails a loss of 40 per cent. in the yield. The average curative pigeon-day dose, by injection, is stated to be 0·0024 mg.

The sulphur content of Jansen and Donath's crystalline hydrochloride was subsequently demonstrated, and the identity of the crystalline antineuritic preparation from rice with that obtained from yeast seems to be established [Tscheche, 1932].

The acid clay preparation from rice polishings, which is the starting-point of Jansen and Donath's process, is largely used in Java for the cure of beri-beri and forms a satisfactory stable preparation of vitamin B_1. This preparation has been adopted by the (League of Nations) International Vitamin Conference, 1931, as the provisional standard for the antineuritic vitamin B_1 (see Appendix II).

Administration of Compounds of Known Chemical Composition.

Several compounds of known chemical composition have been administered to animals suffering from polyneuritis in the hope, which has not been fulfilled, that some known pure substance might prove to be identical with the antineuritic vitamin. As has already been noted, deprivation of vitamin B_1 is not the only cause of the classical symptom of acute polyneuritis (head retraction) in birds. In view of later experiments the claims as to the alleviation of polyneuritic symptoms by some of the earlier workers must therefore be accepted with caution.

Funk [1912–13, 2] investigated a number of purine and pyrimidine compounds and found that certain of them, adenine, allantoin, yeast- and thymus-nucleic acids among others, exercised a marked though temporary alleviation of the symptoms in polyneuritic pigeons. Schaumann [1908] and Grijns [1909] had failed to find any benefit from the administration of nucleic acid prepared from yeast or katjang-idjo beans. Similarly Drummond [1917] found yeast-nucleic acid and adenine to be inactive.

R. R. Williams [1916; 1917] observed that certain hydroxypyridines exerted an antineuritic effect, but passed readily into a stable isomeric form which was inactive. Trigonelline, betaine, and nicotinic acid, which Funk had suggested to be a decomposition product of the vitamin, produced a similar curative effect. He found, however, that the activity of his preparations disappeared on keeping, and he was unable to reproduce the curative effect in birds receiving a second treatment. Moreover, protective tests with these compounds failed. R. R. Williams and Seidell [1916] obtained from yeast a crystalline material which exerted antineuritic properties. On attempting to purify the substance the antineuritic properties were lost, and the resulting product was found to be identical with adenine. R. R. Williams advanced the theory that the vitamin was liberated in an unstable form by acid and alkaline hydrolysis and readily passed into a stable isomer which was inactive. Reinvestigation of these claims failed to confirm them [Voegtlin and White, 1916–17; Harden and Zilva, 1917; McCollum and Simmonds, 1918; Guha and Drummond, 1929].

Sahashi [1926, 1, 2; 1927] isolated from oryzanin a substance identified as 2 : 6-dihydroxyquinoline, which he found to possess temporary antineuritic properties in doses of 4–15 mg. Later [1928] he stated that 4 (or 5)-glyoxaline methylethylcarbinol in doses of 6 mg. temporarily cured polyneuritic pigeons, although the birds invariably died within 7–10 days. Gulland and Peters [1929] have examined a number of glyoxaline and pyrimidine compounds including those investigated by Sahashi, but with negative results. From its behaviour to silver nitrate and baryta, and from the fact that it gave a strong Pauly reaction, Jansen and Donath [1927] had suggested that their crystalline preparation of vitamin B_1 might contain a pyrimidine or glyoxaline ring. On the other hand Kinnersley and Peters [1928] found that the substance responsible for most of the red coloration with Pauly's reagent did not follow the curative activity on fractionation. Comparative tests of the Jansen-Donath crystals and an active antineuritic yeast preparation showed that both gave an orange colour considerably less intense than that given by an equal amount of histidine [Jansen *et al.*, 1930].

Danysz-Michel and Koskowski [1922] claimed that symptoms of polyneuritis could be suppressed in rice-fed pigeons by the administration of histamine. Abderhalden [1923] and Peters [1924] have also reported cases in which histamine exerted a temporary curative effect, but they lay stress on the fact that the results differ quantitatively and usually qualitatively from the effects produced by the administration of extracts of the antineuritic vitamin. Thus histamine given in daily doses exercised no protection against polyneuritis.

Properties of Vitamin B_1.

The preparation of highly active concentrates of vitamin B_1 in a relatively pure state has clearly emphasized the profound influence exerted by the presence of extraneous material on many of the properties of the vitamin. This is exemplified in its solubility, its stability to heat, and to alkalis, and its relation to various adsorbents. It follows, therefore, that any statement as to chemical or physical properties is valid only in so far as the conditions under

which these properties have been determined are accurately defined. Nevertheless, the results of the earlier investigators on the properties of the vitamin as it occurs in natural foodstuffs or in crude extracts have a practical value in problems of dietetics.

Solubility. Eijkman [1906] found that the antineuritic substance was soluble in water, and was not precipitated by moderate concentrations of alcohol. This solvent in varying concentrations has been widely used to extract the vitamin from various natural sources. For the extraction of yeast and rice polishings a 70 per cent. concentration of alcohol was the most effective [Funk, Harrow and Paton, 1923], 95 per cent. alcohol being only an indifferent solvent [Funk, 1912–13, 1; Vedder and Williams, 1913]. The insolubility of crude preparations of the vitamin in the higher concentrations of alcohol has been utilized by Osborne and Wakeman [1919], and Seidell [1926], who employed 80 per cent. alcohol to precipitate their active fractions. Kinnersley and Peters [1927] have demonstrated that while the vitamin is precipitated by alcohol during the early stages of purification, fractions with an activity of 1–3 mg. (pigeon day-dose) which have been freed from alcohol-insoluble material are soluble in absolute alcohol. They also showed that the solubility of the impure vitamin in the higher concentrations of alcohol is largely determined by the hydrogen ion concentration of the solution. Jansen and Donath [1926, 1, 2] found that their crystalline preparation was also soluble in absolute alcohol, and a similar conclusion has been reached by Guha and Drummond [1929], and by R. R. Williams, Waterman and Gurin [1930].

Vitamin B_1 can be readily extracted from yeast and rice polishings by 70 per cent. acetone [Funk, Harrow and Paton, 1923], but is insoluble in pure acetone [Seidell, 1926; Jansen and Donath, 1926, 2; Kinnersley and Peters, 1928]. It cannot be extracted by benzene from wheat embryo or white beans, or from their alcoholic extracts if these are freed from water and alcohol [R. R. Williams and Waterman, 1926], but is removed by benzene from the alcoholic extract if this is not perfectly dry [see McCollum and Simmonds, 1918].

Cooper [1912; 1914, 3] was able to detect distinct traces of the antineuritic factor in an ether extract of egg-yolk, but concluded that this was due to the presence of an ether-soluble substance having a marked adsorptive affinity for the vitamin. This explanation may account for the observation of Myers and Voegtlin [1920] that olive oil and oleic acid removed the antineuritic factor from a solution of autolysed yeast. Vitamin B_1 present in a purified yeast concentrate is not dissolved by ether, ethyl acetate, chloroform, or carbon tetrachloride [Kinnersley and Peters, 1928]. It is soluble in glacial acetic acid [Levine, McCollum and Simmonds, 1922], and in 70 per cent. acetic acid [Funk, Harrow and Paton, 1923].

Stability to heat. There is general agreement that the resistance of vitamin B_1 to heat in faintly acid or acid media is considerable. Most observers have detected little or no destruction of the vitamin after exposure to 100° C. Eijkman [1906] found no destruction of the antineuritic properties of unmilled rice after heating to 100° C. for three hours, and Drummond [1917] observed that yeast or marmite heated for 30 minutes at 100° C. at their natural acidity (pH 4·0–5·0)

showed no deterioration of growth-promoting properties for the rat. Chick and Hume [1917–19, 2] made a systematic examination of this question, in which they were careful to avoid certain errors into which some investigators have fallen. In particular they recorded the temperature of the interior of the foodstuff as well as the general temperature of the steamer or autoclave. The former is frequently lower than the latter. They compared the minimal amounts of the unheated and the heated substance required for the cure of polyneuritis in pigeons. Using wheat embryo and yeast extract they concluded that only slight destruction of the antineuritic vitamin could be detected after heating at 100° C. for 1–2 hours. With higher temperatures there was a progressively increasing degree and rate of destruction, the original potency being reduced to one quarter or less after 2 hrs. at about 129° C. Grijns [1901 ; see also Eijkman, 1906 ; Weill, Mouriquand and Michel, 1916] found that 1–2 hours' exposure to a temperature of 120° C. destroyed the antineuritic properties of unmilled rice, katjang-idjo beans, and buffalo meat, but Eijkman [1906] did not succeed in entirely destroying the protective value of horseflesh under these conditions. Marked destruction of the antineuritic properties of various foodstuffs, such as meat, eggs, yeast extract, and wheat flour when heated for varying periods at temperatures of 110–124° C. has been noted [see Holst, 1907 ; Vedder, 1918 ; Chick and Hume, 1917–19, 2], but dried peas and whole barley appeared to suffer no loss at 118° C. for ten minutes [Holst, 1907].

As most people derive their supply of vitamin B_1 from the cereals in their diet, the practical conclusion to be drawn from the evidence cited is that in the baking of bread or biscuit, a relatively short operation during which the temperature of the interior of the material does not rise above 100° C., no serious diminution in vitamin B_1 content need be apprehended. This proved to be true in the case of wheaten biscuit made from whole flour on which pigeons throve well [Chick and Hume, 1917–19, 2]. Continued heating of foodstuffs at 100° C., however, leads to some loss of the vitamin. In preserving and canning foodstuffs the temperatures employed are frequently much higher than 100° C., and canned foods of all descriptions may contain very little or no vitamin B_1, and, as a practical rule, should therefore be regarded as free from it, unless they have been specially investigated and found to contain it.

Influence of hydrogen ion concentration on the stability of vitamin B_1. Vitamin B_1 resists hydrolysis with acids ; thus there was no diminution in the antineuritic properties of pressed yeast after heating with 20 per cent. sulphuric acid for 24 hours [Cooper and Funk, 1911], and Vedder and Williams [1913] claimed that, after hydrolysis with 50 per cent. sulphuric acid, an extract of rice polishings had an enhanced curative potency. The stability of this vitamin towards acids has been confirmed by many workers [McCollum and Simmonds, 1918 ; R. R. Williams, Waterman and Gurin, 1929]. Kinnersley and Peters [1928] found that crude antineuritic concentrates stored in 99 per cent. alcohol at pH 1·0–2·0 kept their activity at room temperature for at least three years, but some highly purified preparations gradually lost their activity under these conditions.

The action of alkalis, on the other hand, leads to ready destruction of vitamin B_1 even at low temperatures. Schaumann [1910] and Vedder and Williams [1913] investigated the effect of strong caustic soda; Osborne and Leavenworth [1920–21] showed that the growth-promoting value of yeast was destroyed by treatment with 0·1 *N* caustic soda for 90 hours at room temperature, whilst Cooper [1913] found that the antineuritic potency of horseflesh was destroyed by treatment with 10 per cent. ammonia at room temperature for 24 hours.

Sherman and Burton [1926], using the rat-growth test, have noted a progressive destruction of the vitamin B complex on heating at 100° C. at decreasing hydrogen ion concentrations. Kinnersley and Peters [1928] found that on heating an antineuritic concentrate (pigeon day-dose 0·059 mg.) for one hour with 0·5 *N* caustic soda, there was complete destruction of the vitamin. Heating a preparation of activity 3·0 mg. for one hour with 0·1 *N* soda in the presence of oxygen reduced its activity by approximately 80 per cent., whereas heating it for ten minutes with 0·1 *N* soda in nitrogen reduced the activity by about 30 per cent. The sensitivity of the vitamin was found to be much increased in the presence of alcohol.

Adsorption of vitamin B_1. Chamberlain and Vedder [1911, 1, 2] were the first to show that the antineuritic vitamin present in an aqueous extract of rice polishings was adsorbed by bone black, and was not removed from the adsorbate by absolute alcohol, water, or ether. Cooper [1913] found that not more than 30 per cent. of the vitamin was removed by this adsorbent, even after filtering an aqueous extract six times through it. Later Peters [1924] showed that the vitamin adsorbed on norite charcoal could be eluted by extraction with 50 per cent. acid alcohol.

Seidell [1916] found that vitamin B_1 was adsorbed by fuller's earth or Lloyd's reagent from a solution of autolysed yeast, and the 'activated solid' resulting from this treatment was found to be highly active in curing avian polyneuritis. Later [1926] he showed that as much as 90 per cent. of the vitamin was adsorbed. Harden and Zilva [1918, 1] also reported a quantitative adsorption of the vitamin by fuller's earth, and by 'dialysed iron' in marked contrast to the behaviour of vitamin C. Jansen and Donath [1926, 1] used acid clay to adsorb the vitamin from an acidified extract of rice polishings in the first stage of their purification process.

A more systematic study has demonstrated the important influence of the hydrogen ion concentration of the medium and of the presence of colloidal impurities on the adsorption of vitamin B_1 by different adsorbents. Emmet and McKim [1917] found that kieselguhr did not adsorb the vitamin, while Zajdel and Funk [1926] obtained only a very incomplete adsorption by colloidal ferric hydroxide at varying hydrogen ion concentrations.

An examination of the adsorption of the vitamin by Lloyd's reagent and silica gel has been made by Levene and van der Hoeven [1924]. Optimum adsorption occurred at pH 4·0. Partial extraction of the adsorbed vitamin was obtained at pH 3·0, but elution was much more complete at pH 9·0. These authors found that silica gel was a much more effective adsorbent for vitamin B_1 than for B_2 and claimed that by the use of this adsorbent in small

quantities they were able to prepare antineuritic concentrates virtually free from vitamin B_2. Similar results were obtained by Salmon, Guerrant and Hays [1928, 1, 2]. They showed that the maximum adsorption of vitamin B_1 by fuller's earth was obtained, under their conditions, within the range pH 3·0–5·0 with optimum adsorption at pH 4·0. Increasing alkalinity up to pH 9·0 rapidly diminished the amount of vitamin held in adsorption, while at still more alkaline reactions adsorption was negligible. In this respect the antineuritic vitamin was found to behave like an organic electrolyte such as quinine, in contrast to vitamin B_2 which resembled an organic non-electrolyte, showing optimal adsorption at pH 0·08. These workers found evidence of inactivation of vitamin B_1 amounting to as much as 50 per cent. in alkaline solutions, thus emphasizing the importance of employing only weakly alkaline eluents. Kinnersley and Peters [1925; 1927], who use norite charcoal in their preparation of antineuritic concentrates, find that adsorption of the vitamin after the treatment with mercuric sulphate is most complete within the range pH 5·0–7·0, and optimal at pH 7·0. They find, however, that the pH of optimal adsorption of the vitamin from crude preparations is modified by the presence of impurities. In the more highly purified fractions the vitamin is most completely adsorbed at pH 9·0 [Peters, 1930, 1, 2].

Miscellaneous properties. The antineuritic vitamin withstands desiccation for long periods of time [Jansen, 1923; G. M. Findlay, 1923, 1] as is shown by its abundant presence in dried foodstuffs. It does not appear to be susceptible to oxidation by atmospheric oxygen, in this respect differing sharply from vitamins A and C. Exposure to ozone [Zilva, 1922] has no deleterious effect upon it. Zilva [1919, 1] claimed that exposure to ultra-violet radiation did not destroy the vitamin, a conclusion supported by Hogan and Hunter [1928]. On the other hand, R. R. Williams [1924], Kennedy and Palmer [1929], and Chick and Roscoe [1929, 2] found that longer exposures result in some destruction. Kinnersley and Peters [1928] find that the vitamin in acid solution is stable to hydrogen peroxide, or potassium permanganate, or to heating with 5 per cent. nitric acid. Treatment with nitrous acid is without effect on the vitamin [McCollum and Simmonds, 1918; Peters, 1924; Levene and van der Hoeven, 1926]. This fact is regarded as strong evidence that the vitamin is neither a primary nor a secondary amine. Vitamin B_1 passes through a parchment membrane [Eijkman, 1906; Chamberlain and Vedder, 1911, 2; Shiga and Kusama, 1911; Drummond, 1917; Seidell, 1922, 1; Zilva and Miura, 1921, 1]. These last observers believe that the vitamin has a molecular weight of the order of 300–350, or that it forms a complex of about this molecular weight with some other substance.

Methods of Detection and Estimation of Vitamin B_1.

No satisfactory colour reaction, or other chemical test, for vitamin B_1 has yet been discovered, in spite of much effort in this direction. Funk [1913, 1] and Funk and Macallum [1913] found that a pronounced blue colour was given by Funk's crystalline preparation from

rice polishings and by various alcoholic extracts of the vitamin in the presence of the uric acid and phenol reagents of Folin and Denis. Eijkman [1927] found that the reaction with the uric acid reagent was given by Jansen and Donath's crystalline preparation of vitamin B_1, but that it was much weaker than that produced by a solution of uric acid of the same concentration. The lack of specificity of this test renders it valueless in identifying vitamin B_1. The same criticism has been made by Bezssonoff [1924, 2] and by Levine 1924–25] of the colour reaction proposed by Jendrassik [1923] as a test for the vitamin.

The biological method of testing for the presence of the antineuritic vitamin, while open to many objections, remains the only one that is as yet satisfactory. The animals which have been most usually employed for this purpose are the fowl, the pigeon, and the rat. The rice bird (*Munia maia*) has also been used.

Experiments with Fowls and Pigeons.

The principal methods adopted in experiments with these animals have been of three types, viz. the protective, the weight maintenance, and the curative.

Protective test. The protective test, in the form originally employed [Eijkman, 1897, 3; Vedder and Clark, 1912; Cooper, 1912; 1914, 1, 2; Chick and Hume, 1917–19, 1; Jansen and Donath, 1926, 1; and later workers], involves an estimation of the daily ration of a particular foodstuff necessary to prevent polyneuritis when added to a vitamin-free diet such as polished rice. The test is open to several objections unless very large numbers of birds are used. In the first place difficulties arise owing to the variability of the times of onset of polyneuritis in different individuals. If a given bird fails to develop symptoms within a specified time, it is always possible that the bird in question might not have become polyneuritic in this time even upon polished rice alone [Peters, 1924], or that polyneuritis might have developed later if the experiments had been prolonged. Thus Vedder and Clark [1912] reported a case of polyneuritis which developed in a pigeon after one hundred days on the experimental diet. There is also the disadvantage that relatively large amounts of the test substance are required and that the experiments are time consuming.

Weight-maintenance test. Another test for the presence of vitamin B_1 [R. R. Williams and Seidell, 1916; Seidell, 1922, 1] is one in which the criterion adopted is the maintenance of the body-weight of the animal on a diet of polished rice, or other vitamin-free diet, when supplemented by the substance under test. Peters [1924] offered the criticism that this criterion is based on the assumption that the factors necessary for the cure of polyneuritis and for weight maintenance are identical. This is now known not to be the case (see p. 121). A further disadvantage of this test for vitamin B_1, when employed over a considerable period of time, as in the experiments of Plimmer and his co-workers [1927], is that the animal may become depleted of some other essential factor which may be absent or deficient both in the test substance and the basal diet.

Curative test. In the curative test the experimental animal is fed on a vitamin B_1-free diet until severe symptoms of polyneuritis have developed. The foodstuff or vitamin concentrate under consideration is then administered in varying doses to separate birds, and determination made of the minimal dose required to effect recovery. In a successful test there is a dramatic recovery from a condition of complete helplessness (see Figs. 26, 27). It is not unusual to see normal gait and flight restored within an hour or two of the administration of the vitamin.

The discovery by Funk [1912–13, 2], and R. R. Williams [1916], that the administration of a number of known chemical substances may be followed by a complete, though temporary, recovery from the classical symptom of head retraction led to the criticism and abandonment of the curative test by various workers. Moreover, head retraction has been shown to develop in certain cases in starvation, even when extracts of the antineuritic vitamin are administered, as a result of 'washing out' the tissues of starving birds by giving large amounts of water by mouth [Chamberlain and Vedder, 1911, 2; Chamberlain, Bloombergh and Kilbourne, 1911; Eijkman and Hoogenhuijze, 1916; Marrian, Baker, Drummond and Woollard, 1927], and in other conditions. The spontaneous cure of head retraction in rice-fed pigeons has been reported by Kon and Drummond [1927]; temporary recovery has also been observed after artificially raising the body temperature of the polyneuritic animal, and as a result of the administration of glucose [Peters, 1924; Roche, 1925].

This test has been reviewed by Kinnersley, Peters and Reader [1928], who have defined the conditions necessary for obtaining reliable and quantitative results. They find that, subject to these conditions, any particular bird tends to develop symptoms of polyneuritis at a constant time (within narrow limits) after the commencement of rice feeding, while the duration of the cure in experiments with their antineuritic concentrates has been found proportional to the dose of vitamin B_1 administered. Although the onset of symptoms varies somewhat from bird to bird, they consider that the test provides a method which is economical in time and material, and which may be relied upon to give fair accuracy in the standardization of antineuritic concentrates. When the curative method is used for the estimation of the vitamin content of natural foodstuffs other difficulties may arise. Thus when the test foodstuff itself is given by mouth after the development of acute symptoms, slowness of digestion or absorption from the alimentary canal may delay the recovery for several days and complicate the interpretation of the experiment. If an extract of the foodstuff is prepared, the inevitably large losses of the curative factor incurred in the extraction process may result in considerable errors. Thus in the case of dried peas the curative dose of the natural material was 10 g., whereas that of the alcoholic extract was the equivalent of 40 g. [Chick and Hume, 1917, 2; 1917–19, 2; see also Cooper, 1912; 1914, 1].

Experiments with Rats.

Growth test. The rat-growth test for water-soluble vitamin B introduced by McCollum and Simmonds [1918], which has been widely employed, particularly in America, involves the determination of the minimum ration of a foodstuff required for normal growth or maintenance when added to a diet complete in all other essential dietary constituents. Many of the earlier workers contented themselves with ascertaining whether the experimental material, when constituting a certain percentage of the diet or a certain daily dose, did, or did not, satisfy the requirements of the animal. Their results, therefore, at best give only a rough idea of the quantitative distribution of what we now know as the antineuritic vitamin in different foodstuffs. Moreover, the failure to recognize the multiple nature of the vitamin B complex frequently led to erroneous or misleading conclusions.

A further source of error which complicated the results of earlier workers with the rat was the failure to prevent coprophagy (see p. 138). It is now usual in the study of the B-vitamins to limit the access of the experimental rats to their own faeces by employing cages with bases made of wide-meshed wire.

The principles underlying the rat-growth test as a quantitative method for the estimation of vitamin B_1 have been examined and defined by Sherman and MacArthur [1927], and by Chick and Roscoe [1929, 1].

Curative test. M. E. Smith [1930] and Kinnersley, Peters and Reader [1930] have introduced a curative test for vitamin B_1, using respectively the young and adult rat as experimental animals. Smith's method consists in the determination of the minimal dose of a test material required to effect a cure of acute polyneuritis in young rats which have been reared after weaning on a basal diet free from the vitamin B complex, supplemented by autoclaved brewer's yeast to provide vitamin B_2 and by cod-liver oil. Under these conditions the animals grow for a time, but subsequently decline in weight and finally, in from 50–80 days, develop typical paralytic symptoms characterized by lameness of the hind- and fore-limbs, inco-ordination, spastic gait, convulsions, and cartwheel and rolling movements. The antineuritic vitamin was usually administered intravenously, and after an injection improvement was noticeable within 3–5 hours and was followed by a cure in 18–24 hours. The duration of the cure appeared to be related to the size of the dose. Subsequently the animal declined in weight, but could be restored by a second injection. The author claimed that the method gave quantitative results when comparing the minimum doses of various antineuritic fractions. Moreover, the vitamin appeared to be specific in relieving the polyneuritic symptoms in the rat, since none of the pure substances tried which have been claimed to produce 'cures' in pigeons was found to be effective on the rat.

In Kinnersley, Peters and Reader's test, adult rats are allowed to fall in weight on a vitamin B_1-free diet (to which vitamin B_2 has been added as alkalized marmite). Upon appearance of symptoms, a dose of vitamin B_1 is given by mouth which takes effect within 30 minutes; the authors claim that the duration of cure is proportional to the dose

administered. Care must be taken to distinguish the symptoms of vitamin B_1 deficiency from those of vitamin B_4 deficiency (p. 167) with which they may be confused.

Vitamin B_2 (Antidermatitis Vitamin[1]).

Symptomatology of Vitamin B_2 Deficiency in the Rat.

Human pellagra is a disease which produces a wide variety of symptoms. To give a rough description, the typical pellagrin shows dermatitis on the face and hands and those parts of the body exposed to sunlight, accompanied by inflammation of the alimentary tract from the mouth to the rectum, and often, in the later stages, by mental disorder and lesions of the central nervous system.

The symptoms observed by Goldberger and Lillie [1926] in albino rats fed on diets containing the antineuritic vitamin but devoid of the heat-stable P-P (pellagra-preventive) factor resembled human pellagra in that certain rats developed symmetrical dermatitis, particularly of the paws, fore-arms, neck, and ears. Depilation occurred in almost all cases. In addition to the skin lesions, the authors describe a tendency for the lids of one or both eyes to adhere together, with an accumulation of dried secretion on the margin of the lids in some instances. Ulceration of the tip of the tongue and at the angles of the mouth occurred in some rats, and diarrhoea in rare cases. The shortest period taken for symptoms to develop was seven weeks. The condition noted by Chick and Roscoe [1927] among rats receiving Peters's antineuritic concentrate from yeast as the sole source of 'vitamin B' appears to be substantially the same. Very similar skin and eye lesions were observed, developing in some animals after about six to eight weeks. Neither stomatitis nor diarrhoea was recorded, but post-mortem examination indicated 'a very unhealthy condition of the whole alimentary tract, especially in the small intestine, which showed signs of inflammation with atrophy of the mucous membrane and often contained blood-stained mucus'.

G. M. Findlay [1928] and Stern and Findlay [1929], who produced a similar 'pellagrous' condition in rats by the method of Chick and Roscoe (i.e. using Peters's concentrate as a source of vitamin B_1), made histological studies of the skin, alimentary tract, adrenals, liver, pancreas, and central nervous system, for the full details of which the original papers should be consulted. The skin condition showed congestion, with invasion of small round cells, followed by the production of flakes of keratinized material on the free surface of the epidermis. Occasionally the whole epidermis was destroyed. Examination of the spinal cord revealed a vacuolation and swelling of the anterior horn cells. No very definite or characteristic lesions were found in other organs of the body.

Sherman and Sandels [1928–29] observed in rats on a vitamin B_2-free diet a somewhat similar condition, but clearly defined dermatitis of the fore and hind paws did not occur. The symptomatology of vitamin B_2 deficiency as recorded by Salmon, Hays and Guerrant

[1] Vitamin G of some American writers.

[1928] agrees well with that described by Goldberger and Lillie and by Chick and Roscoe. Salmon and his colleagues found a 'large coccus' constantly associated with the skin lesions of vitamin B_2 deficiency in the rat, and put forward the idea, which has not been confirmed, that rat 'pellagra' needs a microbic in addition to a dietary cause for its development.

Bing and Mendel [1929], using an extract of rice polishings as the sole source of the vitamin B complex, induced a scabby dermatitis in mice, mostly on the medial surface of the fore and hind legs. Apart from Goldberger's work on black-tongue in dogs, a disease which stands in a somewhat doubtful relation to vitamin B_2 deficiency, this is the only attempt which has been made to investigate the effects of lack of vitamin B_2 in any animal other than the rat.

To sum up, it may be said that skin lesions, often symmetrical, sometimes occur in rats and mice fed on diets devoid of vitamin B_2 but otherwise complete. The symptoms appear after about 6–10 weeks on the deficient diet, during which period growth ceases. Severe conjunctivitis or ophthalmia is constantly recorded, stomatitis occasionally. Red-stained urine is noted by some investigators. Diarrhoea, so marked a symptom in human pellagra, is exceptional in the vitamin B_2-deficient rat. All lesions are curable by foods containing vitamin B_2.

It may be remarked that dermatitis associated with vitamin B_2 deficiency appears to be produced in the rat with some uncertainty. In none of the experiments just recorded did more than a proportion of animals show skin lesions, and several investigators have failed to observe any characteristic symptomatology beyond, perhaps, a condition of general wretchedness. Those who have done much work on vitamin B_2 are puzzled by the changeable nature of the lesions occurring in the vitamin B_2-deficient animal and by the frequent failure to produce any characteristic skin symptoms. Complete cessation of growth without any accompanying decline in weight is, however, a constant feature of young rats provided with vitamin B_1 but deprived of vitamin B_2.

Separation of Vitamin B_2 from Vitamin B_1.

Naturally occurring substances containing one B-vitamin tend as a rule to contain the other, though in some there are marked quantitative differences. An exception exists, however, in egg-white, which, according to Chick and Roscoe [1929, 1], contains vitamin B_2 without admixture of vitamin B_1. In order to investigate the properties of either vitamin it has usually been necessary to work with material containing both, such as yeast, and attempts have been made to obtain a separation of vitamin B_2 from vitamin B_1 without loss of potency.

Separation by heat. The relative stability of vitamin B_2 to heat enables fractions free from vitamin B_1 to be easily prepared, but the application of heat sufficient to destroy the antineuritic vitamin does not leave vitamin B_2 unimpaired [Chick and Roscoe, 1929, 2].

Separation by adsorption. Salmon, Guerrant and Hays [1928, 1] attempted to effect a separation of the B-vitamins by selective

adsorption on fuller's earth. When an extract of soya-bean leaves was treated with a very small quantity of fuller's earth, vitamin B_1 was adsorbed with a very small admixture of vitamin B_2; according to Narayanan and Drummond [1930], however, fuller's earth effectively adsorbed vitamin B_2 also, from a solution containing very little vitamin B_1, at pH values from 6·8 to 0·01. The removal of the vitamin became more nearly complete as the acidity was raised.

At neutral or slightly alkaline reactions vitamin B_1 is probably more strongly adsorbed by norite charcoal than vitamin B_2 [Peters, 1924; Narayanan and Drummond, 1930].

Separation by lead acetate precipitation. The antineuritic concentrate prepared from yeast by Peters's method [1924] is free from the vitamin B_2 present in abundance in the original yeast. Chick and Roscoe [1929, 2] attempted to determine at what stage of the process vitamin B_2 is removed. It was found that during precipitation of the original aqueous yeast extract by lead acetate a considerable amount (roughly one-half) of the vitamin B_2 was carried down with the precipitate, when the precipitation took place at a pH of about 4·5–4·7. At neutral or slightly alkaline reactions precipitation with lead acetate removed vitamin B_2 more nearly completely from solution, but the further the reaction was carried towards alkalinity the more vitamin B_1 was precipitated also.

Properties of Vitamin B_2.

Heat-stability. Almost all the investigators who have attempted to unravel 'water-soluble vitamin B' into its component parts have used autoclaved yeast as a source of vitamin B_2, it being assumed that the antineuritic vitamin is destroyed by being exposed for a few hours to temperatures of 120°–130° C. [Grijns, 1901; Eijkman, 1906; Chick and Hume, 1917–19, 2]. Vitamin B_2 is often referred to as the 'heat-stable factor' in the vitamin B complex. Its heat-stability is, however, only relative, depending largely on the reaction at which heating takes place. Chick and Roscoe [1927] found that brewer's yeast when heated at 120° C. for five hours at its normal pH of about 4·5–5·0 loses half its vitamin B_2 potency. Subsequently, R. R. Williams, Waterman and Gurin [1929] investigated the destruction of vitamin B_2 in brewer's yeast heated for six hours at different reactions at 120° C. They concluded that at this temperature much vitamin B_2 survived if the reaction were that natural to yeast (pH about 4·5), and more if it were more acid (pH 1·6–2·0). If the reaction were acid some vitamin B_1 was also found to survive this temperature. At pH 8–14 both vitamins were destroyed. Similar results have been recorded by Chick and Roscoe [1930]. At pH 5·0 about 50 per cent. of the original vitamin B_2 was lost when the yeast was heated for 4–5 hours at 123° C. When an aqueous yeast extract, at a pH of 8·3–10, was subjected to 122°–125° C. for 4–5 hours, between 75 and 100 per cent. of the original vitamin B_2 was lost; on heating for 2 hours at 98°–100° C. (pH 8·3) the loss was about 50 per cent.; at room temperature for ten days (at pH 8·3), 30 per cent.

The constituent of the B-vitamin complex which is able to correct the deleterious effect of excessive protein (70 per cent.) in a diet

[Hassan and Drummond, 1927 ; Hartwell, 1928] is now generally supposed to be identical with vitamin B_2. This factor, as contained in marmite, was found to survive heating in 'strongly alkaline' solution for 1 hour at 120° C. (Hassan and Drummond), a result which has been confirmed by Reader [1929, 2].

The discrepancy between this result and that obtained by Chick and Roscoe suggests that the stability of vitamin B_2 to high temperatures may be influenced by other factors than the pH, for example, by the salt content of the solution.

Since a number of investigators have shown that there are other factors in the vitamin B complex in addition to vitamins B_1 and B_2, factors which are necessary for the continued growth of rats or for the maintenance of weight in pigeons [R. R. Williams and Waterman, 1927–28 ; 1928 ; Hunt, 1928, 2 ; Reader, 1929, 2] the sensitivity of vitamin B_2 to heat and alkali under various conditions is of considerable importance. If it be assumed that vitamin B_2 survives unchanged on heating, whatever the reaction, the danger arises that an unrecognized shortage of this vitamin may be mistaken for lack of an unknown factor. The existence of other B-vitamins cannot be satisfactorily proved unless the most rigid precautions are taken to exclude a quantitative deficiency of vitamins B_1 and B_2.

Solubility. The antineuritic vitamin has long been known to be soluble in alcohol in strengths ranging from 88 per cent. to absolute [Fraser and Stanton, 1911]. Drummond [1917] found that 'water-soluble vitamin B' could not be satisfactorily extracted with absolute alcohol, but succeeded with 70 per cent., an observation readily explained in the light of later knowledge that the second factor in the vitamin B complex is relatively insoluble in strong alcohol.

Goldberger, Wheeler *et al.* [1926] in the course of their demonstration of the dual nature of vitamin B, found that an 18 per cent. alcoholic extract of whole maize was deficient in vitamin B_2, though rich in the antineuritic factor. It was observed by Salmon, Guerrant and Hays [1928, 1] that the vitamin B_2 contained in an extract from soya-bean leaves was precipitated by 82·7 per cent. alcohol. Similarly, Sherman and Sandels [1928–29] found vitamin B_2 to be insoluble in 95 per cent. alcohol, an observation confirmed by Chick and Roscoe [1929, 2]. It was noted by both these latter groups of workers that the vitamin B_2 recovered from the residue insoluble in strong alcohol was reduced in amount, a fact suggesting that alcohol in high concentrations had a destructive effect upon it. Narayanan and Drummond [1930], on the other hand, found vitamin B_2 to be very soluble in 50 per cent. alcohol, which extracted about 88 per cent. of the vitamin B_2 originally present in a yeast fraction.

Dialysis. Vitamin B_2 dialysed through a cellophane membrane, permeable also to vitamin B_1, at a pH of about 3·0 [Chick and Roscoe, 1929, 2]. Zilva and Miura [1921, 1] observed that 'water-soluble vitamin B' passed a collodion membrane of such permeability as to permit diffusion of semi-colloidal substances such as methylene blue and safranine.

Susceptibility to ultra-violet irradiation. Hogan and Hunter [1928] reported that the vitamin B_2 present in yeast was destroyed by an exposure to ultra-violet irradiation which left vitamin B_1 unimpaired.

In attempting to repeat this work, Chick and Roscoe [1929, 2] confirmed the susceptibility of vitamin B_2 to damage by ultra-violet radiation, but found that irradiation of a yeast extract for six hours at a distance of 40 cm. destroyed only about half the vitamin B_2 originally present. Its content of vitamin B_1 suffered also, so that ultra-violet irradiation does not provide a satisfactory method of separating vitamin B_1 from vitamin B_2.

Action of nitrous acid on vitamin B_2. Vitamin B_2, like vitamin B_1 [Peters, 1924], does not appear to be susceptible to the action of nitrous acid, so that its activity cannot depend on the presence of amino-nitrogen. Levene [1928] reported that vitamin B_2 was destroyed by de-amination, but the results of other workers are opposed to this view. Chick [1929] found that de-amination had no effect on the vitamin B_2 activity of a yeast extract, and later Narayanan and Drummond [1930] recorded a similar result, which is in accord with the observation of McCollum and Simmonds [1918] that 'water-soluble vitamin B' is resistant to the action of nitrous acid.

It will be seen that the literature concerning the properties of vitamin B_2 is full of contradictions. The probable explanation lies in the fact that, in common with the other recently described members of the vitamin B complex, vitamin B_2 is not yet a well-defined chemical entity. Its solubility and precipitation limits, and also its stability to heat, are influenced greatly by the circumstances under which the tests are made, including not only the pH, but also the presence of other substances, mineral salts, adsorbents, &c. Doubtless these discrepancies will be cleared up as the result of further investigations.

Methods of Estimation of Vitamin B_2.

Two methods have been described for the estimation of vitamin B_2. In both the rat is used as the experimental animal; in one the criterion adopted is the cure of dermatitis developed in absence of this vitamin, and in the other the restoration of growth. Only the latter can as yet be used with any degree of accuracy.

Curative test. Goldberger and Lillie [1926] and Chick and Roscoe [1927] showed that the dermatitis which developed in rats on diets deficient in vitamin B_2 could be cured by the administration of this vitamin, and the latter workers [1928] investigated the dermatitis-curing effect of the substances tested by them for content of vitamin B_2 by other methods [see also Aykroyd and Roscoe, 1929; Chick and Copping, 1930, 1; Chick, Copping and Roscoe, 1930; Roscoe, 1930]. It is, however, difficult to obtain quantitative results, as there is a great variation in the time elapsing before symptoms develop and in their type and severity, as well as in the general condition of the animal. It was found, however, that, with the substances tested, the curative value ran parallel with the growth-promoting potency, slightly less of any material being needed to cure symptoms than to promote good growth.

Leader [1930], Sherman and Sandels [1931] and Sure and Smith [1930–31] obtained symptoms of dermatitis with greater frequency

when the diet contained some vitamin B_2 than when it was entirely deficient. When the dermatitis developed on diet containing only a small amount of vitamin B_2 it seems possible that the symptoms may have been due to a partial lack of the vitamin; but when, as in the cases observed by Sure and Smith, the rats received as much as 10 per cent. of autoclaved yeast in their diet, grew normally, but yet developed symptoms of dermatitis, it would seem that the symptoms were not the same as those observed by other workers with vitamin B_2-deficient animals, or else that the factors preventing dermatitis and promoting growth were not identical. In either case experiments based on the cure of symptoms so obtained cannot be used for accurate quantitative estimation of vitamin B_2.

Growth test. In this test comparison is made between the amounts of different materials needed daily by young rats to promote growth, at a given standard rate, when these are administered as additions to basal diets adequate in all respects and lacking only in vitamin B_2. These methods differ one from another in the form in which the vitamin B_1 is supplied. Both yeast and the cereal embryos (which contain relatively more vitamin B_1 than B_2) have been used as the starting-points for purified vitamin B_1 concentrates suitable for use in these tests.

Goldberger and Lillie [1926] used an alcoholic extract of corn meal and also an adsorbate on fuller's earth from yeast extract [Seidell, 1922, 3] as sources of vitamin B_1. The former contained only small amounts of vitamin B_2 but the latter contained sufficient B_2 for good growth if the dose was large.

Chick and Roscoe [1927] used the vitamin B_1 concentrate prepared from yeast by the method of Peters [1924; Kinnersley and Peters 1925], which is obtained by extraction with acid alcohol from a charcoal adsorbate. This extract which is free from vitamin B_2 was also used by Narayanan and Drummond [1930].

In the method of Chick and Roscoe, young rats about 40 g. in weight, immediately after weaning, receive an experimental diet in which the caseinogen providing the protein is specially purified. Vitamins A and D are supplied by a daily ration of cod-liver oil and vitamin B_1 by 0·1 c.c. ($\equiv$ 0·6 g. dried yeast) of Peters's concentrate. On this diet the body-weight should remain constant indefinitely, but on addition of a source of vitamin B_2 growth is immediately resumed. Determination is made of the minimum dose required daily to restore growth to the extent of about 12 g. weekly increase in weight, over a period of about five weeks.

Evans and Burr [1928,1] and Hetler, Meyer and Hussemann [1931] used 'tiki-tiki', an alcoholic extract prepared from rice polishings for the treatment of beri-beri in the Philippines (p. 127). The extent of growth which occurred when more than a minimal amount of this extract was given showed, however, that vitamin B_2 was present. The former workers considered that this was contained in the unpurified caseinogen and starch of the basal diet, but the latter workers that it was contained in the tiki-tiki.

Guha and Drummond [1929] prepared vitamin B_1 concentrates using adsorbates on charcoal and fuller's earth from an alcoholic extract of wheat germ. These were used by Guha [1931, 2, 3] in

vitamin B_2 tests, as also was an aqueous alcoholic extract of yeast fractionated with normal lead acetate [Guha, 1931, 1]. The latter preparation, however, contained some vitamin B_2.

R. R. Williams, Waterman and Gurin [1929] in their tests for vitamin B_2 used as source of vitamin B_1 an activated fuller's earth preparation from yeast, described by them in 1928 and shown to contain no vitamin B_2.

Distribution in Foodstuffs of the Vitamin B Complex.

The (Antineuritic) Vitamin B_1.

The distribution of the antineuritic (antiberi-beri) vitamin B_1 was first studied systematically by Cooper [1912; 1914, 1, 3] at the Lister Institute, using as criterion the prevention or cure of polyneuritis in pigeons on a diet of polished rice. In response to war-time needs this work was rendered more complete by other workers in the same Institute [Chick and Hume, 1917, 1, 2; 1917–19, 1]. A summary of the chief results obtained is given in Tables IX and X.

Table IX.

Showing approximately relative values of the more important foodstuffs (weight for weight) in natural condition for prevention of beri-beri.
(Value of wheat germ taken as equal to 100.)

Substance.	*Value.*	*Approximate water content. Per cent.*
Wheat germ	100	10 to 13
Wheat bran	25	10 to 13
Rice germ	200	10 to 13
Yeast (pressed)	60	70
Peas (dried)	40	12
Lentils	80	10
Egg yolk	50	70
Ox liver	50	70
Ox muscle	11	75
Potatoes	4·3	80

Of subsequent work using the growth of rats as criterion, only that which has been done since the discovery of the complex nature of vitamin B and in which due provision of vitamin B_2 has been made in the basal diet can now be accepted to indicate the distribution of the antineuritic vitamin apart from other members of the vitamin B complex [Salmon, 1927; Aykroyd and Roscoe, 1929; Roscoe, 1930; 1931, 1, 2; Aykroyd, 1930, 2].

Vitamin B_1 is widespread and has been detected in almost all the natural foodstuffs examined except egg white, honey, and the endosperms of cereals. Yeast, eggs, liver, and the embryos of cereals are the richest known sources, and green-leaf vegetables must be regarded as potent if dry weights are considered. Milk, meat, and fish are poor in this vitamin; the roes of fish, however, like the eggs of birds, are rich sources.

Cereals. The most important practical result emerging from the work on vitamin B_1 is the fact that among its chief sources are to be

Table X.

Preventive Experiments.

Minimum Daily Ration which must be added to a Diet of Polished Rice to prevent Onset of Polyneuritis (Beri-beri) in a Pigeon of 300 to 400 g. Weight.

Substance.	*Daily Ration.* *Natural Foodstuff.* (*g.*)	*Daily Ration.* *Dry Weight.* (*g.*)	*Observer.*	*Notes on Results.*
Wheat germ, sample R.I., free from bran .	1·5	1·3	Chick and Hume [1917, 1, 2; 1917–19, 1]	Complete protection.
Wheat bran, sample R.I., free from germ .	More than 1·5	More than 1·4	,, ,,	No protection with 1·5 g. daily. Polyneuritis occurred in same time as in control birds.
,, ,, ,, .	More than 2·5	More than 2·2	,, ,,	Small degree of protection. Mean time of onset delayed about 2 weeks.
Yeast extract, commercial sample A . .	0·5	0·35	,, ,,	Protection not secured in all cases.
	1·0	0·7	,, ,,	Protection.
Pressed yeast	2·5	0·5	Cooper [1912, 1914, 1, 3]	,,
Lentils	—	3·0	,, ,,	,,
Barley, unhusked	3·7	3·2	,, ,,	,,
Barley, husked	5·0	4·5	,, ,,	,,
Egg yolk	3	1·5	,, ,,	,,
Ox muscle	20	5·0	,, ,,	,,
Ox heart muscle	5	1·7	,, ,,	,,
Ox brain	6	1·2	,, ,,	,,
Ox liver	3	0·9	,, ,,	,,
Sheep brain	12	2·5	,, ,,	,,
Fish muscle	More than 10	More than 2	,, ,,	Protection not secured with these amounts.
Cheese	More than 8	More than 5·6	,, ,,	
Cow's milk	More than 35	More than 3·5	,, ,,	

numbered the cereals and edible pulses. In the case of the cereals an interesting differentiation has been established [Chick and Hume, 1917–19, 1] between the different constituents of the grain, the largest deposit of the antineuritic factor being found in the embryo or germ, the bran (*pericarp* and *aleurone layer*) coming next in order of importance (see Fig. 29*a*, *b*). The endosperm, especially when deprived of the aleurone layer (as is customary in the preparation of white wheaten flour or 'polished' rice), is deficient in the antineuritic factor, and if employed too exclusively as a diet will occasion polyneuritis in birds or beri-beri in man [see also Edie and Simpson, 1911]. The experimental work on cereals has been done chiefly with rice and wheat, but there is confirmatory evidence in the case of maize and rye, and no reasonable doubt exists that, as a rule, among cereals, the anti-beri-beri factor is chiefly concentrated in the embryo.

The potency of the cereal embryo in curing avian polyneuritis was found to be very great. Chick and Hume found that 2·5 g. (and occasionally 1 g.) of wheat germ was sufficient to cure a pigeon showing fully developed symptoms; in case of rice and maize embryo, cures were obtained with amounts varying from 0·5 to 1 g. and from 1 to 3 g. respectively.

The prevailing opinion, based on observation of rice-eating populations consuming decorticated rice, has located the principal deposits of the substance preventing beri-beri in the wall of the husked grain, and in the outer layer (aleurone layer) of endosperm, which is removed during steam milling. This view was based upon the connexion between beri-beri and decorticated rice, and the prevention or cure of the disease effected when rice bran or its extracts were added to the diet. A truer explanation recognizes the richer source of the vitamin to be the germ removed with the bran. In the case of wheat and rice the germ, weight for weight, was found to be respectively five times and ten times as potent as the bran in the cure of polyneuritis.

The wheat grain has also been investigated by Bell and Mendel [1921–22; 1922; see also Osborne and Mendel, 1919, 2] who used growth of mice as criterion for the presence of the vitamin B complex and found that the embryo contained about one-sixth of the total amount in the grain. White flour (American patent flour) contained no appreciable amount of vitamin B_1; low grade flour and bran were about twice as rich, and standard middlings, which contained most of the embryo, four times as rich as the unmilled grain; the remaining grades (first and second clear) were about equal to the unmilled grain. It must be remembered that in the preparation of bread yeast is added, and this will introduce a small amount of vitamin B_1; the amount of yeast added is usually less than 1 per cent. of the flour.

Pulses. The foodstuffs next in importance to the cereals in prevention of beri-beri are the pulses. Grijns [1901] and others have demonstrated the value of certain beans ('katjang-idjo', *Phaseolus radiatus*) in prevention and cure of avian polyneuritis or human beri-beri. Dry peas and lentils were found to occupy a high place in the series of foodstuffs collected in Table IX. Experiments with rats confirm the results which have been obtained with pigeons [see

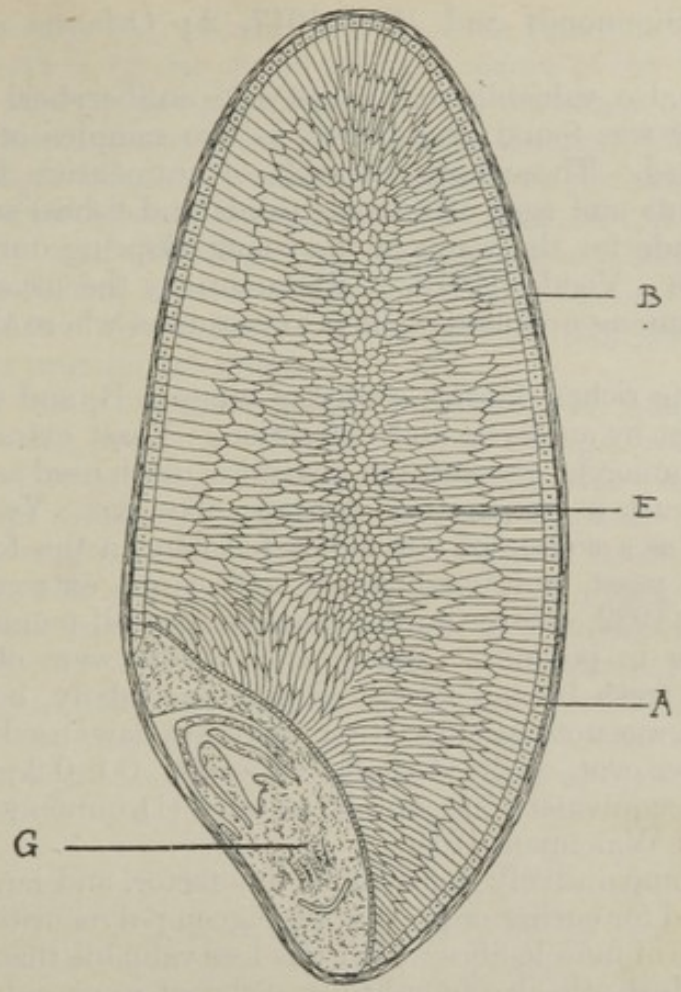


FIG. 29*a*.[1] Diagram of a longitudinal section through a grain of wheat, showing: *B*, pericarp, forming the branny envelope; *A*, aleurone layer of cells forming the outermost layer of the endosperm removed with the pericarp during milling; *E*, parenchymatous cells of the endosperm; *G*, embryo or germ.

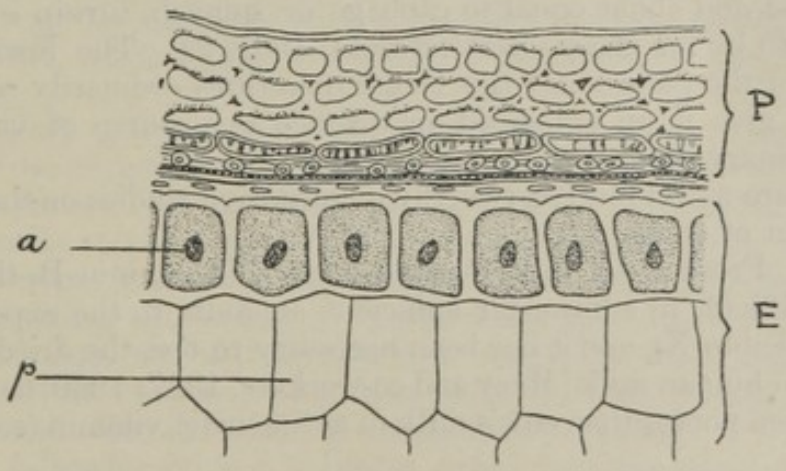


FIG. 29*b*.[1] Cross-section through the branny envelope and outer portion of the endosperm of wheat grain, showing: *P*, the pericarp; *E*, endosperm, consisting of *a*, layer of aleurone cells, and *p*, parenchymatous cells.

[1] Reproduced, with the permission of the Controller of H.M. Stationery Office, from Figs. 1 and 2 in Dr. J. M. HAMILL's 'Report to the Local Government Board on the nutritive value of bread made from different varieties of wheat flour', 1911.

McCollum, Simmonds and Pitz, 1917, 2; Osborne and Mendel, 1919, 1].

Eggs are also valuable sources of the antiberi-beri factor, and this property was found unimpaired in two samples of dried hen's eggs examined. These large deposits of antineuritic factor in the seeds of plants and eggs of animals (birds and fishes) suggest a due provision made for the wants of the young offspring during the early period of life. Vedder [1913, p. 61] mentions the usual prevalence of beri-beri among pregnant women in countries where the disease is endemic.

Yeast is the richest known source of vitamin B_1 and is unaffected in this respect by autolysis and extraction. Yeast extract A (Table X) was a commercial preparation (marmite) much used as a substitute for meat extract in preparation of soup cubes, &c. Yeast has been largely used as a source of vitamin B for rats, in the form of fresh yeast, dried yeast, autolysed yeast, and yeast extract. Kennedy and Palmer [1922, where the literature is quoted] found yeast to be very variable in potency. Satisfactory results were obtained with about 0·6 g. fresh brewer's yeast administered daily, but even then reproduction was not normal. Other observers have used such quantities as 3·6 per cent. of dried yeast in the diet, 0·1–0·2 g. dried yeast daily, or an equivalent amount of extract [Drummond, 1917; see Osborne and Wakeman, 1919].

Meat is comparatively deficient in this factor, and large quantities were required for curing or preventing pigeon polyneuritis. This was specially true of muscle-fibre which was less valuable than heart, liver, or brain. Meat was also found to be deficient in vitamin B as tested on rats [Osborne and Mendel, 1917, 2], and in this case also the heart, liver, and brain were found to be more efficacious.

Vegetables and Fruits. Of the green-leaf vegetables tested, watercress and lettuce proved to be about twice as rich as cabbage and spinach and not inferior to egg-yolk or the embryo of cereals, if dry weights are compared. Of the 'root vegetables' examined, carrot is the richest and about equal to cabbage or spinach, turnip is less rich and potato by far the poorest [Roscoe, 1931, 2]. The lower water-content of the potato and the larger quantities ordinarily consumed, however, give it an enhanced importance as a source of vitamin B_1 in an ordinary diet.

There are as yet few trustworthy quantitative studies on the vitamin B_1 content of fruits.

Milk. Fresh cow's milk is often so poor in vitamin B_1 that it has proved difficult to administer sufficient amounts to the experimental animals (Table X), and it has been necessary to test the dried product. Studies on human milk [Macy and co-workers, 1927; 1930] have shown it to be even poorer than cow's milk in antineuritic vitamin (see p. 164).

The (Antidermatitis) Vitamin B_2.

In general vitamin B_2 has been found to accompany vitamin B_1 in ordinary foodstuffs. Hen's egg white, which contains the former and is devoid of the latter [Chick and Roscoe, 1929, 2], appears to be unique in this respect. The quantitative distribution of these two

vitamins, however, shows many differences and these were put forward as an argument in favour of the multiple nature of the 'water-soluble B' vitamin many years before the separate existence of vitamins B_1 and B_2 was demonstrated [see for example Mitchell, 1919].

Vitamin B_2 is frequently found to accompany the proteins of 'high biological value', probably in an adsorbed state, and in some cases is removed only with difficulty, e.g. from caseinogen [Chick and Roscoe 1927]. The richest known sources are liver, yeast, milk, and green-leaf vegetables if dry weights are compared. Egg yolk, egg white, and meat (ox muscle) are inferior.

Meat and milk are much richer in vitamin B_2 than in the anti-neuritic vitamin, but the reverse is true of egg yolk and green vegetables. The potency of green vegetables in this vitamin appears to run parallel with the degree of greenness of the leaf. Egg white has proved very useful in the study of the vitamin B complex as providing a source of vitamin B_2 unaccompanied by vitamin B_1.

In the cereals and their components there are also marked differences in the distribution of vitamins B_1 and B_2. Wheat embryo, one of the richest sources of the former, is relatively poor in the latter, inferior in this respect to meat and milk. The endosperms of wheat and maize on the other hand, while devoid of vitamin B_1, have been found to contain small but appreciable quantities of vitamin B_2.

The distribution of vitamins B_1 and B_2 is shown in Table XI. The vitamin B_1 content of a foodstuff, as there stated, is estimated from protective and curative pigeon tests (see p. 147) or from growth experiments on rats in which the remainder of the vitamin B complex was supplied and vitamin B_1 was the only limiting factor. The results given for the vitamin B_2 contents depend on experiments expressly designed to estimate the vitamin B_2 content of a foodstuff. The distribution of Goldberger's P-P and the blacktongue-preventive factors is not included here (see p. 183).

Most of the work on the distribution in nature of the B-vitamins has been concerned with the whole complex. In column 6 of Table XI is given an estimate of the richness in this multiple factor of foodstuffs whose relative content of vitamins B_1 and B_2 is still unknown.

The values given refer to substances in the fresh natural state. It should be noted that the drying of natural foodstuffs which have a high water-content, e.g. fruits and vegetables, increases their content of B-vitamins relative to bulk. The temperature required to preserve foods by canning may seriously affect vitamin B_1. The heat necessary for cooking, e.g. boiling of vegetables or stewing of meat, on the other hand, does not of itself as a rule impair the vitamin B_1 potency of the foodstuff concerned, but loss tends to occur through diffusion of the vitamin into the cooking water [Roscoe, 1930].

In the following table o indicates either that the vitamin in question has not been detected, or that it is present in very small amount; + that the material contains the vitamin; + + that it is a good source; and + + + that it is a rich source.

Table XI.

Distribution of Vitamins B_1 and B_2 in Foodstuffs.

Substance.	*Vitamin B_1.*		*Vitamin B_2.*		*Water-soluble Vitamin B.*	
Cereals and Cereal Products.		*References.*		*Refs.*		*References.*
Barley (unhusked)	++	1, 2	.	.	+	3, 4, 5
,, (husked)	++	1				
Buckwheat	+	2				
Custard powders and egg substitutes	.	.	.	.	o	6
Dari	++	2				
Maize, whole	++	2, 85	+	7, 85		
,, embryo	+++	20, 21	+	7		
,, endosperm	o	2	o	7		
Gluten	o	2				
Malt	+	4	.	.	+	4, 9, 5, 11
,, extract (commercial)	+	12, 13, 14	.	.	+	11, 13, 14
Millet	++	2	+	15		
Oats, whole	+	2, 16, 17	.	.	+	17, 18
Rice, whole	++	22	+	15		
,, embryo	+++	20, 21				
,, bran	++	19, 20, 21				
,, polished	o	.	o			
,, starch	o	.	o			
'Parboiled' rice, whole	++	22				
,, ,, polished	++	22				
Rye	++	2				
Wheat, whole	++	2, 84, 85	+	7, 84		
,, germ	+++	2,20,21,75	++	7		
,, bran	++	2,19,20,21	++	7		
,, middlings	++	2	++	7		
,, flour, refined (endosperm)	o	21, 22, 23	o	7		
Legumes.						
Beans, green string	.	.	.	.	+	24
,, haricot	++	2				
,, kidney	.	.	.	.	++	25
,, mongo	.	.	.	.	++	26
,, Soy	+	2, 27	+	27		
,, velvet	++	27	+	27		
,, katjang-idjo (*Phaseolus radiatus*)	+++	19				
Lentils	++	1, 2	.	.	++	28, 48
Peas, fresh	.	.	.	.	++	29
,, dry	++	2, 21, 30	++	7		
Other Seeds.						
Coffee beans, fresh	+	2				
Cotton seed	.	.	.	.	+	
Nuts.						
Almond	+	2	.	.	+	32
Brazil-nut	.	.	.	.	+	32
Chestnut	+	2	.	.	+	32
Coco-nut	.	.	.	.	+	32
Filberts	++	12	.	.	+	32
Hazel	++	2	.	.	+	32
Hickory-nut	.	.	.	.	++	32
Pea-nut	++	2, 30	.	.	+	34
Pecan	.	.	.	.	++	32
Pine	.	.	.	.	++	32
Walnut, black	.	.	.	.	+	32
,, English	.	.	.	.	+	32

Substance.	*Vitamin B_1.*		*Vitamin B_2.*		*Water-soluble Vitamin B.*	
		References.		*Refs.*		*References.*
Vegetables.						
Artichokes	·	·	·	·	++	35, 36
Asparagus	·	·	·	·	++	38
Bamboo shoots	·	·	·	·	o	
Beet, leaves and stem	·	·	·	·	+	37
,, root	·	·	·	·	+	37
,, sugar, root	·	·	·	·	o	39
Bilimbi (*Averrhoa carambola*)	·	·	·	·	+	6
Cabbage, green leaves	++	40	++	40		
,, etiolated ,,	++	40	+	40		
Carrots	++	41	+	41		
Cauliflower	·	·	·	·	+	42
Celery	·	·	·	·	+	38
Clover	·	·	·	·	++	37, 38
Dandelion	·	·	·	·	++	38
Dasheen (*Caladium colocasia*)	·	·	·	·	+	39, 43
Duhat (*Eugenia jambolana*)	·	·	·	·	+	6
Lettuce	++	40	+	40		
Lucerne (Alfalfa)	·	·	·	·	++	37, 38
Mushroom	·	·	·	·	+	44
Okra	·	·	·	·	+	35
Onion	+	40	+	40		
Parsley	·	·	·	·	++	38
Parsnips	·	·	·	·	++	29
Pollen	++	45				
Potatoes, raw	++	41	+	41		
,, peel	++	21				
Pumpkin	·	·	·	·	+	46
Radish	·	·	·	·	+	47
Rape leaves	+	27	++	27		
Spinach	+	40	++	40		
Sugar cane	·	·	·	·	o	59
Swede	·	·	·	·	+	39
Sweet potato	·	·	·	·	+	49
,, leaves	·	·	·	·	+	35
Timothy grass	·	·	·	·	+	37, 38
Turnips	+	41	+	41		
,, greens	·	·	·	·	++	50, 51
Velvet bean, leaves	+	27	++	27		
Watercress	+	40	++	40		
Fruits.						
Alligator pear (Avocado)	·	·	·	·	+	35, 52
Apple	+	41	+	41	+	53, 29
Banana	+	54	+	54		
Breadfruit	·	·	·	·	+	59
Cherry	·	·	·	·	+	54
Currant	o	21				
Date	+	21				
Grape	·	·	·	·	+	29, 53
Grape fruit	·	·	·	·	+	53
Green pepper	·	·	·	·	+	55
Lemon juice	·	·	·	·	+	29, 53
Lime juice	+	56				
Melon, cantaloup	·	·	·	·	+	29, 51
Orange juice	+	8	+	·	+	9, 29, 53, 58
Paw-paw (Papaya)	·	·	·	·	+	60
Peach	·	·	·	·	+	29, 61
Pear	·	·	·	·	+	54, 53
Prune	·	·	·	·	+	53, 29
Raisin	·	·	·	·	+	62
Strawberry	·	·	·	·	+	63
Tomato	+	·	+	·	++	29, 53, 64, 65

Substance.	*Vitamin B_1.*		*Vitamin B_2.*		*Water-soluble Vitamin B.*	
Meat and Fish.		*References.*		*Refs.*		*References.*
Oysters	.	.	.	.	++	68
Clams	.	.	.	.	o	69
Blood	o	70	+	66		
Fish muscle (hake)	+	1	.	.	o	67
,, roe	+	21				
Meat, lean beef, mutton and pork	++	1, 30, 75	++	7		
Ox brain	++	12, 56				
,, heart	++	1				
,, kidney	.	.	+++	74		
,, liver	++	12. 75	+++	7		
,, testes	+	19				
Pig brain	.	.	.	.	+	72
,, heart	.	.	.	.	+	72
,, kidney	.	.	.	.	+	72
,, liver	.	.	.	.	++	71, 72
Sheep brain	++	12				
,, pancreas (sweetbreads)	.	.	.	.	++	73
Milk and Milk Products.						
Milk, cow's, fresh	+	12,30,76,79	++	7, 79		
,, ,, dried	.	.	.	.	++	29
,, ,, sterilized	.	.	.	.	+	6
,, ,, boiled	.	.	.	.	+	6
,, ,, pasteurized	.	.	.	.	+	82
,, ,, condensed	+	83	.	.	+	6
,, ,, skimmed (dried)	+	77	.	.	+	82
,, goat's	.	.	.	.	+	
,, human	+	80, 81	+	80, 81		
Cheese	o	12	+	7		
Eggs, Hen's.						
,, White	o	86	++	86		
,, Yolk	++	1, 86, 75	+	7		
Miscellaneous.						
Beer (stout)	o	57	+	57		
Sugar	.	.	.	.	o	6
Tea	.	.	.	.	o	6
Honey	o	45, 88	o	88		
Molasses, beet	.	.	.	.	+	87
,, cane	.	.	.	.	++	87
Yeast, brewer's	+++	.	+++			
,, dried	+++	.	+++			
,, extract (marmite)	+++	.	+++			
,, autolysed	+++	8				
,, autoclaved	o	.	++			

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4. Harden and Zilva, 1924
5. Southgate, 1924, 1
6. Med. Res. Coun. Report, 1924
7. Aykroyd and Roscoe, 1929
8. Kinnersley and Peters (unpublished)
9. Cooper, 1914, 1
10. Stammers, 1924, 2
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12. Cooper, 1914, 1
13. Randoin and Lecoq, 1927, 2
14. Randoin and Lecoq, 1926, 2
15. Aykroyd, 1930, 1
16. McCollum and Kennedy, 1916
17. Smith and Hendrick, 1926
18. McCollum, Simmonds and Pitz, 1917, 1
19. Schaumann, 1910
20. Chick and Hume, 1917–19, 1
21. Chick and Hume, 1917, 1
22. Aykroyd, 1932
23. Ohler, 1914
24. Quinn, Butris and Milner, 1927
25. McCollum, Simmonds and Pitz, 1917, 2
26. Santos, 1922
27. Salmon, 1927

28. Jones and Murphy, 1924
29. Sherman, 1926
30. Vedder and Clarke, 1912
31. Osborne and Mendel, 1917, 1
32. Cajori, 1920
33. Johns, Finks and Paul, 1919
34. Daniels and Loughlin, 1918
35. Santos, 1922
36. Morgan and Stephenson, 1923
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42. Russell, Morrison and Ebling, 1926
43. Miller, C. D., 1927
44. Orton, McCollum and Simmonds, 1922
45. Dutcher, 1918, 2
46. Morgan and Francis, 1924
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48. Eddy, Kohman and Carlsson, 1926
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50. Burton, 1928
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52. Weatherby and Waterman, 1928
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56. Funk, 1912–13, 1
57. Aykroyd (unpublished)
58. Willimott, 1928
59. Miller, C. D., 1929
60. Miller, C. D., 1926
61. Kohman, Eddy, Carlsson and Halliday, 1926
62. Dutcher and Outhouse, 1922–23
63. Kohman, Eddy and Halliday, 1928
64. Sherman and Grose, 1923–24
65. House, Nelson and Haber, 1929
66. Kollath, 1930
67. Drummond, 1918, 2
68. Jones, Murphy and Nelson, 1928
69. Jones, Nelson and Murphy, 1928
70. Hoagland, 1923
71. Osborne and Mendel, 1917, 3
72. Osborne and Mendel, 1918
73. Eddy, 1916
74. Hoagland and Snider, 1930
75. Chick and Copping, 1930, 2
76. Gibson and Concepcion, 1916
77. Johnson and Hooper, 1921
78. McCollum and Davis, 1915, 2
79. Hunt and Kraus, 1928
80. Macy, Outhouse, Graham and Long, 1927, 2
81. McCosh, Macy and Hunscher, 1931
82. Johnson, 1921
83. Daniels and Brooks, 1927–28
84. Sherman and Axtmayer, 1927
85. Hunt, 1928, 1
86. Chick and Roscoe, 1929, 1
87. Nelson, V. E., Heller and Fulmer, 1925
88. Hoyle, 1929

Vitamins B_3 and B_5.

The literature of the last 20 years dealing with the behaviour of pigeons fed on polished rice supplemented with yeast extracts and other sources of the antineuritic vitamin exhibits much disagreement as to whether full maintenance of weight can be induced by addition of the antineuritic vitamin alone. Thus Emmett and McKim [1917] showed that there were quantitative differences between the amounts required to cure polyneuritis and to give full nutrition when the birds were treated respectively with a yeast concentrate or the original yeast. Evidence related to the same question was early presented by Cooper [1913], who found that potency to prevent polyneuritis and to protect against loss of weight did not run parallel in a series of foodstuffs. Hence some workers held that curative and weight-maintenance factors in the pigeon were different, though others [e.g. Seidell, 1922 1,] used weight maintenance upon polished rice as a means of testing for 'vitamin B'. Kinnersley and Peters [1925] found that one of the most potent antineuritic concentrates obtained by them up to that time (daily dose 0·08 mg.) did not arrest loss of weight in pigeons fed upon polished rice when given in sufficient amounts to protect against polyneuritis for at least 44 days. Somewhat later Jansen and Donath [1926, 2], finding that their crystalline vitamin B preparation could protect against loss of weight as well as against polyneuritis if supplemented by meat powder and cod-liver oil, considered that any factor required, other than the antineuritic vitamin, was protein in character. A step towards clearing up these difficulties

was taken when R. R. Williams and Waterman [1927–28; 1928] showed that pigeons upon an artificial diet or on polished rice did not gain in weight when vitamin B_1, adsorbed upon fuller's earth, was administered, or when this was supplemented with autoclaved yeast containing vitamin B_2. When, however, instead of the latter, air-dried yeast was given, the birds at once improved in condition and attained a maximum weight-level. The same effect was produced by whole wheat. They interpreted this to show that yeast contained in addition to the antineuritic vitamin B_1 a thermolabile factor which was not adsorbed by fuller's earth. Special experiments were made to show that the unknown factor in unheated yeast was not vitamin A, D, or E. The essential facts have been confirmed [Peters, 1930, 1, 2] and vitamin B_3, as it is now called, has been found to be present in whole wheat, in wheat germ and in other foodstuffs, as well as in yeast [Eddy, Gurin and Keresztesy, 1930].

It can be considered an established fact that a pigeon whose weight has fallen upon a polished-rice diet does not rise in weight even when receiving very large doses of vitamin B_1 concentrates. Many such concentrates, though not all, however, have been found to stabilize the weight at about 80 per cent. of the maximum possible. The recognition of the special entity, vitamin B_3, described above as necessary for rising nutrition under these experimental conditions, does not clear up completely the discrepancy between the effect of different vitamin B_1 concentrates. It appears that for maintenance at some intermediate weight, vitamin B_1 alone is not sufficient. Experiments by Carter, Kinnersley and Peters [1930, 1, 2] and simultaneous work by R. R. Williams, Waterman and Gurin [1930], using both their own preparations and one of Jansen and Donath, indicate that some highly purified vitamin B_1 preparations in comparatively large doses cannot alone maintain weight in pigeons feeding upon polished rice. The missing entity, present in some vitamin B_1 preparations from yeast and absent from others, is considered by Carter, Kinnersley and Peters [1930, 2] to be distinct from the other factors of the vitamin 'B complex' including vitamin B_3 and vitamin B_4. It has been provisionally termed vitamin B_5 and is present in whole wheat, yeast and marmite, and in the acid alcoholic extracts from the norite charcoal in the Kinnersley-Peters method of extraction of vitamin B_1 from yeast. It is adsorbed upon fuller's earth together with vitamin B_1 and is more stable in alkaline solution than vitamins B_1 and B_4 under the conditions so far investigated. Its characteristic effect is that given together with vitamin B_1 to pigeons losing weight on a diet of polished rice, it arrests this loss, but does not produce an increase, for which vitamin B_3 is also necessary. Vitamins B_3 and B_5 appear to be more readily stored than vitamin B_1.

Randoin and Lecoq have repeatedly claimed that pigeons need in addition to the antineuritic vitamin a factor *d'entretien*, thought to be more stable to heat than the antineuritic. As the pigeons were not allowed to feed naturally, it is difficult to relate this work with the foregoing, but it would appear that the vitamin B_5 factor is here indicated, though Lecoq [1931] considers this factor *d'entretien* to be identical with vitamin B_3 [see also Hauge and Carrick, 1926, 1].[1] The stability of

[1] It is possible also that the claim of Rosedale [1929], that a curative factor for

vitamin B_5 to heat and alkali suggests a possible identity with factor Y (see below). We may summarize briefly by pointing out that though knowledge about these newer vitamin B factors has not yet reached the same level of certainty as that about those which have been longer known (there are as yet no definite pathological lesions known to be attached to their deficiency), it is impossible to ignore the cumulative evidence in favour of their existence available at the present time.

Vitamin B_4.

Reader [1929, 2] showed that rats on a basal diet with the addition of alkaline autoclaved marmite (120° C. for 1 hour at pH 9) as a source of vitamin B_2 did not grow at the normal rate when vitamin B_1 was added in the form of Peters's concentrate, but did so when unheated marmite was added. The existence of a thermolabile factor in addition to vitamin B_1 was thus indicated. This factor [Reader, 1930, 1, 2] was found to accompany vitamin B_1 in the Kinnersley and Peters process for the concentration of that vitamin as far as the stage at which mercuric sulphate is added. The precipitate then produced, which was almost free from vitamin B_1, contained the vitamin B_4. This could be further concentrated by decomposing the mercury precipitate with sulphuretted hydrogen, concentrating *in vacuo* and removing the precipitates formed by the successive addition of 50 per cent. alcohol at pH 4·5 and of 80 per cent. acetone to the concentrated filtrate from the former.

Vitamin B_4 can be estimated by a curative method on adult rats which have been made polyneuritic by deprivation of vitamins B_1 and B_4 and have then received doses of vitamin B_1 free from vitamin B_4 (see p. 140) [Reader, 1930, 2]. In these circumstances the polyneuritic symptoms disappear, but the animal remains in a condition of muscular weakness, with spastic gait, swollen red paws, and a tendency to sit in a hunched position, these symptoms forming the syndrome characteristic of vitamin B_4 deficiency. Administration of vitamin B_4 causes the disappearance of these symptoms and restores growth. The daily dose required of the purest preparation so far obtained was 0·4 mg., but a crystalline specimen of vitamin B_1 from rice polishings [Jansen *et al.*, 1930] was found to be effective as B_4 in a dose of 0·002 mg. per diem.

Factor Y.

The existence of Factor Y [Chick and Copping, 1930, 2] was deduced from the results of comparative experiments on the growth of young rats receiving a basal diet in which vitamin B_1 was provided by minimal doses of Peters's purified concentrate from yeast, while vitamin B_2 was supplied respectively as an autoclaved yeast extract or a preparation from egg white. Growth was consistently found to be superior in the former case. This fact indicated that a heat-stable dietary factor, in addition to vitamin B_2, was present in autoclaved yeast extract more abundantly than in egg white; this factor was provisionally called factor Y. Its distribution was studied by a series of comparative tests similar to the above in which the vitamin B_1 in

intestinal stasis is present in the lead acetate precipitate from rice-polishing extract, is to be explained in terms of one of these factors.

the diet was provided by minimal doses of various foodstuffs [Roscoe, 1930; Chick and Copping, 1930, 2]. Factor Y was found to be absent from, or present in small amounts in, onion, meat (ox muscle), wheat embryo, and egg white and present more abundantly in yeast and watery yeast extracts (even after strongly heating at 120–125° C. in alkaline solution for 4 hours at pH 9–10), ox liver, egg yolk, and green-leaf vegetables, i.e. in foods known to possess the highest nutritive values.

Its great resistance to heat and alkali distinguishes this dietary factor from all other known constituents of the vitamin B complex.

Growth Factors for Yeasts and Bacteria, and their Relation to the Vitamin B Complex.

The evidence which is available seems to suggest the existence of a number of growth factors for yeasts and bacteria. In certain species growth appears to be dependent on the presence of the appropriate factor, whereas in others growth can occur in a synthetic medium containing only a small number of known chemical compounds. It appears, further, that certain organisms possess the ability to synthesize certain of the B-vitamins as judged by animal tests, whereas in other cases no such synthesis can be demonstrated.

Growth Factors for Yeasts.

Wildiers [1901] first observed the fact that when a synthetic medium containing only well-defined substances was inoculated with yeast no growth occurred unless the inoculation were a large one. He explained the phenomenon by supposing that the yeast required some unknown substance for its development which was not present in synthetic media, but was present in ordinary beer wort and in all growing cultures of yeast. He termed this substance 'bios', and made a number of observations on its chemical and physical properties. He believed that yeast did not itself produce bios but was entirely dependent on outside sources for its necessary supply.

In order to avoid confusion of thought in this field, it has been decided to retain the term 'bios' for growth-promoting factors for yeast. Even this limitation leaves the difficulty that the term is still used in a different sense by different workers; some imply a growth stimulant, others an essential factor for growth, while others again refer to a factor stimulating the production of carbon dioxide. As these effects are not necessarily physiologically equivalent, caution is required in interpreting the literature. In addition it is not clear that due attention has always been paid to the need for accurate adjustment of media, temperature, &c., and the distinction of the effects of such factors from that of bios.

Interest in this question has centred mainly round three problems, viz:

The relation of bios to the growth of the yeast cell.

The relation of bios to vitamin B_1, and the use of yeast growth as a criterion in tests for vitamin B_1.

The synthesis of vitamin B_1 by yeast.

The Relation of Bios to the Growth of the Yeast Cell.

For a discussion of the early literature the review of Tanner [1925] should be consulted.

Pringsheim [1906] examined the phenomenon discovered by Wildiers critically and came to the conclusion that the failure of small inoculations to grow in mineral solutions is due to the inability of the yeast to adapt itself rapidly enough to the new medium. When a larger inoculation is made some of the cells bud at the expense of material derived from other cells which die, and at the same time partially make use of the inorganic constituents of the medium. Subsequently they become adapted to assimilate this, and thereafter grow normally.

According to Wright [1922] yeast, growing in a synthetic medium to which lemon juice has been added, multiplies at the expense of the lemon juice until a certain concentration of cells is attained, after which the ammonium salt of the medium is utilized. Wildiers's bios, on this view, would be simply a source of suitable nitrogen. Certain yeasts, according to Ide [1906], never seem to acquire the power of rapid growth in synthetic media.

A fact of fundamental importance is that different species and strains of yeast behave very differently in respect to their need for bios. This may be deduced from the work of Bachmann [1919], Lucas [1924], Copping [1929], and Fulmer, Nelson and White [1923], the last named having used a yeast which grew well on an artificial medium in which a synthetic sugar, methose, replaced sucrose.

The answer to the question whether bios acts simply as a stimulant to growth [Macdonald and McCollum, 1920–21; Macdonald, 1923] or is indispensable for growth [Funk and Dubin, 1921; Willaman and Olsen, 1923] is probably different for different species of yeast. Peskett [1927–28] found that there appears to be a critical concentration of bios, in the presence of which a small seeding of yeast will attain the same ultimate cell concentration as a large seeding. In the presence of concentrations of bios below this critical value this is not the case.

There seems, therefore, no reason to suppose, at any rate in the case of certain strains of yeast, that bios is a substance '*indispensable au developpement de la levure*', as believed by Wildiers, but rather that it acts as a stimulant to the growth of the yeast cell. For some yeasts, however, bios appears to be an essential factor for growth.

Concentration of Bios.

Meanwhile much effort has been devoted to the isolation of bios and to the study of its chemical properties. Recent work has tended to emphasize the complexity of the bios effect, and evidence, still somewhat conflicting in character, has been forthcoming in support of the view that more than one factor is concerned.

Thus it was found by Fulmer and Nelson [1924], and Fulmer, Duecker and Nelson [1924] that, in alcohol fractionation of an aqueous extract of alfalfa, the fraction obtained with 40 per cent. alcohol and the residue obtained after repeated extraction with 95 per cent. alcohol

provided a much better growth stimulus to *S. cerevisiae* when both were present than when either was present alone.

Much work has been done on the fractionation of bios [W. L. Miller, 1924; Lucas, 1924; Deas, 1924; Sims, 1924; Eastcott, 1924; 1928]. Two fractions, bios I (precipitated by baryta) and bios II (not precipitated; adsorbed by charcoal and yeast) have been obtained from malt combings and rice polishings, and found to be separately inactive but active in combination. Neither promoted the growth of rats or cured avian polyneuritis. Bios I, which has also been isolated from tea-dust, was found to be inactive inositol. Whether this substance bears a relation to yeast growth similar to that claimed by Reader [1929, 1] for mannitol to the growth of *Streptothrix corallinus* remains uncertain. Narayanan [1930] failed to obtain any growth stimulus for *S. cerevisiae* with inositol.

A crystalline material, melting at 223° C. and having bios activity, has been isolated from yeast extract by a method involving its selective adsorption or precipitation by ferric oxide hydrosol at pH 5·3, and subsequent removal of the iron by baryta [Eddy, Kerr and Williams, 1924]. The crystals had an empirical formula $C_5H_{11}ON_3$, were soluble in water, acids, and alkalis, and in alcohol up to 80 per cent. concentration. Later, by conversion into the inactive sulphonamide and hydrolysis of this back to the original active compound, these authors brought further evidence that the activity of their factor, which they termed α-bios, was not due to traces of an adherent potent substance [Kerr, Eddy and Williams, 1925–26]. By the use of phosphotungstic acid and subsequent treatment with fuller's earth, two fractions were obtained from yeast extract [R. J. Williams, Wilson and Ahe, 1927] which exhibited marked bios activity in combination, but little or no action independently. In later work, Kerr [1927–28], by a method of electro-dialysis, succeeded in separating a β-bios and a γ-bios which resembled the bios II of Miller and Lucas.

Narayanan [1930] found that samples of Eddy's α- and β-bios were considerably less active in stimulating the growth of *S. cerevisiae* than his own preparations. He observed that the bios activity was precipitated by phosphotungstic acid and platinic chloride, but not by lead acetate. In agreement with Peskett and O'Brien [1930] no evidence was obtained in favour of the complex nature of bios.

The Relation of Bios to Vitamin B_1, and the Use of Yeast Growth as a Test for Vitamin B_1.

The employment of yeast growth as a test for the antineuritic vitamin was based on the assumption of the identity of this vitamin with Wildiers's bios. R. J. Williams [1919] was led to this view by his observation that the growth of yeast was markedly stimulated by the addition to his synthetic medium of extracts known to contain the antineuritic vitamin, and his suggestion was supported by the close similarity of these two factors in their behaviour to phosphotungstic acid, to acids and alkalis, and to heat.

Several modifications of the test were proposed [Bachmann, 1919; R. J. Williams, 1920; Funk and Dubin, 1920; Swoboda, 1920; Eddy and Stevenson, 1919–20, 1, 2, 3; 1920], but its reliability was not firmly established. Thus Funk and Dubin found that the

growth of yeast varied directly with the dose of the antineuritic factor when small concentrations were employed, but that with higher concentrations of vitamin this relationship did not hold good, suggesting the presence of inhibitory factors. E. W. Miller [1920] reported large variations in the growth of colonies maintained under identical conditions, while Whipple [1920] found that salts and nitrogenous organic substances present in the vitamin extract introduced complicating factors.

A further study of the question revealed many discrepancies between the results of tests for vitamin B_1 obtained by animal experiments and by yeast growth respectively. These concerned the heat-stability of the factors involved [see E. W. Miller, 1920; R. J. Williams, 1919; Eddy and Stevenson, 1920; Emmett and Stockholm, 1920; Souza and McCollum, 1920; Funk and Paton, 1922], their occurrence in various materials such as commercial glucose and hydrolysed meat extract [Souza and McCollum, 1920] and various vegetables [R. J. Williams, 1921; Eddy, Heft, Stevenson and Johnson, 1921], behaviour to adsorbents [Funk and Dubin, 1921; Fränkel and Scharf, 1921], production by moulds [Hoet, 1922], and presence in the tissues of animals deprived of vitamin B [Heaton, 1922]. It was also found that the bios I and II of Miller and his colleagues had no antineuritic potency [Deas, 1924].

While the evidence is overwhelmingly against the suggested identity of bios and vitamin B_1, the possibility raised by Eddy that vitamin B_1 might be one factor related to the growth of yeast has not yet received a final answer. According to R. J. Williams, Warner and Roehm [1929] certain yeasts require for growth one factor only which is not adsorbed by fuller's earth or precipitated by phosphotungstic acid, while other species need at least two factors, one of which resembles vitamin B_1 in its chemical and physical properties, the other being similar to or identical with bios. In the Jansen-Donath technique for the isolation of vitamin B_1, the activity at various stages up to the final precipitation by acetone as judged by the yeast test gave good agreement with the results of animal tests, but beyond this stage discrepancies appeared [R. J. Williams and Roehm, 1930; Narayanan, 1930]. The final crystalline product, however, showed a high degree of potency in stimulating yeast growth.

Synthesis of Vitamin B_1 by Yeast.

The synthesis of vitamin B by yeast was demonstrated by V. E. Nelson, Fulmer and Cessna [1921], who found that after subculture for a year in a purely synthetic medium the yeast thus obtained promoted growth of young rats. Harden and Zilva [1921] also showed that various strains of yeast grown under similar conditions cured polyneuritis when administered to pigeons. Eijkman, Hoogenhuijze and Derks [1922], on the other hand, found that yeast grown on a synthetic medium had no curative action on avian polyneuritis whereas yeast cultured on natural wort was highly active. The liquid separated from the yeast at the end of fermentation was inactive. It was concluded that yeast does not synthesize the antineuritic factor but that it abstracts it from the medium when this contains the

factor or the products of its break-down. Similar results were obtained by Southgate [1924, 2].

Funk and Freedman [1923] concluded that the synthesis of the antineuritic vitamin by yeast is determined by the presence of bios in the medium. This has been confirmed by Macdonald [1922], Heller [1923], Hawking [1927], and Peskett [1927].

Hoet, Leclef and Delarue [1924] found that of the organisms *Monilia candida*, *Torula rosea*, and *Mycoderma cerevisiae* only the first is capable of synthesizing the antineuritic factor. The observation that different strains may vary in their ability to form vitamin B_1 may account for the negative results of Eijkman and his co-workers, and of Southgate.

Bacterial Reproduction and Growth Factors.

Much discussion has taken place with regard to the question whether bacteria require growth stimulants of the nature of vitamins, and further whether they are capable of synthesizing vitamins. The general question can only be dealt with summarily here and for further details the reader is referred to the Medical Research Council *System of Bacteriology*, vol. 1, p. 252; vol. 2, *passim*.

The conclusion to be drawn from the large amount of experimental work which has been done on this subject may be summarized briefly as follows.

Some bacteria (Group 1), e.g. *B. coli*, *B. pyocyaneus*, &c., can be cultivated continuously on purely synthetic media, whilst others (Group 2), e.g. *Staph. pyogenes albus*, *C. diphtheriae*, *Meningococcus*, &c., can only be cultivated continuously in such media when certain pure amino-acids are added.

The *haemolytic streptococci and pneumococci* (Group 3), on the other hand, do not grow on synthetic media and require a growth-promoting factor. This occurs in ordinary beef broth and in many animal and vegetable extracts, fruit juices, &c. [see Hosoya and Kuroya, 1923, 1, 2; Weichardt, 1920; Davidsohn, 1924; Leichtentritt and Zielaskowski, 1922; Reader, 1928]. This growth factor appears to be more stable to heat and alkali than vitamin B_1, less so than bios.

Finally the *haemophilic bacteria*, e.g. *B. influenzae* Pfeiffer (Group 4), require a still more complex medium containing two unknown factors [Fildes, 1921; Thjötta and Avery, 1921]. The first of these (the V or vitamin factor) is very easily inactivated by heat or alkali, occurs in many vegetable extracts and is synthesized by many bacteria. The second (the X factor) contains iron and occurs in blood, but is also present in potato and is produced by certain bacteria; in blood it is thermostable.

The interesting observation has been made by Reader [1927] that *Sarcina aurantiaca* and two strains of streptothrix can be grown on a suitable synthetic medium, but that growth is greatly enhanced by the addition to the medium of traces of a tryptic digest beef broth. The growth stimulant present has been shown to be an organic, water-soluble, and dialysable substance which is not precipitated by either normal or basic lead acetate. It is found in muscle, serum, wheat embryo, and in some antineuritic yeast concentrates having an

activity of 0·01 mg. (pigeon day-dose). The factor is not inactivated by alkali [Reader, 1928; Peters, Kinnersley, Orr-Ewing and Reader, 1928], indicating that it is not identical with vitamin B_1 though possibly related to it. It has been shown that the Jansen-Donath crystals of the antineuritic vitamin produce maximal growth of the streptothrix in concentrations as low as $4{\cdot}2 \times 10^{-4}$ mg. per 100 c.c. of medium [Jansen, Kinnersley, Peters and Reader, 1930]. Reader [1929, 1] observed that the addition of mannitol to the synthetic medium containing a small amount of an antineuritic concentrate produced a marked stimulant action on the growth of the streptothrix. Other polyhydric alcohols, including inositol, were inactive. It was concluded that mannitol acted as a specific source of food supply rather than as an additive growth stimulant.

It was further shown [Orr-Ewing and Reader, 1928] that the meningococcus could be grown successfully on a medium devoid of the streptothrix factor, but that in the course of its growth this factor was synthesized.

There is therefore no conclusive evidence to support the view that bacteria require the presence of vitamin B_1 as such for normal growth. On the other hand the synthesis of the vitamin by many, though not by all, species of bacteria has been attested by a number of observers. The subject is of much interest with respect to the possible synthesis of B-vitamins in the alimentary canal of animals on vitamin-free diets. There is some doubt about the synthesis of vitamin B_1 by *B. coli* [see Damon, 1921; Kuroya and Hosoya, 1923], but positive results have been obtained with *B. influenzae* Pfeiffer, *Mycobact. smegmatis*, *Mycobact. phlei*, and *Mycobact. moelleri* [Damon, 1923; 1924], *B. vulgatus* [Scheunert and Schieblich, 1923], negative with *B. paratyphosus*, *B. subtilis* (a large amount of which was administered) [Damon, 1921], *B. adhaerens* and *B. friedländer* [Damon, 1923; 1924].

Sunderlin and Werkman [1928] found that the growth of rats was stimulated by the addition to the diet of cultures of *Torula rosea*, *Oospora lactis*, *B. adhaerens*, *B. coli*, *B. subtilis*, *B. mycoides*, *Azotobacter chroococcum*, *Rhizobium leguminosarum*, and by *Actinomyces*.

Bechdel, Eckles and Palmer [1926] found that calves could be grown to maturity on a diet carrying insufficient vitamin B to support the growth of rats. This fact was later shown by Bechdel, Honeywell, Dutcher and Knutsen [1928] to be due probably to the synthesis of vitamin B by a flavobacterium present in the rumen of the animals. Both the alcoholic extract of the contents of the rumen of a cow fed on a diet low in vitamin B and cultures (on a medium free from vitamin B) of a flavobacterium, which was abundantly present in the rumen contents, protected rats against deficiency of the vitamin B complex.

Refection.

Refection is the term used to describe a remarkable condition in which rats can exist and grow normally without any supply of the vitamin B complex in their diet [Fridericia *et al.*, 1927; Roscoe, 1927, 2]. The condition may occur spontaneously in rats on synthetic diets containing the B-vitamin complex, or on those deprived of it (Fridericia *et al.*) or of its constituents (Roscoe). In the last instance

the symptoms of deficiency of vitamin B_1 (polyneuritis) or of vitamin B_2 (dermatitis) are rapidly cured. Normal growth to maturity may take place and breeding for two generations of refected rats has been recorded (Fridericia *et al.*).

The condition is infectious. It can be transmitted by feeding the faeces of refected rats and may also spread in the absence of direct contact, unless the refected animals are kept strictly segregated. These facts have led Fridericia to postulate a virus of refection.

The most noticeable feature of refected rats, apart from their satisfactory growth and generally good condition, is the presence in the faeces of a large proportion (up to 60 per cent.) of undigested starch. In some cases as much as 45 per cent. of the starch of the diet may be thus excreted in the faeces. The growth of the animal appears to some degree to be related to the amount of starch excreted, and this starch has been found to be very resistant to the action of amylase or ptyalin. The phenomenon of refection has only been recorded with diets containing raw starch; when the carbohydrate is present as cooked starch (Roscoe) or as sugar (Fridericia) refection has not been observed.

The faeces of refected rats appear to be richer in B-vitamins than those of other rats deprived of these vitamins. This suggests that in the refected state the vitamin B complex is synthesized in the intestinal tract, owing (according to Fridericia) to the presence of an infecting micro-organism, probably identical with that transmitting the condition from rat to rat. All attempts to isolate any micro-organism, however, have so far been unavailing and attempts to induce refection by systematic feeding with small doses of various living yeasts have also been unsuccessful [Roscoe, personal communication].

Mendel and Vickery [1928–29] have reported their repeated failure to produce refection in rats and the condition has not as yet been observed in the United States. It seems possible that its occurrence in European laboratories may be due to a chance infection of the intestine with some wild yeast derived from the particular sample of starch incorporated in the diet. The use of potato starch appears to favour the occurrence of refection [Kon and Watchhorn, 1928; Roscoe, personal communication], but in the United States this was also used without success.

The non-specific nature of the protection afforded in refection is shown by the power to correct a deficiency of the complete yeast B-vitamin complex and is another reason for supposing that the protective mechanism may be concerned with growth of a yeast or other micro-organism in the animal's intestine. An interesting case is described by Boas-Fixsen in her investigation of the pathological state induced by feeding young rats on dried egg white as sole source of protein (Boas, 1924, 1, 2; 1927; Boas Fixsen, 1931). This condition, which includes severe dermatitis, as well as nervous affections, develops on diets containing marmite, but can be cured or prevented by whole yeast and by many foodstuffs known to be rich in the vitamin B complex. In the course of her work spontaneous cures took place resembling refection, marked, in this case also, by the excretion

of bulky faeces containing undigested starch. The refected condition could be transmitted to unrefected rats on the egg white diet by feeding them with these faeces or equally with the white faeces from rats which had become refected on diets deprived of all B-vitamins.

A condition resembling refection has been noticed in pigeons fed on a diet deprived of B-vitamins consisting of autoclaved rice and dhal (legumes) ; it differs, however, from that described by Fridericia and his co-workers and by Roscoe in the fact that it was developed on a cooked diet and could not be transmitted to unrefected birds by feeding the bulky faeces excreted [Taylor and Thant, 1928].

CHAPTER VII

PELLAGRA AS A VITAMIN DEFICIENCY DISEASE.

History.

The records of pellagra do not extend far back into medical history. The disease, which possesses very characteristic and easily recognized symptoms, was apparently unknown to the physicians of antiquity, a fact ascertained and used in controversy many years ago. Its scientific history dates from the treatise written by the Spaniard, Casal, in 1735, which was not fully published until 1763. It is interesting to note that Casal gave a full description of the very poor diets customary in the pellagrous districts of Andalusia, and actually ascribed the disease to faulty nutrition.

A few years later pellagra was recognized and received its name in Italy, where it has ever since been prevalent. Early in the nineteenth century it was observed in France and subsequently it spread eastward across Europe, afflicting Hungary, Rumania, Turkey, and Greece. In 1847 pellagra was recorded in Egypt by Pruner, and towards the end of the century Sandwith [1898] gave a full account of the condition in that country. Recently the disease has been reported from more southerly countries in the African Continent, such as Rhodesia and Nyasaland.

In the United States, pellagra first became seriously evident about 1909 [Wood, 1920]. Within a few years of its recognition it became a terrible scourge among the negroes and poor whites of the Southern States, 170,000 cases being recorded in 1917, with a high mortality [McCollum and Simmonds, 1928]. Of recent years the American outbreak has become milder in form and somewhat lessened in prevalence, but in the cotton-growing districts of the Southern States pellagra remains a very serious menace.

Vedder [1916], in supporting the dietetic origin of pellagra, which was suggested by Funk [1912], pointed out some suggestive analogies between that disease and beri-beri. It is interesting to note that in both cases the theories of their aetiology have run a similar historical course. Before the time of Pasteur and the germ theory of disease many physicians unhesitatingly ascribed to beri-beri and pellagra an origin in poor and monotonous diet. Later, when microbes were at the height of their popularity, a new bacterial cause of both scourges was discovered by enthusiasts almost annually. Of recent years it has again become clear that the origin of beri-beri and pellagra must be sought in faulty nutrition, but while there is good evidence that beri-beri is related to deficiency of vitamin B_1, the exact relation between pellagra and dietary error still remains to be demonstrated beyond question.

An enormous amount of human effort has been expended in the study of this formidable disease, and some early work on the subject has considerable interest and value. The voluminous early literature has been collected by Harris [1919] to whose book those interested should refer. Among a number of shrewd investigators

the French physician Roussel [1845; 1866] stands out. Roussel realized clearly the value of milk in the treatment of pellagra, a fact recorded by Casal and many others, and recommended that pellagrins should be given broth, meat, eggs, and fish. He pointed out the very important fact, which can be further developed to-day, that the history of the spread of pellagra in Europe is the history of the introduction of maize as a staple food. Brought from America, maize reached Spain first, then Italy, and later France, and thence its use spread gradually eastward. Pellagra followed it across Europe into Africa, where maize, first used in Egypt, is now tending to displace millet as the most widely used cereal. On the other hand, the apparently sudden outbreak of pellagra in the United States, where maize has long been used, has never been quite satisfactorily linked to a change of dietary habits either as regards maize or other foodstuffs.

A connexion between pellagra and chronic alcoholism has been noted by several observers [Klauder and Winkelman, 1928; Sweitzer, 1928; Kumer, 1931].

The Occurrence of Pellagra on Diets consisting chiefly of Cereals other than Maize.

Many observers in the last century were impressed by the intimate connexion between maize and pellagra. Pellagra has been called 'zeism', and at one period research resolved itself into an effort to demonstrate a pellagra-producing poison in maize. There are, however, a number of well-authenticated instances of the appearance of the disease on other staple foods. Stannus [1911–12; 1913–14] and Sheppard [1912] have reported its occurrence on rice, W. H. Wilson [1921] on millet, and Siler, Garrison and McNeal [1913] recorded its prevalence in a district where wheat flour was the most widely used cereal. A few cases [Sambon, 1913] in persons who had never eaten maize have been reported in the British Isles.

Such observations must be taken into account in an aetiological inquiry, but perhaps too much can be made of them. It should never be forgotten that pellagra is *almost* exclusively a maize eater's disease and that its occasional appearance among a population consuming other cereals attracts attention by its very rarity.

The Prevention of Pellagra by Dietary Alterations.

Goldberger was appointed in 1913 by the U.S.A. Bureau of Public Health to investigate the outbreak of pellagra in the Southern States, which was just then assuming grave proportions. He appears [1914] to have been struck at the outset by two observations, which he made the basis of his researches. One was the prevalence of the disease among the very poor, the other, the immunity of doctors and attendants in asylums where pellagra was rife.

The latter fact at once excluded the theory that pellagra is primarily an infectious, microbe-borne disease. With regard to diet, however, the patients were definitely worse off than the staff. Goldberger gained the impression that their dietary, and the dietary

of other pellagrins in the cotton-milling districts, differed from that of individuals and populations without pellagra in being mainly composed of cereals and almost devoid of animal protein.

An example on a small scale of the incidence of the disease throughout the general population was discovered at an orphanage in Missouri in which pellagra was found to be confined to one age group, which, in comparison with the rest, had a diet very short of fresh meat and other animal protein [Goldberger, Waring and Willets, 1915]. Acting on the observation that pellagra does not occur when liberal amounts of animal protein are eaten, Goldberger and his colleagues attempted preventive experiments, made possible by the recurrent nature of the disease. In two orphanages containing large numbers of pellagrous children recurrence was prevented by adding milk and eggs to the poor diet, composed mainly of maize and other cereal foods. In other similar institutions under observation no dietary change was made, with the result that pellagra recurred in 40 per cent. of the former year's victims.

Goldberger, Wheeler and Sullivan [1915] restricted 11 convicts working on a prison farm, who were offered a pardon on the conclusion of the experiment, to a diet mainly composed of maize endosperm for 6 months. After 5½ months, 6 were found to be suffering from a condition closely resembling pellagra. In the following year Goldberger [1916] and a number of his colleagues in reply to critics, demonstrated on themselves the impossibility of transmitting pellagra by inoculation with the secretions of pellagrins. In support of the dietetic theory a careful study of the epidemiology of the disease in certain villages in South Carolina [Goldberger, Wheeler and Sydenstricker, 1920] showed that 'increasing supplies of milk and fresh meat were associated, one independently of the other, with a decreasing pellagra incidence'.

Simultaneous work by W. H. Wilson in Egypt confirmed Goldberger's observation that dietary measures can prevent pellagra. Wilson [1921], adopting the hypothesis that the cause of pellagra is to be found in a shortage of proteins of high biological value according to Thomas's [1909] figures, showed clearly that pellagra may be cured and prevented by the addition of milk or meat to the high cereal diet with which he found the disease associated. Boyd and Lelean [1919], working in co-operation with Wilson in Egypt, reached conclusions similar to his with regard to the causation of pellagra.

The Amino-Acid Deficiency Theory.

There is thus sound evidence that certain types of foodstuffs, taken in adequate amounts, will avert pellagra. Goldberger [1922], following Wilson and others, at first identified the preventive agent with proteins of 'high biological value,' a value connected with their content of the more important amino-acids. There was considerable evidence in favour of the amino-acid deficiency theory of pellagra. Kossel and Kutcher [1900], Osborne and Harris [1905], and Wilcock and Hopkins [1906] had demonstrated that zein, the principal protein of maize, is deficient in lysine and tryptophan.

Thomas [1909] defined 'biological value' as the number of parts of body nitrogen replaceable by 100 parts of the nitrogen of the foodstuff tested, and by experiments on himself worked out values for the proteins of a number of common food substances. His method consisted in consuming a nitrogen-free diet for a few days until his nitrogen output fell to a low figure, taken to represent only endogenous metabolism, and then testing the power of various foods, fed as additions to the nitrogen-free diet, to replace the nitrogen lost in tissue destruction. Thomas assigned to maize the low value of 30 as compared with rice 88, wheat flour 39·5, ox muscle 105, and milk 100.

On the basis of Thomas's figures W. H. Wilson [1921] showed that diets associated with pellagra in Egypt and elsewhere usually contained only protein of low biological value. He suggested that pellagra is liable to occur when the biological value of the protein consumed daily falls below a value equal to that of 40 to 45 g. of meat protein, and demonstrated that the diets of pellagrins are usually below this figure.

The amino-acid deficiency theory rests, however, on shaky foundations. It is difficult to accept Thomas's results as absolute, since they have not been confirmed either for man or the lower animals. Martin and Robison [1922] pointed out the difficulties underlying determinations of biological value and subjected Thomas's results to criticism, for the details of which their original paper should be consulted. Taking great care to avoid fallacies they attempted to estimate by experiments on themselves the biological value of the proteins of milk and whole wheat. Their value for wheat (35, C.J.M., and 31, R.R.) agreed fairly well with that of Thomas for wheat flour (39·5), but in the case of milk the difference was large (50, C.J.M., and 100, Thomas).

McCollum [1911], using Thomas's method and working with pigs, had found previously that zein could cover 73 per cent. of the animals' nitrogen expenditure. Using the pig as the experimental animal Hart and McCollum [1914] found the growth value of the proteins of the wheat, maize, and oat kernels roughly equal. These growth experiments, being probably complicated by unrecognized vitamin deficiences, are, however, difficult to interpret. It may be remarked that Thomas's work, which was performed before the importance of the vitamins was realized, is also subject in this respect to criticism.

Later work of McCollum, Simmonds and Parsons [1921], in which young rats were reared to maturity on diets containing protein from one source only, assigned to maize a low value and showed that the foodstuffs may be arranged as follows in descending order as regards the biological value of the constituent proteins:

(1) Ox kidney;
(2) Whole wheat;
(3) Milk; ox liver;
(4) Ox muscle; barley; rye;
(5) Maize; oats;
(6) Soy bean; navy bean; pea.

In this series the low position of maize is noteworthy, and a

similar result was obtained by Mitchell [1924], who also used the rat as experimental animal, but employed the method of nitrogenous exchange for determining the biological value of the proteins investigated.

At present a fresh survey of the 'biological value' of proteins, with fuller recognition of the role played by the vitamins, is required. Until this is available and more trustworthy figures have been obtained for the biological value of the proteins of maize compared with those of other cereals, it is difficult to assess the importance of amino-acid deficiency as a factor in the causation of pellagra.

The Pellagra-preventive Factor.

Funk [1912; 1913, 2] was the first to suggest that pellagra, like beriberi, might be due to a vitamin deficiency, and advanced the theory that in the milling of maize a dietary factor is removed whose absence leads to pellagra.

Voegtlin, Neill and Hunter [1920] administered to pellagrins a 95 per cent. alcoholic extract of ox liver in daily doses equal to 1 kg. of fresh liver. They claimed that the extract, which was almost free from amino-nitrogen, produced as good a therapeutic effect as 'a diet containing a considerable quantity of milk, eggs, and meat'. It was recorded by the same observers that alcoholic extracts of rice polishings and yeast failed to produce parallel results, which suggested that the curative substance was not 'water-soluble vitamin B'.

Goldberger [1922] at first believed that amino-acid deficiency was the cause of pellagra, but, in the course of clinical observations to discover which foods could prevent pellagra, he obtained evidence pointing in another direction. These experiments consisted in making clear-cut additions to the dietary in certain asylums in which pellagra recurred regularly, a dietary mainly composed of maize and short of animal protein, and observing the effect of these additions on the incidence of pellagra in the following spring. By this work the value of milk and meat in both prevention and treatment was further confirmed [Goldberger and Tanner, 1924]. Gelatin, butter, and cod-liver oil were found to be without preventive effect. Supplements of dried soy beans and cowpeas, legumes containing a high proportion of vegetable protein, did not lower the proportion of recurrences [Goldberger and Tanner, 1925]. Addition to the diet of a liberal allowance of caseinogen, previously purified by repeated washings in acidulated water, had no effect in preventing the systemic lesions of pellagra, though it appeared to have some power to avert the appearance of the characteristic dermatitis, an observation difficult to reconcile with the theory that animal proteins, or proteins of supposed high biological value, are all-important in the prevention of pellagra. Having at about this time accidentally discovered that yeast was effective in preventing and treating experimental blacktongue in dogs, a condition thought to be analogous to pellagra, Goldberger and Tanner tried the effect

of yeast as a prophylactic in the human disease. It was found that 30 g. daily of dried brewer's yeast gave complete protection. This would supply less than 15 g. of protein, and later it was discovered by Goldberger and his co-workers [1926] that a commercial preparation made from an acid extract of yeast, which contained very little protein-nitrogen, given in doses of 15 g. per diem, was equally effective. This confirmed the earlier observation of Voegtlin, Neill and Hunter [1920] referred to above (p. 180). A non-protein 'P-P' (pellagra-preventive) factor was therefore postulated in yeast and other foodstuffs possessing similar preventive properties.

Subsequently the 'P-P' factor was found by Goldberger and Wheeler to be present in tomato juice [1927, 1], wheat germ [1927, 2] and canned salmon [1929]. Carrots and turnips [1927, 1] and Virginian cowpeas [1927, 2] were found to be poor sources of the factor.

As the result of the extension of these investigations to experimental work on rats, Goldberger and his colleagues were able to identify their pellagra-preventive dietary factor with a vitamin contained in the yeast vitamin complex, differing from the anti-neuritic vitamin in its stability to heat and in its distribution in foodstuffs. This vitamin is now known as vitamin B_2 and by some American authorities as vitamin G. The therapeutic value of yeast in pellagra has since been confirmed by other observers in U.S.A. and elsewhere. In Russia, Tcherkes [1929], investigating a severe outbreak of pellagra in the Ukraine, recorded that a daily allowance of 45 g. of yeast, 1·5 litres of milk, or 200 g. of meat was of great effect in clearing up the symptoms of pellagra.

Carley [1930] tried the effect of dried brewer's yeast on a number of negro pellagrins in Mississippi. He states that 1 oz. per diem assisted in alleviating the symptoms, and that a similar daily dose, continued over a period of six months, was extremely effective in preventing recurrence.

Experimental Blacktongue in Dogs.

Chittenden and Underhill [1917] observed in dogs given a diet composed of wheat-flour biscuits, peas, and cotton-seed oil, a pathological syndrome which they suggested might be the analogue of human pellagra. In periods ranging from one to six months the dogs showed necrosis and gangrene of the tongue and buccal mucous membrane, accompanied by a bloody diarrhoea. Death usually ensued. The inclusion in the diet of large amounts of peas or small amounts of lean meat delayed the appearance of these symptoms.

Wheeler, Goldberger and Blackstock [1922] suggested that the disease produced experimentally by Chittenden and Underhill was identical with 'blacktongue' (canine typhus, Stuttgart epizootic, &c.), a canine disease well known to veterinarians, so named because the affected animal develops necrotic areas on the tongue. Pellagra and blacktongue appear to have a similar geographical distribution in the United States, and Wheeler and his colleagues

further suggested that blacktongue occurring spontaneously is, in reality, canine pellagra.

Later, Goldberger and his collaborators [1925; 1926; 1928, 1, 2] produced in dogs by dietary means a disease which they called 'blacktongue'. The diets of the dogs were modelled on those found to be associated clinically with human pellagra, and on that used in the prison farm experiment (p. 178) [Goldberger, Wheeler and Sullivan, 1915]. It was found that blacktongue and pellagra, developing on similar diets, could be prevented by similar foodstuffs, and the two conditions were accepted as analogous by the American workers. A wide study of the distribution of the blacktongue-preventive factor was undertaken with the purpose of acquiring practical information of use in the prevention of pellagra [Goldberger, Wheeler, Lillie and Rogers, 1928, 2; Goldberger, Wheeler, Rogers and Sebrell, 1930]. It was found that the blacktongue-preventive factor in yeast was thermostable [Goldberger, Wheeler, Lillie and Rogers, 1928, 1], and this observation was used to link up human pellagra with rat 'pellagra', the latter being associated with lack of the relatively thermostable vitamin B_2.

Though the possibility of producing a pellagra-like disease in dogs was first suggested to Goldberger by Chittenden and Underhill's experiments, it soon appeared that the two groups of investigators were dealing with different conditions. Underhill and Mendel [1925] discovered that their syndrome could be relieved by a number of substances containing carotene. Carrots and yellow butter, which Goldberger had found lacking in the pellagra and blacktongue-preventive factors, cured their experimental disease, while yeast, though rich in the P-P factor, was without effect. Underhill and Mendel [1928] later recorded that crystallized carotene possessed curative properties, a significant observation in the light of the discovery that carotene and vitamin A have similar physiological actions (see p. 39). The food-mixture given by Underhill and Mendel to their dogs lacked vitamin A, and this deficiency may have caused many of the symptoms observed, while the diets employed by Goldberger, modelled on those associated with human pellagra, contained that vitamin. It would seem that the two groups of investigators had been dealing with only superficially similar pathological conditions, which were associated in reality with a deficiency of different food factors. It is possible that Underhill and Mendel's syndrome may have been caused by lack of vitamin A and by other serious dietary deficiencies, though on the other hand it was recorded that cod-liver oil would not cure the affected dogs [Underhill and Mendel, 1925].

The Relation between Vitamin B_2, the P-P Factor, and the Blacktongue-preventive Factor (Goldberger).

Aykroyd and Roscoe [1929] pointed out that the distribution in foodstuffs of the P-P factor, the blacktongue-preventive factor, and vitamin B_2 as determined by themselves showed fair agreement. Table XII shows the correspondence.

TABLE XII.

Comparison of the Preventive Value of Various Foodstuffs for Vitamin B_2-Deficiency in Rats, for Pellagra in Man, and for Blacktongue in Dogs.

The percentages refer to the proportions present in the diet.

Material.	*Vitamin B_2. Rat.*	*P-P Factor. Man.*	*Blacktongue-preventive Factor. Dogs.*
Maize endosperm	Very poor.	Associated with pellagra in U.S.A. and Italy.	. . .
Wheat flour	Very poor.	Associated with pellagra in U.S.A.	Used in basal diet to produce the disease.
Whole maize	Poor. Growth less than half the normal.	Traditionally associated with pellagra.	No preventive action; used in basal diet to produce the disease.
Whole wheat	Poor, but better than maize.	. . .	66 % delayed onset of the disease; no protection.
Peas	30–40 % needed for good growth ('Clipper' dried peas).	30–35 % (168 g. daily), incomplete protection (Virginian cowpea).	58 %, slight protection; 73 %, almost complete protection (Virginian cowpea).
Wheat germ	15–30 %, good growth.	30 %, protection almost complete.	31 %, protection almost complete.
Fresh milk	5–10 % milk solids, good growth.	20 % solids (1,200 g. buttermilk daily) protected.	250–300 c.c. skim milk daily protected.
Lean meat	7–10 % (dry weight), good growth.	10 % (dry weight, 50 g. daily) protected.	11 % (dry weight) protected.
Dried yeast	1–3 % good growth,	7 % (30 g. daily) protected.	5 % protected.
Liver	1–2 % (dry weight) ox liver, good growth.	Alcoholic extract of ox liver effective in treatment.	11 % dried pork liver protected.
Dried egg yolk	7–15 %, good growth.	. . .	17 % protected.

Apart from similarity of distribution, other evidence points to the identity of vitamin B_2 and the P-P factor. As previously recorded, a diet deficient in vitamin B_2 often produces a pellagra-like syndrome in rats and mice. Experimental blacktongue in dogs (also a pellagra-like condition) develops on diets similar to those associated with human pellagra and the factor which prevents its occurrence is, like vitamin B_2, relatively thermostable.

It is not justifiable, however, to assume that pellagra and vitamin B_2 deficiency are synonymous. Evidence against the identity of vitamin B_2 with the P-P factor, and the analogy between human pellagra and rat dermatitis, is the fact that this last disease requires a highly purified diet for its development. Again, it has been shown by Aykroyd [1930, 1] that maize, millet, and rice, the last two of which are rarely associated with pellagra, are all three equally low in their vitamin B_2 content. This observation weakens the theory that pellagra is due solely to a deficiency of vitamin B_2.

Pellagra and Beri-beri.

Diets composed almost exclusively of milled rice or refined wheat flour are commonly found among populations liable to beri-beri, but this disease is rarely recorded among maize-eaters. It is possible that there is enough vitamin B_1 in their diet to enable them to subsist long enough on this defective cereal to develop pellagra, supposing the latter disease to be due to vitamin B_2 deficiency and to occur after a longer depletion period than beri-beri. It could also be argued that the exclusive eater of milled rice and refined wheat flour, whose diet is deficient in both B vitamins, does not get pellagra because he succumbs first to beri-beri. Against such a theory is the observation that 'parboiled' rice, which contains vitamin B_1 [Plimmer *et al.*, 1927], usually confers immunity from beri-beri [Fraser and Stanton, 1911] and is almost never associated with pellagra, though it does not compare favourably with whole maize and maize endosperm as a source of vitamin B_2 [Aykroyd, 1930, 1].

The Maize Toxin Theory of Pellagra.

Lombroso's theory that a poison produced in deteriorated maize is the cause of pellagra—a theory still held officially in Italy—was the starting-point of numerous investigations in the last century. In spite of considerable effort, however, no conclusive experiment in proof was put forward. At a somewhat later period the 'photodynamic' theory attracted adherents. It has long been known that the skin lesions of pellagra occur on parts of the body exposed to sunlight, and Aschoff [1905] suggested that this might be explained by the presence of a photo-sensitizing substance in maize analogous to that supposed to exist in buckwheat. It is said that white hogs and sheep which consume buckwheat develop skin lesions on the parts exposed to sunlight.

Horbaczewski [1910; 1912] attempted to demonstrate the presence of a photo-sensitizing substance in maize by experiments on white mice and rats. White mice fed on maize polenta and milk developed dermatitis, when exposed to sunlight, in periods ranging from two weeks to six months. Dermatitis was also recorded in a number of rats given a diet of wheat flour and milk to which an alcoholic extract of maize was added. Subcutaneous injection of maize extracts into mice resulted in death in two or three hours in animals exposed to light, while in the dark they survived for twenty to thirty hours. An attempt to repeat Horbaczewski's observations on rats has proved unsuccessful [Aykroyd, 1931, unpublished results].

It is difficult to assess the value of Horbaczewski's work. His papers contain some contradictions, and some experiments are imperfectly controlled and need confirmation. Nevertheless, the possibility that maize contains a positive 'pellagra-producing' factor, perhaps operative only in the absence of the P-P factor, should not altogether be lost sight of. It is conceivable that the action of the P-P factor might be to neutralize a toxin rather than to make up a deficiency, just as vitamin D 'neutralizes' the anti-

calcifying effect of certain cereals though it also makes up a deficiency. Since it has been found that maize does not greatly differ from other cereals as regards its vitamin B_2 content, the epidemiology of pellagra cannot at present be explained in terms of a deficiency of this vitamin. If, however, a positive toxic factor could be demonstrated in maize, this difficulty would be overcome.

The Present Position of Pellagra Research.

Though a considerable number of points remain obscure, from the practical point of view much useful knowledge about pellagra has been obtained in the last twenty years. Pellagra is unquestionably associated with a poor and monotonous diet, and its appearance can be prevented by suitable dietary alterations. The observations of Goldberger, Wilson, and others should be of the utmost value to administrators and medical men in actual contact with the disease. The efficiency of yeast, for example, in prevention and in cure of milder cases, has been conclusively demonstrated. Yeast is cheaper than milk and meat, also efficient pellagra-preventives, but is often a difficult addition to make to a human dietary. The important question of the attitude to be adopted towards the consumption of maize by pellagrins or potential pellagrins remains unsettled. It is, however, interesting to recall that in the last century the French physicians concerned with pellagra directed their efforts mainly to the elimination of maize from the diet of the people. Their campaign was ultimately successful and the use of maize in France was discontinued. The simultaneous disappearance of pellagra naturally appeared to be a complete justification of the policy.

CHAPTER VIII

VITAMIN C, THE ANTISCORBUTIC VITAMIN.

SCURVY has for many centuries been regarded as a disease due to dietetic errors, and rightly so. It was common knowledge in olden times, especially among seafaring folk, that scurvy occurred after deprivation for long periods of fresh foodstuffs, and that it could be prevented and rapidly cured when fresh vegetables and fruits were available. Thus Bachstrom in 1734 (*Observationes circa scorbutum; eiusque indolem, causas, signa et curam*) wrote as follows:

'From want of proper attention to the history of the scurvy, its causes have been generally, though wrongfully, supposed to be cold in northern climates, sea-air, the use of salt meats, etc., whereas this evil is solely owing to a total abstinence from fresh vegetable food and greens; which is alone the true primary cause of the disease. And where persons, either through neglect or necessity, do refrain for a considerable time from eating the fresh fruits of the earth, and greens, no age, no climate or soil are exempted from its attack. Other secondary causes may likewise concur, but recent vegetables are found alone effectual to preserve the body from this malady; and most speedily to cure it, even in a few days, when the case is not rendered desperate by the patients being dropsical or consumptive' (translation given by Lind [1757, p. 394]).

Other theories of the aetiology of scurvy dating from more recent times include, firstly, that of bacterial origin, which has been frequently advanced from different sources, and, secondly, the suggestion that the cause is chronic poisoning from putrefying meat or fish. Many adherents of the former theory [e.g. Coplans, 1904; Jackson and Moody, 1916] have brought forward some experimental support for their view, the explanation being, doubtless, that any animal in a scorbutic condition, due to dietetic deficiency, will be a ready prey to a secondary infection. The second view, first advanced by Torup [quoted by Jackson and Harley, 1900, 1] and supported by the work of Jackson and Harley [1900, 1, 2], received wide acceptance in recent times, especially among the leaders of some Arctic expeditions, with the result that the nature of the stores taken and diet arranged was modified accordingly. There is, however, little, if any, support for this view in the final experience of the expeditions, and the interpretation placed by Jackson and Harley upon their experimental results has not been accepted by their scientific colleagues [Holst and Frölich, 1912, p. 42].

A diet deprived of all antiscorbutic foods is generally one which affords an acid ash, and it was suggested by Wright [1900; 1908] that scurvy was caused by a condition of acidosis brought about by a diet deficient in organic salts of sodium and potassium. Wright found a decrease in the alkalinity of the blood of scurvy patients, some of whom responded favourably to treatment with alkalis, whereas Lamb [1902] was unable to confirm this. The

bicarbonate of the blood and plasma of scorbutic guinea-pigs is somewhat lower than that of normal guinea-pigs [Hess and Killian, 1918–19; Lepper and Zilva, 1925], but can be brought up to normal by the addition of sodium citrate to the diet without alleviation of the scorbutic symptoms. Moreover, scurvy can be cured by the administration of daily doses of concentrated fractions of lemon juice which contain only about 1 mg. of solid matter.

The pH of the blood of guinea-pigs does not decrease in scurvy (Lepper and Zilva), thus showing that acidosis does not occur.

The idea that experimental scurvy in guinea-pigs was due to chronic constipation [McCollum and Pitz, 1917; McCollum, 1918; Pitz, 1918] has been found to be erroneous [Chick, Hume and Skelton, 1918, 1, 2; Cohen and Mendel 1918; Cohen, 1917–18; Hess and Unger, 1918, 1; Harden and Zilva, 1918, 3] and has been abandoned by its originators.

Systematic experimental work on scurvy dates from the early part of the present century, when Axel Holst and his colleagues at the University of Christiania made a careful study of guinea-pig scurvy, its aetiology, symptoms, and methods of prevention and cure, and decided that it offered a complete analogy with the human disease. One or two centuries previously, when scurvy was an everyday occurrence and the common dread of all seamen, rough 'experiments' were from time to time made upon the human material so unfortunately provided. Thus Lind, in the middle of the eighteenth century, published the following account of an 'experiment', which expresses, as the result of his own careful observation, what became the common knowledge of his own day.

'On the 20th May, 1747, I took twelve patients in the scurvy, on board the "Salisbury" at sea. Their cases were as similar as I could have them. They all in general had putrid gums, the spots, and lassitude, with weakness of their knees. They lay together in one place, being a proper apartment for the sick in the fore-hold; and had one diet common to all, *viz.*, water-gruel sweetened with sugar in the morning, fresh mutton-broth oftentimes for dinner; at other times light puddings; boiled biscuit with sugar, etc., and for supper, barley and raisins, rice and currants, sago and wine, or the like.

'Two of these were ordered each a quart of cyder a day. Two others took twenty-five drops of *elixir vitriol*, three times a day, upon an empty stomach; using a gargle strongly acidulated with it for their mouths. Two others took two spoonfulls of vinegar three times a day upon an empty stomach; having their gruels and their other food well acidulated with it, as also the gargle for their mouths. Two of the worst patients, with the tendons under the ham rigid (a symptom none of the rest had), were put under a course of sea-water. Of this they drank half a pint every day, and sometimes more or less, as it operated by way of gentle physic. Two others had each two oranges and one lemon given them every day. These they ate with greediness, at different times upon an empty stomach. They continued but six days under this course, having consumed the quantity that could be spared. The two remaining patients took the bigness of a nutmeg three times a day of an electary recom-

mended by an hospital-surgeon, made of garlic, mustard-seed, *rad-raphan*, balsam of *Peru*, and gum myrrh; using for common drink, barley-water well acidulated with tamarinds; by a decoction of which, with the addition of *cremor-tartar*, they were gently purged three or four times during the course.

'The consequence was, that the most sudden and visible good effects were perceived from the use of the oranges and lemons; one of those who had taken them being at the end of six days fit for duty. The spots were not indeed quite off his body, nor his gums sound; but without any other medicine, than a gargarism of *elixir vitriol*, he became quite healthy before we came into Plymouth, which was on the 16th June. The other was the best recovered of any in his condition; and being now deemed pretty well, was appointed nurse to the rest of the sick.

'Next to the oranges, I thought the cyder had the best effects. It was indeed not very sound, being inclinable to be aigre or pricked. However, those who had taken it were in a fairer way of recovery than the others at the end of the fortnight, which was the length of the time all these different courses were continued, except the oranges. The putrefaction of their gums, but especially their lassitude and weakness, were somewhat abated, and their appetite increased by it.

'As to the *elixir of vitriol*, I observed that the mouths of those who had used it by way of gargarism, were in a much cleaner and better condition than many of the rest, especially those who used the vinegar; but perceived otherwise no good effects from its internal use upon the other symptoms. I indeed never had a great opinion of the efficacy of this medicine in the scurvy, since our longest cruise in the "Salisbury" from the 10th August to the 28th October, 1746; when we had but one scorbutic case in the ship. The patient was a marine (one Walsh), who after recovering from a quotidian ague in the latter end of September, had taken the *elixir vitriol*, by way of a restorative for three weeks; and yet at length contracted the disease, while under a course of medicine recommended for its prevention.

'There was no remarkable alteration upon those who took the electary and tamarind decoction, the sea-water, or vinegar, upon comparing their condition, at the end of the fortnight, with others who had taken nothing but a little lenitive electary and *cremor-tartar*, at times, in order to keep their belly open; or a gentle pectoral in the evening, for relief of their breast' [Lind, 1757, p. 56].

Holst and Frölich [1907; 1912] showed that scurvy could be induced in guinea-pigs by removing the greenstuff from the ordinary diet of grain and cabbage leaves, and by giving a diet consisting of grain and water only. It proved immaterial what grain was used, and the experiments included uncooked maize, oats, barley, and rice, as well as wheat and rye in the form of bread. In all cases this diet caused severe scurvy from which the animals died in from twenty to forty days, showing the haemorrhages and bony changes characteristic of the disease. By supplementing the scurvy diet with fresh vegetables, fruits, and fruit juices, these observers were able to maintain the animals in health. For example, scurvy was

prevented by a daily ration of 30 g. of fresh raw cabbage, dandelion leaves, sorrel, carrot, or cranberries. When these antiscorbutic materials were heated, their value was reduced by an amount depending on the temperature and time of heating; after heating at 100° C. for one hour (and in some cases for half an hour) the same ration was found inadequate to prevent scurvy. A similar result was obtained with dried materials; rations equivalent to 30 g. of raw carrot, dandelion leaves, or cabbage leaves failed to prevent scurvy when in the dried condition, and equally disappointing results were obtained with dried potatoes. The antiscurvy substance originally present in the expressed juices of vegetables suffered a similar fate rapidly on keeping, but the expressed juices of acid materials, e.g., lemons or sorrel leaves, were found to be more stable in this respect.

A further result of first-class importance was obtained by Fürst [1912] also working in Holst's laboratory, He found that, whereas dry cereals or pulses, e.g., oats, barley, lentils, peas, beans, were unable to prevent scurvy in the dry condition, if soaked in water and allowed to begin germination for 2–3 days they acquired antiscurvy properties. This result was confirmed by Chick and Hume [1917, 1], and Chick and Delf [1919] as regards pulses. In the case of cereals it was called in question by Weill, Mouriquand and Péronnet [1918], but has since been frequently confirmed (see Table XIII, p. 205).

Even this important result had been anticipated as long ago as 1782, for we find in 'An account of the Diseases of India as they appeared in the English Fleet' (by Charles Curtis, formerly Surgeon of the Medea Frigate, Edinburgh, 1807) the following passage (p. 41):

'With regard to sea-scurvy, reports and accounts have been published, as if this had been cured at sea, by lime or citron juice, lemon rob, nitre dissolved in vinegar, nitric acid and all which I would suspect to be rather something hyperbolical. But there is a plan for this purpose recommended by a *Mr. Young of the Navy*, which from its practicability and perfect conformity to known and established principles, is highly deserving of attention, and of being recorded in such a publication. It proceeds upon the well-known fact, that nothing more is necessary for the cure of this disease in any situation where there is tolerably pure air, than—not dead and dried, but fresh vegetable diet, greens or roots, in sufficient quantity. To be sure, we cannot have a kitchen garden at sea, and a short and scanty crop of greens can only be raised on board ship; *but beans and pease, and barley and other seeds brought under the malting or vegetating process, are converted into the state of a growing plant, with the vital principle in full activity throughout the germ and pulp* and if eaten in this state without any sort of preparation, except that of separating or rejecting the husks, cannot fail to supply precisely what is wanted for the cure of scurvy, *viz.*, fresh vegetable chyle.

'For this purpose, besides the articles mentioned, which may all be employed, he gives the preference for gram, an East Indian grain, chiefly used in the feeding of horses. The malting process is to be performed in shallow frames of wood, constructed so as to preserve

the water for successive operations; and a little experience will readily lead to the proper degrees of heat and moisture for conducting it successfully.'

From the researches of Holst and his colleagues we are enabled to make the following generalization. The antiscorbutic accessory factor is found in nature associated primarily with living plant tissues in which active metabolic processes are still proceeding. When these active processes cease or are greatly reduced, as in seeds, the antiscurvy vitamin also disappears. In the case of seeds it is created anew during germination. The distribution of this factor thus presents a marked contrast with that of the anti-beri-beri factor, of which one of the principal sources is found in dry seeds.

Effects of Vitamin C Deficiency.

Guinea-pigs as Experimental Animals.

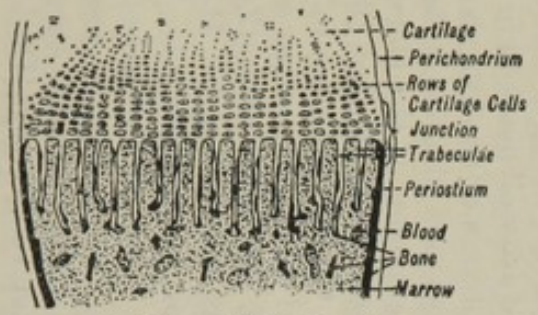
The symptoms of severe scurvy in guinea-pigs are briefly the following. Tenderness and swelling of the joints are the earliest sign, and the animal will frequently adopt a position (scurvy position), in which it rests on its side, while the affected member is held twitching in the air. In other cases the animal lies down with the side of its face upon the floor of the cage indicating that its gums and jaw have become tender ('scurvy face-ache position', see Fig. 30). Later on the teeth become loose, solid food is refused, and death may occur a few days later. In cases where the antiscorbutic given is only adequate for partial protection, the animal will usually live on for a considerable time, exhibiting swollen and painful joints, but growing up and enjoying a measure of health and spirits depending on the degree of protection afforded [see Delf, 1918].

In animals dying from scurvy the principal lesions at the post-mortem examination show close analogy with those characteristic of the human disease. Haemorrhages occur in any position, but are most frequent in the limbs. In cases where they occur in the intestinal tract blood is frequently passed in life, and death occurs suddenly. Rarification of the long bones is almost invariably present, and these frequently show fracture in the neighbourhood of the junction of shaft and epiphysis. The ribs are swollen and often fractured at the juncture of bone with cartilage. The teeth are loose and can easily be removed.

The histological changes produced at the rib junctions in the guinea-pig are very striking. These changes are gradually produced and become progressively more extensive as the period of deficient diet increases. Tozer [1918; 1921, 1, 2] distinguishes 'incipient', 'mild', 'chronic', and 'acute' scurvy, and these various degrees are illustrated by Fig. 31, ii–vi, the normal condition being shown in Fig. 31, i. No macroscopic changes occur for the first seventeen days, but about this time, even when the rib remains flat and normal in appearance, microscopic changes are apparent. 'The most noticeable departures from the normal are a variable amount of irregularity of the junction as a whole, slight disarrangement, and usually shortening of the rows of cartilage cells and of the trabeculae; an increased amount of blood in the marrow cavity, and a reduction

Fig. 30.

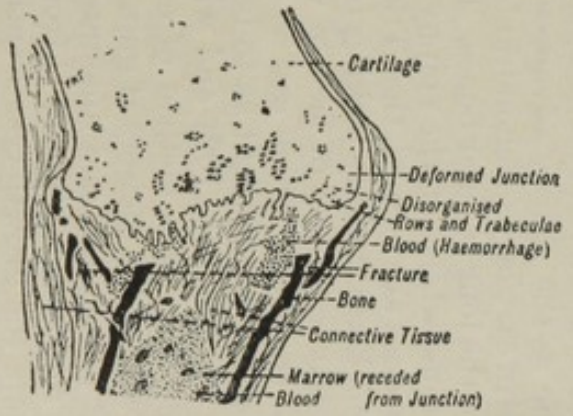
To face p. 190]



i. Normal Rib Junction.

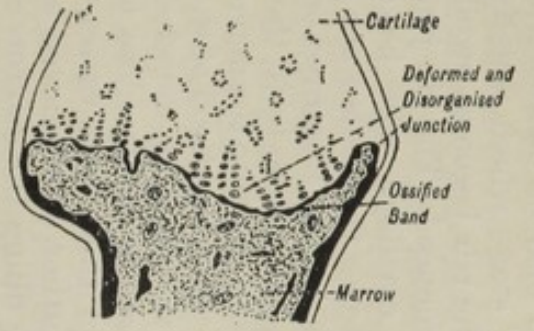
ii. Nearly Normal Rib Junction. 'Incipient Scurvy.'

iii. 'Definite Scurvy.'



iv. 'Acute Scurvy.'

v. 'Chronic Scurvy' (definite).



vi. 'Chronic Scurvy' (acute).

FIG. 31.[1]

[1] Reproduced by permission from the *Biochemical Journal*, 1918, 12, p. 446.

in thickness of the bone, especially near the junction' (Fig. 31, ii). This condition may be characterized as one of 'incipient' scurvy. In the 'acute' stage which usually supervenes after 18–35 days of deprivation the rib becomes swollen and the 'microscopic changes are more pronounced. The rib junction as a whole is disorganized, the rows of cartilage cells have lost their alignment and are scattered, and the trabeculae are shortened. The bone, which has become thin, is usually fractured on one or both sides of the junction, and, as a rule, quite close to it, the appearances suggesting that the cartilage is telescoped into the marrow cavity. There is haemorrhage, usually in the neighbourhood of the fracture, into the marrow cavity, and into the surrounding muscles. There is also a mass of tissue within the marrow cavity close to the junction and the fracture. The amount of this tissue varies considerably, from a few threads between the rather disorganized and possibly fractured trabeculae to a large plug filling the marrow cavity, and displacing the marrow from its contact with the junction for a considerable distance.' This tissue, the 'Gerüst Mark' of the German workers, was described by Tozer as fibrous tissue, but according to Höjer [1924] it is a less differentiated form of bone' (Fig. 31, iv).

Partial deprivation of vitamin C leads either to 'definite' or 'chronic' scurvy, in which the condition is intermediate (Fig. 31, iii, v, and vi). It may be pointed out that almost identical changes may be produced by deprivation of vitamin A when the guinea-pig is well supplied with antiscorbutic, and this renders it impossible in mild cases to ascertain from the histological examination whether the partial deficiency has been one of vitamin C or A. In acute cases, haemorrhage into the marrow cavity, fracture of the bone, and the presence of connective tissue in the region of the junction may be regarded as indicating scurvy, whereas atrophy of the bone marrow is characteristic of a deficient supply of vitamin A.

In general the membrane bone (endosmal bone) formation in scurvy of an early stage is both qualitatively and quantitatively reduced. Osteoporosis follows progressively 'and with that a new formation of a calcified bone of spongy character' which becomes more porotic as the scurvy advances. Analogous changes occur in bone formed in cartilage (enchondral bone) and are well marked in the epiphyses of the long tubular bones. The diaphyses of the long bones become porous, whilst at the epiphyseal junctions shapeless dense columnar masses are formed, the 'Trümmerfeldzone' of the German workers, which are entirely lacking in collagen fibrils [Höjer, 1924].

A very early symptom of lack of vitamin C is the change in the structure of the teeth, which can be detected when the other scorbutic symptoms are so slight as to be almost unrecognizable [Zilva and Wells, 1919] (see p. 102) and which has been used by Höjer [1926] as the basis of a method of estimation of vitamin C. The rate of growth of the incisors of guinea-pigs, which in animals on a normal diet is constant and amounts to 0·75 mm. a day, is greatly affected by removal of vitamin C from the diet and in total absence of the vitamin the growth ceases entirely after 15–20 days [Dalldorf and Zall, 1930].

The characteristic effect of scurvy on the tissues other than bones

is atrophy. Thus a general atrophy of the connective tissue occurs, specially attacking the collagen fibrils, and of the vascular walls, whilst the muscular and lymphoid tissue, the liver, salivary glands, adrenals, and kidneys undergo an atrophy combined with necrosis. These changes may be traced at a very early stage of the latent period of the disease [Höjer, 1924; see also Meyer and McCormick, 1927–28; K. D. Harris and Smith, 1928]. According to McCarrison [1921] and Brouwer [1927], however, the adrenals are enlarged; the lipoid content of their cortex is reduced [Peiper, 1922].

With regard to other histological effects of vitamin C deficiency, it may be noted that no marked change occurs in the blood picture [Bedson, 1921; Herzog, 1921; G. M. Findlay, 1921, 2], while the endothelium of the capillaries becomes swollen and degenerated, and there results a stagnation of blood in the capillaries which appears to be one of the factors leading to deficient oxygenation of the tissues and thus to death [G.M. Findlay, 1921, 1]. Both in acute and chronic scurvy definite lesions of a degenerative character occur in the bone marrow of guinea-pigs, and, possibly owing to these, guinea-pigs with chronic scurvy are less resistant to infection than normal animals [G. M. Findlay, 1923, 2]. On the other hand Zilva [1919, 2] found no diminution in the power to form agglutinins and amboceptor in scorbutic guinea-pigs.

The changes in the bone marrow and liver of scorbutic guinea-pigs persist for a long time after the animal has received a complete diet and consequently the symptoms of scurvy recur very rapidly when such animals are again deprived of vitamin C [Mouriquand, Michel and Bernheim, 1924, 1, 2].

Monkeys as Experimental Animals.

In many ways monkeys are more suitable animals to use than guinea-pigs for testing the antiscorbutic value of foodstuffs, since they are capable of taking much larger doses of substances of low antiscorbutic potency, whilst their daily requirement of antiscorbutic vitamin is almost exactly the same as that of guinea-pigs (see p. 195). Monkeys have proved, for example, of special value in the testing of milk, the protective dose of which for guinea-pigs is just at or beyond the receptive capacity of this animal. A convenient basal diet for monkeys consists of boiled polished rice (100 g. dry weight), wheat germ 30 g. and autoclaved milk 200 c.c. daily [Barnes and Hume, 1919, 1, 2; Harden and Zilva, 1919]. In experiments with milk, part of the autoclaved milk is replaced by the milk to be tested. On this diet monkeys develop scurvy in about 8–10 weeks, with symptoms closely resembling those of human scurvy. Such are sponginess and bleeding of the gums with loosening of the teeth (usually the earliest sign), and haemorrhage under the periosteum, into the orbit, and in almost any part of the body. There is weakness and ultimately almost complete powerlessness of the hind limbs, a condition which is characteristic of infantile scurvy and is apparently the result of haemorrhages into the hip and knee joints. The bones also become fragile and there is enlargement and disruption of the rib junctions. When the disease

is far advanced the whole body and limbs are tender. The effect of antiscorbutic deficiency upon the weight of the monkey is similar to that observed with guinea-pigs. Various genera and species have been successfully employed, but *Macacus rhesus* is probably the most suitable, although there is great individual variation.

Origin and Physiological Function of Vitamin C.

Origin.

The only important fact known about the origin of this vitamin is that it is produced during the germination of seeds [Fürst, 1912]. As regards its function in the plant or the nature of its precursor in the seed nothing has so far been ascertained. Its high concentration in such different parts of the plant as the leaves (cabbage), the fruit (lemons, &c.), and the root (swedes) renders it difficult to draw any definite conclusion either as to its function or mode of origin.

Requirements of Different Animals.

Animals derive vitamin C—directly or indirectly—from the vegetable kingdom. Different species of animal, however, vary greatly in their susceptibility to scurvy and in the amount of antiscorbutic vitamin which they require for growth and maintenance. Thus, while human beings, monkeys, and guinea-pigs are susceptible to scurvy, rats, mice, and adult rabbits do not show the characteristic symptoms of the disease, in spite of deprivation of vitamin C. It appears, however, that the difference between these two classes is one of degree only. It has been shown [Harden and Zilva, 1918, 4; Drummond, 1919, 1] that rats existing on a scorbutic diet do not thrive so well, and are not so fertile, as those which receive some antiscorbutic material, although Hartwell [1930] could not confirm this. Parsons [1920] has shown that the liver of the rat, and, to a much less extent, the muscle, contains vitamin C even when the animal has been kept for a long time on a scorbutic diet, and that this was also the case in the offspring of such rats reared on the same diet [Parsons and Hutton, 1924; Lepkovsky and Nelson, 1924]. It is evident that the rat requires vitamin C, but only in very small amounts compared with such animals as the guinea-pig and monkey. It is of interest that another rodent, the prairie dog of North America (*Cynomys ludovicianus*), resembles the rat, a young animal of 169 g. growing to a weight of 690 g. in six months when maintained on a diet (soy-bean flour and butter) on which guinea-pigs acquired scurvy in 2–3 weeks [McCollum and Parsons, 1920]. The rabbit receiving a scorbutic diet of oats, beans, and water generally loses weight and dies, but does not develop the characteristic symptoms of scurvy. Female rabbits became pregnant, but the young were born dead, and showed typical haemorrhages and bone lesions [G. M. Findlay, 1921, 3]. The addition of swede juice to the diet produced a great improvement in the condition of the animals.

The fowl is also able to subsist on a scorbutic diet and like the rat possesses under these conditions a store of vitamin C in the liver [Carrick and Hauge, 1925, where the literature is quoted; Hauge and Carrick, 1926, 2]. Calves [Thurston, Eckles and Palmer, 1926; Thurston, Palmer and Eckles, 1929] and pigs [Hughes, Aubel and Lienhardt, 1928] also thrive without any obvious source of vitamin C, although well-developed scurvy has been occasionally observed in pigs [Plimmer, 1920; Zilva, Golding and Drummond, 1924]. It is still doubtful whether these animals acquire the vitamin C contained in their livers by the accumulation of traces from their food or by chemical change from some source of which guinea-pigs are not able to avail themselves. Even among the animals which are subject to typical scurvy there are great differences in requirement. Thus, for protection from scurvy, a monkey weighing 2–3 kg. requires about the same daily dose of orange juice, 1·5 c.c. [Harden and Zilva, 1919, 1920, 1], or milk, 100–150 c.c. [Barnes and Hume, 1919, 2], as a guinea-pig of 250–300 g. A child of 8–10 kg. requires about 500 c.c. of milk or 10–15 c.c. of orange juice per day, so that its requirements are approximately the same as those of the monkey per unit of weight, whereas those of the guinea-pig are 5–10 times greater.

Göthlin [1931] has made use of a blood capillary resistance test (p. 266) to ascertain the minimum dose of antiscorbutic substances, such as orange juice, required to preserve a normal capillary resistance in adults. This was found by experiments on patients in an asylum, who were artificially fed, to be about 0·7–1·0 c.c. orange juice per kg. per day; or 42–60 c.c. for an adult weighing 60 kg.

To protect a young guinea-pig from the ordinary symptoms of scurvy a daily dose of 1·5 c.c. orange juice is needed, whilst 3 c.c. are required to prevent degeneration of the dental pulp. Assuming that the dose required to preserve normal capillary resistance is of the same order as that required to prevent degeneration of the dental pulp, it would follow that an average adult would require 14–20 times as much antiscorbutic as a guinea-pig. The requirements of a human adult for other foodstuffs can be roughly calculated by the use of these factors from the experimentally determined values for the guinea-pig, provided that the foodstuffs are such that their vitamin C is as readily absorbed in the human digestive tract as in that of the guinea-pig. On this basis a man would require per diem about 1000 c.c. milk, 200 g. of boiled new potatoes, 350 of old; 400 c.c. of carrot juice, 60 c.c. of tomato juice, &c.

Storage and Excretion.

Although vitamin C is present in muscle and tissue, it does not appear to be stored in the animal body to the same extent as vitamin A. Experiments with guinea-pigs [Hess, 1920, p. 75; Harden and Zilva, 1918, 2] show that a previous period during which large doses of orange or lemon juice (freed from acid) are administered, does not in any way increase the time necessary for the development of scurvy on a deficient diet. Some idea of the relative amounts stored by the guinea-pig and monkey may be gathered

from the fact that although the daily requirements are the same, the guinea-pig develops scurvy in three weeks, the monkey in two months: in the human being the period of development is six to eight months (p. 267).

No evidence of excretion of the antiscorbutic vitamin in the urine has been obtained [Van der Walle, 1922].

Effect of Vitamin C Deficiency on Metabolism.

In spite of much experimental work, it has not been found possible to establish any definite relation between the onset of scurvy and disturbed metabolism. As the disease progresses all the metabolic processes of the animal are disturbed, owing to the state of inanition which supervenes, and the changes reported by many observers must be attributed to this cause. If, however, any metabolic disturbance precedes or accompanies the onset of scurvy it should reveal itself in the guinea-pig in the early stages of the vitamin C deficiency, since scorbutic lesions are sometimes in evidence by the tenth day and microscopic changes in the teeth can be detected still earlier [Zilva and Wells, 1919].

Acting on this principle Shipp and Zilva [1928, 2] and Humphreys and Zilva [1931] have investigated the metabolism of nitrogen, calcium, and phosphorus by making daily observations of the intake and output of these elements by guinea-pigs on a scorbutic diet. The results for these three series of experiments were similar. No definite indications of a disturbed metabolism were found in the early stages of the disease. When the intake of food was diminished as the disease progressed disturbances occurred, the balance in every case becoming negative. Similar results were obtained in Japan by Nagayama and his co-workers, who also found no disturbance of carbohydrate metabolism [Nagayama, Machida and Takeda, 1928; Nagayama and Sato, 1928; Nagayama and Munehisa, 1929]. The experiments of Baumann and Howard [1917] on the metabolism of calcium, phosphorus, and nitrogen are vitiated by the low intake of the elements in question by the experimental animals, so that their conclusion that the negative balance observed is characteristic of scurvy cannot be accepted. Jarussowa [1928], whose animals were forcibly fed, observed a negative balance of nitrogen, but this did not become evident until the 24th day, long after the symptoms of scurvy had been manifested.

The calcium and phosphorus contents of the blood of guinea-pigs are very variable, but no definite change occurs in scurvy [Palladin and Ssawron, 1924; Edelstein and Schmal, 1926; Shipp and Zilva, 1928, 2]. The fall in blood-phosphorus noted during scurvy by Euler and Myrbäck [1925] was probably an accidental variation. No change in the calcium and phosphorus of the serum of guinea-pigs suffering from scurvy was found by Nagayama and Munehisa [1929].

The fatty tissues undergo no marked change in scurvy, but the cholesterol is decreased in lungs and testes, slightly in muscle and largely in the adrenals [Nagayama and Tagaya, 1929; Igarashi and Tagaya, 1929].

The excretion of lactic acid in the urine of scorbutic guinea-pigs was found to run parallel to that in the urine of controls kept on a restricted normal diet so that they lost weight at the same rate as the scorbutic animals, and no specific effect of scurvy was detected [Shipp and Zilva, 1928, 1].

According to Palladin and his colleagues, deprivation of vitamin C is accompanied by a rise in the blood-sugar and blood-amylase, which attains its maximum at the end of the second week and then gradually falls off [Palladin, 1924]. This change in the blood-sugar due to scurvy could not be confirmed by Lepper and Zilva (unpublished results). Other blood ferments (catalase, protease, esterase, and peroxidase) are scarcely affected in the early stages of vitamin C deficiency [Palladin and Normark, 1924]. The creatine of the muscles steadily increases, whereas in starvation it first increases and then decreases [Palladin and Kudrjwzewa, 1924]. At the same time creatine appears in the urine and steadily increases in quantity, as does also the proportion of creatine- and creatinine-N to the total N of the urine. Near the end of the scorbutic period, as death approaches, the muscles contain comparatively little creatinephosphoric acid, whereas the lactacidogen is increased [Palladin and Epelbaum 1929].

Concentration of Vitamin C.

The methods employed are based on the facts that the greater part of the vitamin is precipitated by basic lead acetate [Zilva, 1924, 3], i.e. by lead acetate in the slightly alkaline region, and that impurities can be previously precipitated by treatment with the normal salt [Bezssonoff, 1922, 1; Zilva, 1925], with alcohol [Harden and Zilva, 1918, 2; Zilva, 1924, 3], and by fermentation of the sugars in the solution with yeast [Zilva, 1924, 1; Lepkovsky *et al.*, 1925; Grettie and King, 1929]. Owing to the great instability of the vitamin (see p. 199) and the variations in the character of the medium, preparations of very variable activity are often obtained even from the same natural source.

Lemon juice forms one of the best sources for the concentration of vitamin C. Chemical and physical studies have afforded some information as to the conditions governing the concentration of the active principle [Zilva, 1924, 1, 3; 1925; 1927, 1, 2; 1928; 1929; 1930; Daubney and Zilva, 1926; Hoyle and Zilva, 1927]. The best results were obtained by Zilva by using the following procedure. The bulk of the organic acids are removed from the lemon juice by the addition of an excess of precipitated chalk and the mixture is allowed to stand for about one hour. The liquid is then filtered through a Büchner funnel and a saturated solution of normal lead acetate is added. The proper quantity to be used can only be ascertained by trial and must be such that the final preparation has a reducing power to phenolindophenol (0·02 per cent. solution) of not less than 6 c.c. for an equivalent of 5 c.c. of the original decitrated juice (see p. 199). The quantity varies greatly with the individual sample and unless the right amount of the reagent is used

at this stage, there may be a great loss of activity in the final preparation. After immediate filtration through a Büchner funnel or centrifugation, the liquid (pH 5·0 – 5·4) is brought to pH 7·2 – 7·4 by addition of a 6 per cent. solution of ammonia. The precipitate which contains the vitamin is quickly centrifuged and taken up in 10 per cent. acetic acid. The lead is then removed by the addition of just sufficient saturated magnesium sulphate (3–5 c.c. for 50 c.c. of decitrated juice) and subsequently of 2 volumes of 96 per cent. alcohol. After filtration the solution is concentrated to a small volume in order to remove the greater part of the acetic acid. An equivalent of 1·5 c.c. of the original juice (i.e. a daily protective dose for a guinea-pig), prepared daily in small quantities and administered very soon after preparation to young guinea-pigs, is sufficient to keep the animals alive for periods of about 60 days, although scorbutic symptoms may be present. Considering the risk of loss in activity involved in the manipulation, it would appear that the greater part of the antiscorbutic potency of the juice can be obtained in the final fraction by this procedure.

The active lead precipitate may be dissolved in acetic acid and reprecipitated at pH 7·2 – 7·4 [Grettie and King, 1929; Sipple and King, 1930]. Care must, however, be taken that the minimum amount of acetic acid is used, and it is found that the reprecipitation does not necessarily lead to further concentration, as the loss of activity may more than balance the removal of inactive matter [Zilva, 1931]. Grettie and King [1929] and Sipple and King [1930] have also introduced several minor modifications into the process without materially increasing the degree of concentration.

It is possible by the above method to effect such a concentration that the protective dose for a guinea-pig (the equivalent of 1·5 c.c. of lemon juice) may contain about 0·5–1 mg. of solid matter, whereas 1·5 c.c. of the original juice contains about 135 mg. The result depends on the character of the juice, since the same procedure may yield different figures with different samples.

The purest fraction so far obtained is undoubtedly a complex mixture of substances. Concentrated preparations have been found to contain phosphorus, nitrogen [Zilva, 1924, 3], amino-compounds and amides [Zilva, 1925], iron, sulphur [Hoyle and Zilva, 1927], and iodine, the last of which can be separated from the active principle by dialysis [Daubney and Zilva, 1926]. These preparations possess strong reducing properties (see p. 199), give a faint coloration with ferric chloride and a faint cloudiness with bromine water [Grettie and King, 1929]. The amide nitrogen has no relation to the antiscorbutic activity of the lemon juice [Zilva, 1925] and probably the other substances mentioned above are also impurities.

The process detailed above for lemon juice can be applied to swede juice [Zilva, 1925]. Bezssonoff [1925] used a similar method with cabbage juice in which the active principle was reprecipitated several times with lead acetate and extracted with alcohol and with acetone. He claimed to have obtained a crystalline material which was protective for adult guinea-pigs in doses of 2 mg. Zilva [1930] was unable to confirm this work.

The statement has been made by Rygh *et al.* [1931; 1932, 1, 2;

Laland, 1932] that substances possessing high antiscorbutic activity can be obtained from an opium alkaloid, narcotine, by ultra-violet irradiation or by chemical methods. Unripe *Citrus* fruits are said to contain narcotine, which disappears on ripening, giving rise to vitamin C.

Partial demethylation of narcotine (from opium) yielded an *o*-diphenol which, it is stated, possessed a high degree of antiscorbutic activity.

A repetition of the experiments with partially demethylated narcotine by Smith and Zilva [1932] has yielded negative results and the conclusions of Rygh *et al.* cannot be considered as established.

Conditions of Stability of Vitamin C.

Vitamin C is very sensitive to oxidizing agents and to air [Delf, 1920; Hess, 1920; Hess and Unger, 1920–21; Zilva 1921; 1922; Dutcher, Harshaw and Hall, 1921]. Inactivation takes place rapidly in alkaline solution in the presence of air [Holst and Frölich, 1912; Harden and Zilva, 1918, 6; Hess and Unger, 1919], but not under anaerobic conditions [Zilva, 1923, 1]. Thus at pH 12·5 decitrated lemon juice lost 80 per cent. of its potency on exposure to air for half an hour and the whole of it in 3 hours, whereas when preserved anaerobically for 24 hours it had not appreciably decreased in potency. The antiscorbutic activity of a juice at pH 2·2 (the natural acidity of lemon juice) was decreased by aeration for 1 hr. at 100°, but to a much less extent than that of juice at pH 6·2–6·6.

Evidence is accumulating to show that the inactivation is not direct. Lemon juice, and other sources of vitamin C, and active preparations derived from them, possess reducing properties. Even the purest antiscorbutic fractions reduce ammoniacal silver nitrate in the cold and decolorize potassium permanganate [Zilva, 1924, 3]. Conditions favourable to the preservation of the vitamin are favourable to the preservation of these reducing properties of the active solution, but the destruction of the antiscorbutic activity and of the reducing properties proceed at different rates [Connell and Zilva, 1924, 1]. Phenolindophenol is also rapidly reduced in the air to its leuco-base by decitrated lemon juice and other sources of vitamin C, and by using this indicator Zilva [1927; 2; 1928; 1929; 1930] studied titrimetrically the behaviour of the reducing power of active solutions in relation to their antiscorbutic activity. The reducing capacity, like the antiscorbutic factor, is destroyed in alkaline medium in the presence of air, and when the active solution is aerated. When, however, phenolindophenol is added to decitrated lemon juice until the indicator is no longer reduced and the solution administered to the test animals *immediately*, no very appreciable loss in antiscorbutic activity is detected, but if this solution is administered after a few hours, the presence of the vitamin cannot be detected. If the quantity of the indicator is insufficient completely to destroy the reducing capacity, the leuco-compound formed is re-oxidized in the air, and the resulting colour base is again reduced by the active solution until the reducing power is entirely destroyed.

That this reducing substance is not the vitamin itself is also shown by the fact that the precipitate formed in decitrated lemon juice by normal lead acetate at pH 5·0–5·4 (which is antiscorbutically inactive) reduces phenolindophenol even more readily than the active fraction obtained with the same reagent at pH 7·2–7·4 [1927, 2]. The latter fraction, which has a lower reducing capacity than its equivalent of decitrated lemon juice, is also less stable than the original juice [1928] and when its reducing capacity for the indicator falls below a certain level it loses its antiscorbutic activity [1930].

All this evidence suggests that the 'phenolindophenol-reducing principle' exerts a protective action on the vitamin. It is not yet known whether the inactivation of the antiscorbutic factor which takes place rapidly in the absence of the reducing principle is itself due to oxidation or to some other change. There is the possibility that vitamin C is composite in the sense of an enzyme and co-enzyme, but experimental evidence so far obtained does not support this view. Thus if the 'phenolindophenol-reducing principle' obtained by precipitation with normal lead acetate, which is free from vitamin C, is added to the 'active' fraction in which the reducing power and consequently the antiscorbutic potency have been destroyed by mild oxidation, the vitamin activity is not restored [1930].

Zilva's results with respect to the behaviour of the reducing substance to colouring matters have been confirmed by Tillmans and P. and W. Hirsch [1932, 1, 2], but they claim, in disagreement with him, to have found that the reducing power and antiscorbutic potency of lemon juice and preparations from it are always proportional. When the reducing power is completely removed by the addition of a colouring matter they ascribe the residual antiscorbutic power to the action of the primary oxidation products formed. On standing and on further oxidation these are slowly converted into some substance or substances without antiscorbutic potency. They conclude that the action of vitamin C is due to the reducing substance and its primary oxidation products.

Zilva, however, as explained above, has frequently obtained fractions of high reducing power which were devoid, or almost devoid, of antiscorbutic potency and these observations invalidate the conclusions drawn by Tillmans and P. and W. Hirsch from their experiments.

Another series of observations made by Zilva in these investigations has a bearing on the destruction of the reducing power and the inactivation of vitamin C. If decitrated lemon juice in neutral or acid medium is heated in an autoclave anaerobically at 115° C. for one hour, a certain degree of diminution takes place both in the antiscorbutic activity and in the capacity for reducing phenolindophenol, although the reducing capacity for iodine is increased by this treatment [1929]. Such heated juice when stored under neutral, but not acid, conditions loses its vitamin C and phenolindophenol-reducing capacity much more quickly than unheated lemon juice [1927, 1928]. The autoclaved juices are also capable of oxidizing *p*-phenylenediamine [1929]. The addition of autoclaved to unheated decitrated juice also accelerates the destruction both of the 'phenolindophenol-reducing principle' and of the vitamin, as also does the

addition of an ethereal extract from autoclaved decitrated lemon juice, or of quinol, quinhydrone, benzoquinone [1929; 1930] or catechol (unpublished results). It would therefore appear that in the process of autoclaving a substance is formed, possibly of a phenolic character, which promotes the destruction (either directly or after previous oxidation by exposure to air) of the 'phenolindophenol-reducing principle' and eventually of the vitamin. It is also probable that the reducing properties of antiscorbutic solutions may be due to several substances, some of which, like the 'phenolindophenol-reducing substance', may act as protective agents for the vitamin, whilst others such as the 'phenolic substances' may, under certain conditions, acquire inactivating properties.

Decitrated lemon juice can be preserved aerobically at pH 1·0–0·6 or at pH 7·0 for a week with no significant loss of potency [Zilva, 1930]. It is stated by J. Williams and Corran [1930] that the potency of acidified lemon juice at a lower pH than 1·8 (pH 1·1, 1 and 0·6) disappears completely in 7–8 months at 15–18° C. The latter authors have also found that antiseptics, in concentrations capable of preserving lemon juice at ordinary temperatures from mould growth, &c., have, as a rule, a very deleterious effect on the stability of vitamin C.

Bezssonoff [1921, 2; 1922, 2; 1923] found that vegetable extracts containing vitamin C give with a solution of phosphomolybdotungstic acid a blue coloration which is also given in great intensity by quinol. He traced a marked parallelism between the antiscorbutic potency of such extracts and the intensity of the blue reaction and suggested that the antiscorbutic substances probably contained an unstable radical which could be split off in the form of quinol and that this reaction could be utilized as a test for vitamin C. On the other hand, it was found by Kay and Zilva [1923], that although the parallelism found by Bezssonoff undoubtedly exists in many cases, the substance giving the blue coloration can be removed by adsorption with fuller's earth from lemon juice, leaving a liquid which gives no reaction with the reagent but possesses antiscorbutic properties. Further, substances not containing vitamin C, such as yeast, gave a positive reaction. Wedgewood and Ford [1924] have also found that the colour reaction is not specific for vitamin C. It is more likely that the colour is given by some phenolic substances accompanying the antiscorbutic factor in its natural sources, since the reagent is a modification of Folin's reagent for phenol.

Our present knowledge of the conditions of stability of vitamin C makes it plain that the preparation of active concentrates by chemical fractionation on a large scale and the preservation of the activity of such preparations are at present impracticable. It is, however, possible to concentrate natural antiscorbutic juices under carefully controlled conditions without incurring great losses of the vitamin and also to preserve such preparations for a considerable time (see p. 255).

Chemical and Physical Properties of Vitamin C.

As already mentioned the purest preparation of vitamin C so far obtained is a complex mixture. Information is, however, available concerning the physical and chemical behaviour of the active principle under various conditions.

Vitamin C dissolves readily in water and in all strengths of alcohol [Harden and Zilva, 1918, 1; Hess and Unger, 1918, 2; Hart, Steenbock and Lepkovsky, 1922]; in methyl alcohol, but not in butyl alcohol, light petroleum, &c. [Hart, Steenbock and Lepkovsky, 1922]. It diffuses through a collodion membrane of such permeability that it permits the passage of methylene blue, neutral red, and safranine; membranes of lower permeability do not allow its diffusion [Zilva and Miura, 1921, 1]. The diffusion proceeds differently from that of the sugar and the nitrogenous substances present in decitrated lemon juice [Connell and Zilva, 1924, 2]. The 'phenol-indophenol-reducing substance' dialyses, like the vitamin [Zilva, 1928]. Lemon juice can be filtered through a Berkefeld filter without loss of potency [Harden and Zilva, 1918, 1], but the vitamin C is partially retained by a Chamberland candle [Ellis, Steenbock and Hart, 1921]. It is not adsorbed from neutral solution by fuller's earth [Harden and Zilva, 1918, 1], or by colloidal ferric hydroxide (dialysed iron), but is taken up from orange juice by blood charcoal [Ellis, Steenbock and Hart, 1921]. When decitrated lemon juice is treated with adsorbents such as 'norite' or fuller's earth, a varying degree of diminution takes place in the vitamin C content of the supernatant solution, which is probably due to oxidative changes associated with the adsorbent [Zilva, unpublished results]. No convincing evidence of the existence of more than one antiscorbutic principle has hitherto been advanced, although the early investigators were led by the varying behaviour of different foodstuffs towards heat and drying to suggest that several such substances might exist. The experimental evidence in favour of this idea adduced by Scotti-Foglieni [1926, 1; 1927; 1928], Bezssonoff [1926, 2; 1927], and Randoin and Lecoq [1927, 1] is open to criticism, and has not been confirmed.

The effects of heat and oxidation on vitamin C have already been discussed (p. 199).

Estimation of Vitamin C in Foodstuffs.

The researches of Holst and his co-workers on the distribution of vitamin C were continued in 1916 at the Lister Institute, with the aim of making a more complete survey of the commoner foodstuffs and assigning to each some quantitative value as regards antiscurvy properties [Chick and Hume, 1917, 1]. Young, growing guinea-pigs (about 350 g. weight) were employed, the methods used being in essential those of Holst and his colleagues. The principal modification was the substitution of water in the scurvy diet of oats, bran and water by a daily ration of about 60 c.c. of strongly heated milk (autoclaved at 120° C. for one hour), to supply vitamin A [see Hume, 1921]. This addition of milk very greatly improves

the general condition of the experimental animals, although the onset of scurvy is not thereby seriously influenced.

Another suitable diet is that used by Zilva [Bracewell, Hoyle and Zilva, 1930, 2] consisting of—

Bran	6	parts	by	volume
Barley meal	2	„	„	„
Middlings	3	„	„	„
Fishmeal	1	„	„	„
Crushed oats	4	„	„	„

which was offered *ad lib.*, along with 40-60 c.c. of milk reconstituted from a dried powder and autoclaved.

The use of autoclaved milk in the basal diet in such experiments has been criticized on insufficient grounds by Hess and others for the reason that such milk might retain a small and variable amount of antiscorbutic. To avoid the use of milk Sherman and his colleagues employed a ration of oats, 59 per cent.; skimmed milk powder heated in open trays at 110° C., 30 per cent.; butter fat, 10 per cent.; and salt 1 per cent. This diet they found to be taken well by the animals [Sherman, La Mer and Campbell, 1921; 1922]. Eddy later [1929] substituted 1 per cent. of cod-liver oil for the equivalent amount of butter fat and added yeast, so as to ensure a good supply of vitamins D and B. Other scorbutic diets which have been suggested to avoid the use of milk are those of Lopez-Lomba and Randoin [1923, 1, 2]—meat peptone, starch, yeast, butter fat, salt mixture, and filter paper—and of Bezssonoff [1926, 1]—oats, bran, yolk of egg, and yeast.

On a diet deprived of vitamin C, but containing all other essential elements, the guinea-pig shows normal growth for the first 15–20 days of the experiment; with onset of scurvy symptoms, about the twentieth day, the weight of the animal begins to decline, death from acute scurvy ensuing about the thirtieth to fortieth day.

To obtain quantitative results it is necessary to determine the minimal amount of the various foodstuffs that must be added daily to the 'scurvy' diet in order to prevent occurrence of scurvy. When this minimal amount (or more) is consumed, growth does not cease about the twentieth day, but the animal continues to grow in good health without symptoms of scurvy. As a general rule the animals should be kept under observation during three months.

In assessing the results of such experiments several interdependent factors have to be considered, namely, duration of test period, extent of growth, cause of death, first appearance of scorbutic symptoms, and degree of scurvy at death as determined by post-mortem examination. The relative importance of these observations in the assessment of the potency of a dose often varies with the condition of the individual animals.

Sherman and his colleagues attempted to institute a quantitative estimation of relative antiscorbutic potency based on the amounts of two or more materials required to provide the same *degree* of protection. Their experiments were made with filtered juice of canned tomatoes, and were employed for estimating the loss of

antiscorbutic potency undergone by this on further heating. A detailed tabulation was made of the symptoms produced by the specific doses of the juice and these were compared with the symptoms of the animals receiving the heated juice. Thus 3·9 c.c. of juice heated for an additional hour at 100° C. produced symptoms judged to be of the same degree of severity as those present in the animals receiving 2 c.c. of original juice, so that the relative antiscorbutic power of the former juice was in this case $\frac{2}{3 \cdot 9}$ or about 50 per cent. [see also Kenny, 1926].

The chief source of uncertainty in such quantitative experiments has not been found to lie in the nature of the basal diet used, but in the clinical variations of the individual experimental animals and in the indefinite nature of the 'end-point'. Complete protection from scurvy as used by some observers or a 'definite degree of partial protection', as employed by Sherman, are in practice equally difficult to define with accuracy, and it is rarely that differences of less than about 50 per cent. of the dose can be shown to have a definite effect.

Höjer's method. An entirely different principle is employed by Höjer [1926], who determines the degree of scurvy by means of a histological examination of the teeth. His method consists in giving daily doses of the material to be tested, two control animals being kept on the basal diet alone and two on the basal diet with a fully protective dose of a known antiscorbutic. After a period of 10–14 days all the animals are killed. The lower jaw is examined histologically and from a consideration of the changes (see p. 102) Höjer claims not only to be able to determine the minimum fully protective dose with an error of not more than 10 per cent., but to determine quantitatively with moderate accuracy the degree of protection afforded by doses which only afford partial protection. This method has the advantage that it only requires three weeks for a determination in place of the 2–3 months required by the older method. The dose required for complete protection as judged by this method is about twice as large as that required by the usual method [see Goettsch, 1928].

A modification of Höjer's method has been described by Key and Elphick [1931] in which, using the same criterion, four degrees of scurvy are distinguished, the antiscorbutic potency being determined in terms of the dose of a standard antiscorbutic substance which would induce the same degree of scurvy.

As a material to be used as a standard of reference in the biological estimation of vitamin C, the (League of Nations) International Vitamin Conference [1931] has adopted fresh lemon juice, this source of the vitamin having been found remarkably constant. This should be decitrated and used immediately (see Appendix II).

Distribution of Vitamin C in Foodstuffs.

Tables XIII and XXI (see Appendix I) show the approximate antiscorbutic values of a series of foodstuffs, the values being based upon the minimal protective doses as determined for guinea-pigs.

The sign + indicates that the vitamin is present, + + that the material is a good source, and + + + that it is a rich source of the vitamin; o indicates that the vitamin has not been detected by the method employed.

Table XIII

Distribution of Vitamin C in Foodstuffs.

Foodstuffs.	*Antiscorbutic value.*	*References.*
Cereals.		
Wheat, whole grain	o	1
Germ		
Bran	o	
Endosperm, e.g. white wheaten flour, polished rice	o	
Germinated Kafir corn (*Sorghum vulgare*)	+	2
Barley	o	3
,, sprouted	+	3, 4
Malt, green	+ +	5
,, kilned	o	6
,, extract	o	7, 8, 9
Oats	o	3
,, sprouted	+	3
White flour and bread	o	1
Maize (young cobs, green mealies)	+	10
Vegetables.		
Asparagus, raw	+ + +	11
,, boiled	very low	11
Beetroot	+	13, 32
,, juice	+	12
Cabbage, raw, fresh green leaves	+ + +	14
,, cooked 1 hr. at 100° C.	+	14
,, dried	+	15
,, canned	+ +	11, 16, 17
Sauerkraut	o to + +	11, 42, 43, 44
Carrot, young raw	+ +	11, 18
,, old raw	+	18
,, young cooked	+	18
,, old cooked	+	18
,, dried	+	18
,, juice	+	12
,, young canned	o	19
Cauliflower, raw	+ + +	11
,, boiled	+ +	1, 11
Celery, raw	+ +	11
Cucumber	+ +	20
Dandelion	+	1
Endive	+	1
Lettuce	+ + +	1, 11
Mangold leaves	+ + +	11, 21
Mangold	+	
Onion	+ to + +	22
Pepper, green	+ + +	23
Potato, raw	+ + to + + +	11, 24
,, cooked 1 hr. at 100° C.	+	11, 24
,, cooked 15 mins	+ +	11, 24
Pumpkin, juice and pulp	+	2
Rhubarb	+ +	11, 25
Sorrel	+	1

Foodstuffs.	*Antiscorbutic value.*	*References.*
Vegetables (*cont.*)		
Spinach, raw	+ + +	11, 19, 26, 27, 28
,, cooked	+ to + +	11, 19, 26, 27, 28
,, canned	+ to + +	19, 26, 27, 28
Sugar cane juice	o	2
Swede (Rutabaga) juice	+ + +	12, 29, 30
Sweet potato juice	+ +	2, 41
Turnip tops	+ +	31, 33
,, root	+ +	13, 32, 33
Vegetable marrow juice	+ +	2
Watercress	+ + +	34
Desiccated vegetables as a class	o to +	1
Pickled cabbage	o	22
Legumes.		
Beans, green pods		
,, runner		
,, string	+ to + +	16, 23, 11
,, French		
,, ,, canned	doubtful	16
,, haricot	o	11
,, ,, sprouted	+ +	35
Cow peas (*Vigna sinense*) sprouted	+ + +	2
Lentils soaked	+	36
,, germinated	+ +	36
Peas, green, raw	+ + +	11, 37
,, ,, canned	+ +	11, 37
,, dried and then soaked	+	35, 36
,, germinated	+ +	35, 36
Soy beans	o	3
,, sprouted	doubtful	2
Mushrooms	o	11, 39, 40
Tea infusion, green	+ +	49
,, ,, black	very low	49
Eggs.		
Fresh, whites	o	22, 38
,, yolks	o	22, 38
Dried	o	22
Meat, etc.		
Muscle, lean	very low to +	45, 46
Liver, rabbit or pig	+ +	47
,, chicken	+	48
Meat juice, raw	very low	22
,, canned	o	22
Oysters	+	50
Milk.		
Cow's, whole, raw	+	51, 52, 53, 54, 55
,, whole, pasteurized	probably lower than raw, depending on the method used.	56
,, whole, rapidly scalded	very little less than raw.	58
,, ,, sterilized at 120° C.	about 50% of value of raw milk.	51, 52, 59
,, condensed, sweetened	+	60
,, ,, unsweetened	50% value of raw milk.	59
,, whole, dried	o to +	58, 61, 62, 63
,, ,, raw irradiated	o to +	64, 65
,, ,, dried ,,		
,, skim, raw	+	57
,, ,, dried	o to +	57

Foodstuffs.	*Antiscorbutic value.*	*References.*
Milk (*cont.*)		
Goat's, raw	+	66, 67
Human, raw	o to +	68, 69
Lactic acid milk	+	70, 71
Lactose	o	72
Beer.		
Kafir, as drunk in turbid condition containing residues of malt and yeast	+	2
Spruce infusion	+	73
Pale ale	o	5, 6
Honey	o	75, 76, 77
Fruits.		
Almonds, sprouted	+	35
Apples, raw	+ to + + (depending on variety).	78, 11, 79
,, baked	same as raw	79
,, canned	+ to + +	80
,, dried	very low	78
,, concentrated juice	+	81
Apricot, fresh	o to +	11, 113
,, dried	o	113
,, sulphured and dried	+	113
Bananas, raw	+ +	78, 82
,, baked	+ to + +	78, 83
,, dried	o to +	
Blackberry	+ +	11
Bilberry (*Vaccinium myrtillus*)	+	11
Cherries	+ to + +	11
Cloudberries (*Rubus chamemorus*)	+	1
*Cocum (dried)	+	85
Currants, fresh	+ +	11
Cranberry (juice)	very low	1
Gooseberries	+ +	11
Grape	very low	11, 12
,, juice dried	o	84
,, ,, concentrated	o	2
Grape fruit juice	+ + +	82, 86
,, ,, ,, dried	+ +	84
Lemon juice, fresh	+ + +	87, 88
,, ,, concentrated or dried	+ + +	89, 90
,, ,, preserved	o to + +	88
,, ,, tablets	+ +	91
,, peel (flavedo)	very low	92
Lime juice, fresh	+ +	87, 88
,, ,, preserved	o to +	87
,, ,, concentrated	+	81
Mango, fresh	+ + to + ± +	116
* ,, dried (Amchur)	low	85
Melon (cantaloup)	+ +	105
Mulberry	+	1
Naartje (*Citrus nobilis* Lourerio)	+ + +	2
Orange juice, fresh	+ + +	88, 12, 86
,, ,, dried	+ + +	93, 84
,, ,, concentrated	+ + +	94, 95
,, marmalade	very low	2
,, peel	+ +	96
,, ,, dried	+	96
,, ,, outer	+ +	97
,, cake (dried minced orange)	o to +	2
Paw-paw (*Carica papaya*)	+ + +	2, 98
Peach and peach juice	+ to + +	2, 11
,, canned	+ to + +	99, 100

*Dried fruit much esteemed as antiscorbutic by natives of India.

Foodstuffs.	*Antiscorbutic value.*	*References.*
Fruits (*cont.*)		
Peach, dried	+	101
Pear	+	11, 100
,, canned	o to +	100, 102, 115
Pineapple fruit and juice	+ +	2, 103
,, canned	+ +	103
Plums	very poor to + +	11
Prickly pear (*Opuntia decumana*)	+ +	2
Prunes, fresh	+	113
,, dried	o	96, 113
,, sulphured and dried	+	113
Raisins	o	104
Raspberry	+ +	1, 11
,, juice dried	o	84
Strawberry, raw and preserved	+ + to + + +	11, 106
*Tamarind, dried	low	85
Tomato, raw	+ + +	2, 107, 108, 109, 110, 111
,, dried	+ +	2, 107
,, canned	+ + to + + +	111, 110, 112
Commercial dried fruits, except some peaches	o	101, 113, 114

*Dried fruit much esteemed as antiscorbutic by natives of India.

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64. Hottinger, 1927, 2
65. Supplee and Dow, 1927
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Vegetables. Among *green-leaf vegetables* the cabbage [Delf, 1918] and watercress [Coward and Eggleton, 1928] are two of the richest sources of vitamin C. It is also present in sorrel, scurvy grass (*Cochlearia officinalis*), lettuce, spinach, turnip tops, and other leaves, and in grasses. The perennial rye-grass (*Lolium perenne*) is as good a source as cabbage [Brouwer, 1927], but the antiscorbutic potency to a large extent disappears when it is converted into hay or silage [see also Lepkovsky *et al.*, 1925]. Hay, as a rule, contains but little of the vitamin and has been used by American investigators as one of the components of a control diet on which guinea-pigs acquire scorbutic symptoms in 2–3 weeks [see Hess and Unger, 1918, 1]. Rossi [1918], however, found fresh hay to possess antiscorbutic properties.

Among *root vegetables* there are great differences, raw carrot juice (minimum dose about 20 c.c.) and raw beetroot juice (minimum dose more than 20 c.c.) proving comparatively feeble, while raw swede juice is placed in the front rank, the minimum protective daily dose for a guinea-pig being 2·5 c.c. [Chick and Rhodes, 1918].

Neither the carrot nor its expressed juice can be regarded as a good source of antiscorbutic, but accurate quantitative experiments with the cooked vegetable have not so far been made. Hess and Unger's results [1919] are summarized in Table XIII, but the minimum doses were not ascertained. Young carrots were found to be decidedly more potent than old. Using an extract of fresh carrot made with absolute alcohol the equivalent of 25–50 g. of fresh carrots was required daily for the protection of a guinea-pig [Zilva, 1920, 2].

The potato would appear to occupy a mean position. The most extended investigations of this vegetable are due to Givens and McClugage [1920], who examined it raw, cooked, and dried under different conditions, and to Scheunert [1930]. The latter found 3 g. of raw new white potatoes or 4 g. of the same baked, to be a protective dose, the presence or absence of the peel making no difference. Old potatoes which had been stored for a year were equally good. Drying in the air (35–80° C.) destroyed most of the vitamin, but a considerable proportion of it remained when the

potatoes were first steamed for 4 minutes or baked in their skins for 45–55 minutes, and afterwards dried [Givens and McClugage, 1920].

It is interesting to note that the cabbage and swede are nearly allied species, and belong to the same natural order of plants, viz. the Cruciferae. The old legend teaching that plants with a cruciform arrangement of the flower possess special virtue in the service of mankind thus receives scientific support at this late date.

Fresh fruit. Many fruits have been examined for antiscorbutic potency and in a number of cases the minimum protective dose has been determined, but so far little systematic study has been carried out as to the variation of vitamin C content with degree of maturity, mode of cultivation, variety, &c. Information of this nature is available only in the case of the apple, orange, and grape fruit.

Citrus fruits. Many of the *Citrus* fruits—orange, lemon, grape fruit—are highly active, the minimum protective dose for a guinea-pig being 1 to 1·5 c.c. daily of the juice.

It is surprising to find that the juice of West India limes (*C. medica* var. *acida*) is distinctly inferior to that of oranges and lemons and of the sweet lime (*C. limetta*), but such it has proved to be (see p. 254). The experiments on this point included tests with monkeys as well as guinea-pigs. Ordinary preserved lime juice was found to be almost devoid of antiscorbutic properties [Chick, Hume and Skelton, 1918, 3], but preserved lemon juice appeared to be distinctly more satisfactory. Orange juice retained its antiscorbutic potency well for 16 to 19 months when preserved at room temperature in presence of a small proportion of the oil of the rind, whereas at 37° C. in the same time it became practically inactive. Lemon juice behaved in much the same manner at these temperatures, and also retained its potency in presence of the rind oil at 0° C. Potassium metabisulphite ($K_2S_2O_5$) preserves the juice well against fermentation and mould growth, but at room temperature exerts a destructive action on the antiscorbutic potency, even 0·02 per cent. causing definite deterioration in 2½ months [J. Williams and Corran, 1930] whereas at 0° C. as much as 0·06 to 0·1 per cent. has little effect on the potency [Davey, 1921]. Even in absence of all preservative comparatively little change occurs in expressed lemon juice kept at 0° C. for seven months. Entire oranges and lemons can be kept at 2–5° C. for five or six months without detriment to the potency of their juice [Davey, 1921]. According to J. Williams and Corran [1930] the zone of optimal stability for lemon juice at ordinary temperatures lies in the neighbourhood of the natural acidity of the juice (pH 2·2) and natural juice can be kept without loss of potency for as long as fourteen months at room temperature without any addition. It becomes, however, heavily infected with moulds and yeasts. In general, conditions which preserve sterility tend to cause deterioration of antiscorbutic potency.

Tablets, prepared by evaporating lemon juice over H_2SO_4 at 13·5 to 15·5° C., and mixing the syrup with a mixture of 97 per cent. lactose and 3 per cent. gum tragacanth, contained a large proportion of the vitamin C of the original juice, and retained their antiscorbutic properties for over twelve months at low temperature. Each tablet was the equivalent of half a fresh lemon [Bassett-Smith, 1920; 1921].

A systematic investigation has shown that the vitamin C content of the juice of the orange and grape fruit is remarkably constant whether the fruit be picked at the beginning or end of the season [Bracewell and Zilva, 1931]. The longer the fruit remains on the tree the smaller is the yield of juice, so that the total vitamin C content of the fruit diminishes. There is no correlation between the content of soluble solids or the pH of the juice and its antiscorbutic potency. As far as can be seen at present the conditions of cultivation of the orange, origin of stock, age of tree, or nature of soil do not affect the vitamin content of the fruit.

Apple. Observations on the apple have shown that different varieties vary greatly in antiscorbutic potency [Bracewell, Hoyle and Zilva, 1930, 1, 2; Bracewell, Kidd, West and Zilva, 1931; Bracewell, Wallace and Zilva, 1931]. Thus out of eight varieties grown in England, Bramley's Seedling was found to be much the most potent, the minimum daily protective dose for a guinea-pig being 3 g. of the flesh of the fruit, whilst 20 g. of Worcester Pearmain did not afford full protection. No indications were obtained that the character of the soil, the age of the tree, the season, or state of maturity of the fruit had any influence on its vitamin C content. Apples stored in air at 1° C., 3° C., or 10° C., for periods of three months, showed little loss of antiscorbutic potency. This loss was somewhat greater when the fruit was stored at 10° C., in a mixture of 10 per cent. carbon dioxide, 11 per cent. oxygen, and 79 per cent. nitrogen (gas storage) for three months. Bramley's Seedling apples, whether fresh or stored at a low temperature in air, or 'gas stored', lost only a very small fraction of their vitamin C content when baked in their skins, during which process the temperature of the interior of the apples rose to about 95° C. Bramley's Seedling apples frozen at −20° C., and kept at this temperature for four months, did not lose any antiscorbutic potency. Young apples of this variety about 3·2 cm. in diameter and 23 g. in weight, gathered at the end of July, were not more active per g. of tissue than the apples of the same environment gathered in October; the total amount of vitamin in the apple therefore increases as it grows on the tree. The distribution of the vitamin throughout the fruit is by no means uniform. The concentration increases as the skin is approached from the core and is more than six times as great in the peel as in the flesh near the core, so that apple peel must be considered as one of the richest sources of vitamin C. There are indications that a low nitrogen content may be associated with a high vitamin C content in the apple.

Bramley's Seedling apple genetically considered is a triploid with 57 chromosomes, whereas the other varieties referred to above are diploids with 34 chromosomes. Two other triploid varieties, namely, Belle de Boskoop and Blenheim Orange, have so far been found to possess also a high vitamin C content. The former is as active as the Bramley's Seedling apple and the latter somewhat less active [Crane and Zilva, 1931]. Whether any correlation exists between the genetic constitution and the vitamin C content of the apple cannot at present be stated.

Scheunert [1930] has also observed a varying antiscorbutic potency in different varieties of apple grown in Germany. These observations explain the variable results which were obtained by earlier workers with random samples of apple or apple juice. [See Holst and Frölich, 1912; Robison, 1919; Chick, 1920; Givens, McClugage and Van Horne, 1922; Kohman, Eddy and Carlsson, 1924.]

The pear seems to be definitely inferior to the apple in vitamin C content [Scheunert, 1930; Craven and Kramer, 1927].

Tomato. The tomato is a rich source of vitamin C, often barely inferior to the *Citrus* fruits. In the canned form it is still highly potent, 3 c.c. of juice having been found sufficient as a daily dose to protect guinea-pigs [Sherman, La Mer and Campbell, 1921; see also Hess and Unger, 1918-19]. Tomatoes ripened in ethylene are no richer in vitamin C than those ripened in the air and are less rich than those which ripen on the plant [Clow and Marlatt, 1930; House, Nelson and Haber, 1929]. Tomatoes grown and examined in South Africa were found to be less potent than those grown in England, 3 to 5 c.c. of the raw juice being the minimum dose as against 1·5 c.c. for the English fruit (Delf and Pullinger, unpublished results).

Many of the common fruits are comparatively poor sources of the vitamin, grapes, cherries, plums, and peaches falling within this class. Many 'berries'—blackberries, strawberries, raspberries, currants, and gooseberries—are moderately good sources of vitamin C, about 5 g. being a protective dose for guinea-pigs [Scheunert, 1930].

Certain varieties of the mango (e.g. Alphonso) are even richer in vitamin C than the *Citrus* fruits [Perry and Zilva, 1932.]

Dried vegetables and fruits (see also p. 259). *Dried vegetables*, in confirmation of human experience and of Holst's experimental work, have been found of very little value [Delf and Skelton, 1918; Givens and Cohen, 1918]. *Fruit juices* on the other hand can as a rule be dried, and the dry material can be kept without any great loss of potency. Dried grape juice and raspberry juice have, however, been found to be almost devoid of vitamin C [Givens and Macy, 1921].

Meat. Meat with milk comes last in the order of merit [Chick and Hume, 1917, 1]. Twenty c.c. of raw beef juice daily offered only very slight protection to guinea-pigs; a larger ration was not tolerated. Watery extracts of as much as 20 g. of lean meat offered no protection to guinea-pigs. That fresh meat possesses definite antiscorbutic properties is, however, clearly evident from human experience (see p. 261).

Milk is a variable and by no means rich source of the antiscorbutic vitamin. As much as 100 c.c. of some samples of fresh raw cow's milk daily and more may be required if scurvy in guinea-pigs is to be prevented by its agency alone [Chick, Hume and Skelton, 1918, 1, 2], whilst as little as 20 c.c. of a summer pasture milk has been found to afford protection [Dutcher *et al.*, 1920-21] and 40 c.c. of milk were found sufficient by Schwartze, Murphy and Hann [1929-30].

The dependence of the vitamin C content of milk on the diet of the cow and the effect upon it of heating, drying, &c., are discussed later on in Chapter IX.

Effect of Heating, Drying, and Preserving on the Vitamin C Content of Foodstuffs.

The conditions of stability of vitamin C in lemon juice, &c., have been already discussed (p. 199). The behaviour of foodstuffs towards rise of temperature varies with different materials, owing to the different conditions under which the vitamin exists in them. Oxidation usually plays an important part in the changes which are produced.

Effect of Heating.

Following the experiments of Holst and Frölich [1912] upon the effects of heat, a more complete series of experiments upon this point was carried out by Delf [1918]. Working with raw cabbage leaves, the minimum daily ration required to prevent scurvy in guinea-pigs was determined to be less than 1·5 g. and greater than 0·5 g., i.e., about 1 g. When the cabbage was heated in water at 60° C. for one hour, symptoms of severe scurvy were just prevented by a 5 g. ration. When the temperature was 70°, 80°, 90°, or 100° C. for the same period, scurvy was not satisfactorily avoided. If the time was reduced to twenty minutes, the same ration, 5 g., prevented scurvy when the temperature was 90° C. and just failed to do so when the temperature was 100° C.

The conclusion drawn from these results is that when cooked for one hour at temperatures ranging from 80° to 100° C., cabbage leaves lose about 90 per cent. of the antiscorbutic value originally possessed (antiscurvy value of 5 g. ration reduced by cooking to the equivalent of about 0·5 g. raw cabbage). In a similar manner the loss on heating in water either (*a*) for sixty minutes at 60° C., or (*b*) for twenty minutes at 90–100° C., was estimated at about 80 per cent. of the original (antiscurvy value of 5 g. reduced to the equivalent of about 1 g. raw cabbage).

The destructive influence of heat on vitamin C is thus seen to be enhanced to a comparatively slight degree with rise of temperature. Upon the above estimates, the rate of destruction is accelerated only about threefold (the time required for destruction of 80 per cent. of the original value being reduced to one-third) when the temperature is raised from 60° C. to boiling-point, i.e., by 40° C., so that the temperature coefficient would be about 1·3 for 10° C. rise of temperature.

These results confirm those of Holst and Frölich [1912] in showing the great sensitiveness of the antiscurvy factor in cabbage to temperatures of 100° C. and below. This instability of the antiscurvy factor is a matter of the greatest importance in estimating the antiscorbutic value of cooked vegetables and the relative merits of different methods of cooking, and will be referred to later under that heading (p. 262)

Delf's work was subsequently [1920] extended to the juices of cabbage, swedes, and oranges, and revealed the fact that cabbage juice was much more affected by exposure to 100° C. for an hour than either of the other two under similar conditions of exposure. Her results are summed up in Table XIV, the minimum daily doses being stated which were required before and after treatment for protection of guinea-pigs.

TABLE XIV.

	Minimum doses in c.c.			*Percentage loss of potency.*	
	Before treatment.	*After heating for 1 hour at* 100°	130°	100°	130°
Cabbage juice	1	7·5	—	87	—
Swede juice	2·5	5	10	50	75
Orange juice	1·5	1·5	3	0	50

Sherman, La Mer and Campbell [1921; 1922; La Mer, 1922], who have investigated the effect of temperature and hydrogen ion concentration on the destruction of vitamin C in tomato juice, using the method described on p. 203, confirmed the comparative stability of the vitamin at 100° C. and also the relatively low temperature coefficient found by Delf for cabbage. Their results may be briefly summarized as follows.

TABLE XV.

pH.	*Temp. °C.*	*Time—hours.*	*Percentage Destruction of Vitamin C in Tomato Juice.*
4·3	60	1	25
,,		4	35
,,	80	1	40
,,		4	53
,,	100	1	50
,,		2	58
,,		4	68
5·1—4·9	100	1	58
11—9		1	65 (increasing to 90–95 on preservation for 24 hours at 10° C., in partially filled bottles)
	Temperature coefficient for 10° C. (60–80°) 1·23 (80–100°) 1·12		

These results suggest that the stability increases with the hydrogen ion concentration (acidity) of the vitamin solution. Holst had previously arrived at the same conclusion, his opinion being based mainly upon the fact that acid fruit juices retained their antiscorbutic properties much longer than vegetable juices. Harden and Zilva [1918, 6] have shown, as already mentioned, that the presence of alkalis, even when dilute (1/50 *N* sodium hydroxide) and at room temperature, has a rapidly destructive effect upon the antiscorbutic vitamin. These authors have called attention to the danger involved in the practice of adding sodium carbonate when boiling green vegetables.

It has been suggested that the loss in antiscorbutic value suffered by vegetables during cooking might be lessened if the water in which they are heated were made slightly acid with citric acid. The suggestion was originally made in respect of germinated lentils in order to preserve the antiscurvy value as far as possible where these may form the only source of antiscorbutic material in a diet [Greig,

1918]. It has, however, been shown experimentally by Delf [1918] that, when 0·5 per cent. citric acid is added to the water in which germinated lentils are boiled, the loss in antiscurvy properties is, if anything, greater than when no addition of acid is made.

It follows, therefore, that in cooking vegetables there should be no addition either of acid or alkali to the water in which they are boiled.

The commercial process of canning vegetables also leads to a considerable degree of destruction of the vitamin C in vegetables like cabbage and runner beans, whereas in tomatoes very little destruction occurs unless there is exposure to air. Cabbage heated with water in hermetically sealed tins for 1 hour at 90–100° C. lost about 70 per cent. of its potency; runner beans exposed to 100° C. under the same conditions on two successive days for a total of two hours and twenty minutes lost about 75–90 per cent. [Campbell and Chick, 1919]. Tomato when converted into a purée and canned, during which process it was exposed at 80–100° C. for 100 minutes in an open vessel and concentrated to half its bulk, lost 85 per cent. of its antiscorbutic potency [Delf, 1920].

Actual investigation of products canned commercially has shown that in many cases vitamin C is still present, although a certain amount of loss has usually taken place. This holds for many fruits and vegetables (see Tables XIII and XXI).

Peaches, plums, and apricots, all fruits of comparatively low vitamin C content, lose their vitamin C entirely when dehydrated or sun-dried, but if they are dipped in alkali and then exposed to sulphur dioxide before being dried, they retain it satisfactorily. This effect is probably due to the reducing effect of the sulphur dioxide during the process of drying [Morgan and Field, 1929; Morgan, Field and Nichols, 1931].

Raisins prepared by the hot or cold 'dip' process contain no vitamin C [Zilva, unpublished results].

Effect of Drying and Preservation.

The absence of the antiscorbutic principle from many dried foodstuffs, and its disappearance from some of the most powerful antiscorbutics when these are reduced to the dry condition, is sufficient proof of the sensitiveness of vitamin C to drying, and in this respect the contrast between the antiberi-beri and the antiscurvy factors is very marked.

Holst and Frölich [1912] devoted much attention to the question of the effect of drying on the antiscorbutic principle of cabbage with the object of finding some method of preserving vegetables without destroying their antiscorbutic properties. It was soon found that at least three factors required consideration. (1) The effect of the actual process of drying; (2) the effect of preservation of the dried material; to which was added (3) the effect of cooking the dried material.

These investigators did not determine the minimum dose of fresh cabbage required for the protection of a guinea-pig, but worked on a basis of 30 g. of fresh cabbage or its equivalent in dried cabbage. This is now known to be twenty to thirty times the requisite minimum dose of fresh cabbage. It was found that

after drying in the air at 37° C. for 8 days this dose of cabbage was still sufficient to protect from scurvy, and retained this power even after being boiled for half an hour in 0·5 per cent. NaCl or acetic acid solution. On preservation for 5 weeks of the dried cabbage this dose retained its efficiency when the material was kept dry, but lost it when it was exposed to water vapour. Further experiments confirmed the importance of water vapour in this respect [Holst and Frölich, 1920]. Cabbage dried as above preserved its antiscorbutic properties (in doses equivalent to 30 g. fresh cabbage) for 18 to 26 months when kept at 37° C. over phosphorus pentoxide, but lost them over calcium chloride, a less efficient drying agent. Partial loss resulted from preservation of the dried cabbage at 4° C. in closed vessels, without any desiccating agent; over calcium chloride the loss was less at lower temperatures. More definite results with regard to the loss in the actual process of drying were obtained by Delf and Skelton [1918], who found a loss of more than 93 per cent. of the antiscorbutic potency, when the cabbage was dried at 37° C. and stored at air temperature in a closed bottle for 2–3 weeks. When the cabbage was plunged into boiling water before being dried, to inactivate the enzymes, drying took place much more rapidly and the loss of potency was somewhat less, but still very considerable. Even when dried at 65° C. in an atmosphere of nitrogen [Ellis, Steenbock and Hart, 1921] a loss of the same order was observed.

Fruit juices on the other hand may be dried without serious loss of antiscorbutic potency by evaporation *in vacuo* [Harden and Robison, 1919, 1920; Robison, 1919], or after admixture with corn syrup by the 'spray process' [Givens and McClugage, 1919, 2]. The dry residue obtained from orange juice by Harden and Robison when kept over sulphuric acid for 2 years at air temperature protected guinea-pigs from scurvy in doses of 0·5 g. (equal to 4·5 c.c. fresh juice), whilst another sample, kept in a sealed vessel at 29° C. for 14 months, lost more than 85 per cent. of its power [1921]. The material dried by the 'spray process' was still effective after 14–20 months [Givens and McClugage, 1920-21]. These authors also found that commercially dried lemon juice (raw and neutralized), tomato juice, grape fruit juice, and orange juice were all active after 14–20 months, whereas grape juice and raspberry juice were inactive. Dried tomato was found to be active in doses of 1 g.

When dried in the sun (in India) carrots, egg plant, spinach, turnips, and turnip tops lose nearly all their antiscorbutic power, whilst tomatoes, potatoes, and cabbage retain a certain proportion, which is, however, still further diminished by cooking [Shorten and Ray, 1919; 1921]. In this case what was actually determined was that 50 g. of the fresh vegetable protected guinea-pigs from scurvy for about 3 months, and that after drying the equivalent of 50 g. either did or did not protect. No minimum doses were determined either before or after drying. Commercial mixed dried vegetables were found to be inactive in the same dose. Dried tamarinds, amchur (dried mango), and cocum, three Indian fruits, were found to possess a low antiscorbutic potency [Chick Hume and Skelton, 1919].

CHAPTER IX

SOME NUTRITIONAL ASPECTS OF COW'S MILK WITH SPECIAL REFERENCE TO VITAMINS.

The nutritional value of milk as a corrective to deficiencies of various types in the diet is now generally accepted. It is valuable to the adult and probably indispensable to the child and its increased consumption in this country should be advocated. In many diets it is the chief source of fat-soluble vitamins, of protein of good quality, and of calcium salts. Its importance is well illustrated in the experiments of Mann [1926] who compared the effects of several different additions to the dietary of boys in an Institution under well-controlled conditions. His results are summarized graphically in Fig. 32. The diet of these boys was considered adequate, although they were below the normal standard in height and weight. Mann found that an increase of growth, as measured by both height and weight, was promoted by the addition of one pint of whole milk per diem. The milk used was pasteurized. No effect on the height was produced by addition of caseinogen in amount equivalent to that contained in the milk or of margarine in amount equivalent to the extra calories. An improvement was, however, noticeable with New Zealand butter.

Whatever may be the interpretation of these observations, the results are important. They suggest that among the missing factors supplied by the milk addition were the fat-soluble vitamins, though the possibility cannot be entirely excluded that the extra calcium given may also have influenced the results. As mentioned later (p. 242) a similar result was obtained when additions of whole and separated milk respectively were made to the diets of school-children in different towns in Scotland [Orr, 1928; Leighton and Clark, 1929; Clark, 1929].

Untreated Cow's Milk.

Cow's milk in one form or another is the almost invariable substitute for human milk in cases where breast-feeding has for any reason to be abandoned. Though a discussion of the means to be employed for adapting cow's milk for this purpose lies outside the scope of this report, it is necessary to consider the matter, so that attention to the vitamin issue alone may not give a wrong impression. Some infants may be reared from a very early age upon unmodified cow's milk, but for others some modifications are required to render it more like human milk. The most usual difficulty arises from the excess of caseinogen in cow's milk. Dilution of the milk, a method often employed, while ensuring the right percentage of protein, lowers in similar proportion all the other constituents including fat, carbohydrate, salts, and vitamins. A child therefore fed upon milk diluted with water alone is partially starved of a large number of essential dietary constituents. This important fact must never be forgotten, and in the modification of cow's milk for infant-feeding dilution should always be made with milk products.

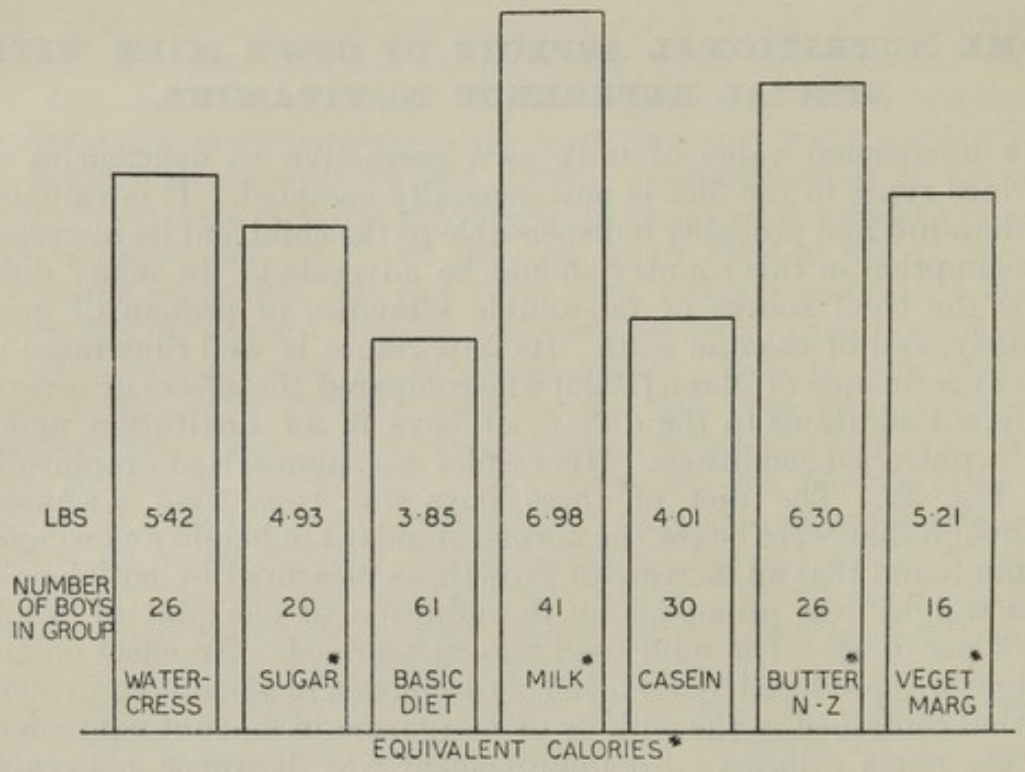

Fig. 32 *a*. **Average gain in weight per boy in one year.**

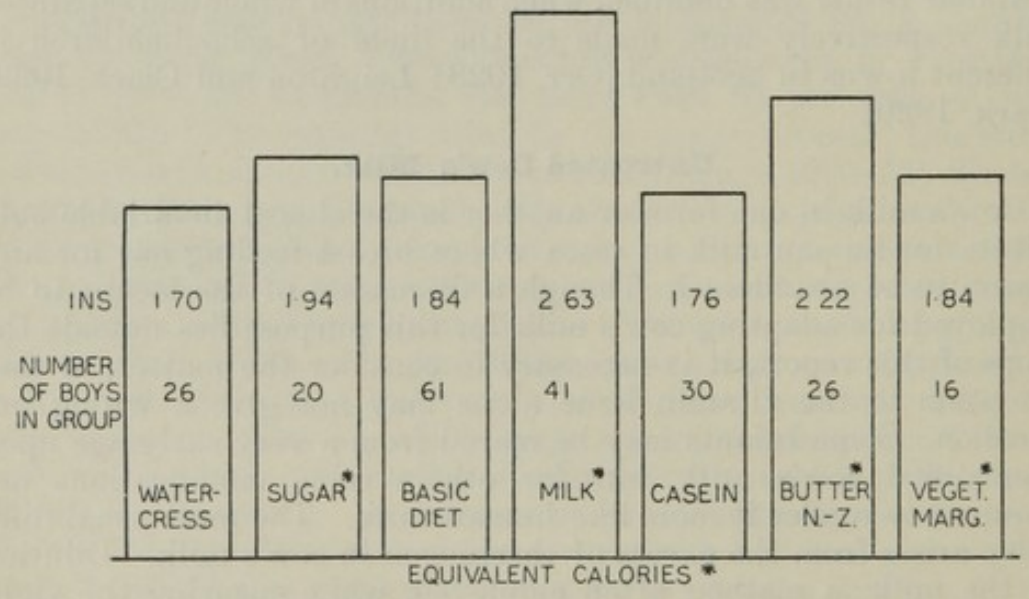

Fig. 32 *b*. **Average increase in height per boy in one year.**
(From Corry Mann (1926) *Sp. Rep. Ser. Med. Res. Counc.*, London, No. 105)

The ideal diluent is the whey left when the casein is removed. If the milk is stirred during the formation of the curd, some of the fat passes into the whey. This method may be too difficult for ordinary use, but it must be remembered that vegetable oils cannot replace cream, and sugar solutions cannot replace whey (see Fig. 33).

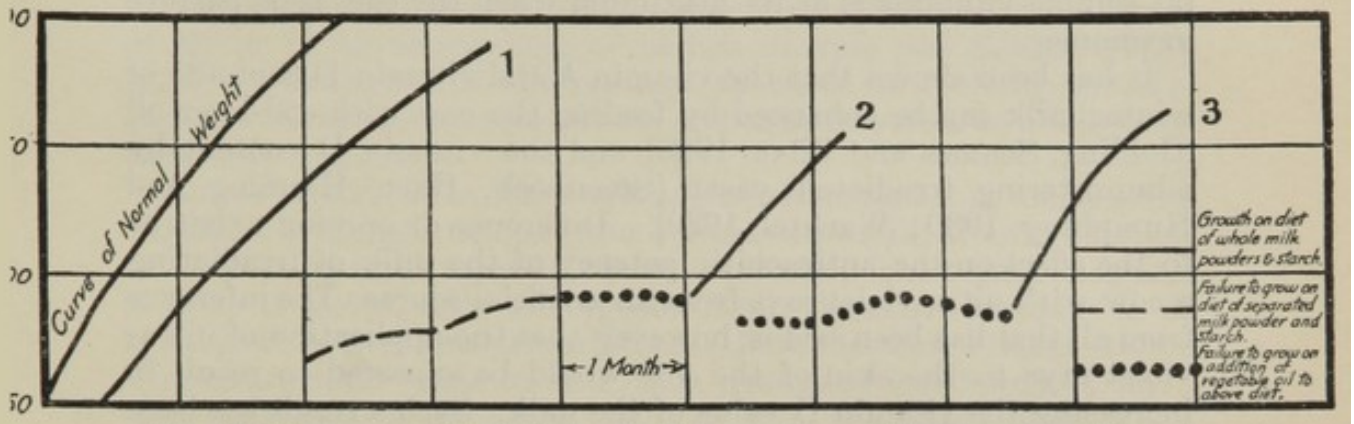

FIG. 33. These curves illustrate the inefficiency of vegetable oils such as linseed and olive oil to replace the fat of milk for promoting growth. Curve 1 represents the growth of a young rat fed upon a food paste composed of whole cream dried milk, and starch. The growth is not quite up to the normal standard, but it is evident that there is no serious deficiency of either vitamin A, D or B. The curve marked (- - -) shows the failure of young rats to grow on a similar paste composed of dried skimmed milk powder and starch. This failure is due to the absence of an adequate amount of the fat-soluble vitamins, and is not made good by the addition to the ration of linseed oil (curve 2 . . .) or olive oil (curve 3 . . .) in an amount equivalent to the deficiency of milk fat. Addition of butter fat, however, at once renders growth possible again (curves 2 and 3 ——).

CONDITIONS DETERMINING THE VITAMIN VALUE OF COW'S MILK.

The vitamin value of raw cow's milk varies greatly according to the diet and management of the cow. There is an extensive literature on the subject, to which only brief reference is here possible. Luce [1924, 1, 2] made an important investigation on the influence of sunlight and diet on the antirachitic and growth-promoting properties of the milk of a cow, and her work was carried still further by Chick and Roscoe [1926, 2]. The cow was in successive periods kept in the light and in the dark and was fed on fresh green grass and on a dry winter feed. It was found that the vitamin A content of the milk was much greater when the cow was receiving fresh green food than when the diet was one of cereals and roots. The vitamin D content was found to depend principally on the degree of insolation of the cow, but there was some indication that insolation accompanied by a diet of fresh green food, i.e., when the cow was on pasture in summer, was more effective than a similar degree of insolation combined with a diet composed only of cereals and roots.

A comparison of the value of human and cow's milk [Outhouse *et al.*, 1927; 1928] when the cows were fed on dry fodder, which, however, included ensilage and alfalfa hay, showed that the vitamin A value of cow's milk was about the same as that of human milk; for B-vitamins the value of cow's milk was slightly better and for antirachitic value also distinctly better, although the cows had little exposure to suushine. The values found for vitamin D did not

approach those found in the investigation of Chick and Roscoe. Outhouse *et al.* [1928] give a summary of the literature pointing out the differing results of various workers according to the diet and environmental conditions of the cow on which the work was done. It may, however, be accepted that the value of the cow's milk in fat-soluble vitamins is at its maximum when the cow is on pasture in summer.

It has been shown that the vitamin A and vitamin D contents of winter milk can be enhanced by feeding the cow with cod-liver oil [Golding, Soames and Zilva, 1926], and the vitamin D content by administering irradiated yeast [Steenbock, Hart, Hanning and Humphrey, 1930; Wachtel, 1929]. Difference of opinion exists as to the effect on the antirachitic potency of the milk of irradiating a cow with ultra-violet rays from an artificial source. The inference from all that has been said is, however, that the application of ultra-violet rays to the skin of the cow would be expected to result in increasing the vitamin D value of the milk. Such a result has been obtained by many workers, but Steenbock, Hart, Riising *et al.* [1930, where the literature is set out and discussed], although finding that the milk of goats could be so affected, were unable to demonstrate an increased antirachitic value in cow's milk by artificial irradiation of the animal. In one of their experiments the milk of cows exposed to sun without change of diet from May 26 to June 16 showed no improvement as the result of insolation, but the milk of the same cows at the end of a summer on pasture was markedly superior.

The vitamin B and vitamin C contents of cow's milk appear also to vary with the diet of the animal. Kennedy and Dutcher [1922] found that 10 c.c. of milk from a cow on an adequate ration would supply an adequate daily provision of B-vitamins for a rat, while 15 c.c. from a cow on an inadequate diet would not. The anti-scorbutic value was found to vary more widely; in experiments with guinea-pigs, 20 c.c. of summer milk were found superior in nutritive value and in antiscorbutic potency to 60 c.c. of winter milk [Dutcher *et al.*, 1920–21]. A similar difference was found by Hess, Unger and Supplee [1920–21].

It must be recognized that cow's milk is a variable foodstuff, of which the vitamin value in any given instance cannot be assumed. Its use in infant-feeding must be specially safeguarded, not only by the use of milk products for the purpose of modification, but by the addition to the child's diet of the same supplements as are recommended for the breast-fed infant (p. 274).

Treated Cow's Milk (Irradiated, Heated, Dried, or Condensed).

Irradiated Cow's Milk.

The vitamin D value of cow's milk, whether raw or dried, can be much enhanced by ultra-violet irradiation; such milk may be of service prophylactically, in protecting children from rickets, but in the present state of knowledge it would be unwise to rely on it to the exclusion of cod-liver oil.

Cowell [1925; see also Daniels, Pyle and Brooks, 1925–26] showed that children could rapidly be cured of rickets by giving them every

day a pint of milk which had been irradiated for 20 minutes. So long an irradiation in presence of oxygen is liable to impart to the milk an unpleasant flavour, which has been ascribed to the oxidation of proteins [Schultz, 1929], and may also damage the vitamin A it contains [Scheer, 1928]. Types of apparatus have now been devised in which the irradiation of milk can be accomplished in the absence of air or in an atmosphere of carbon dioxide [see Scheer, 1928]. Milk irradiated in this way has been shown to be a reliable prophylactic or curative agent for rickets and to be free from any objectionable taste or smell.

Coward [1929, 2], as the result of numerous tests, has found great variation in the antirachitic value of fresh cow's milk after irradiation for periods up to 30 seconds. Such a variation is not surprising and is to be expected, since the ergosterol (provitamin) content of milk is subject to considerable variations [Hentschel and Fischer, 1930]. The antirachitic value of milk thus irradiated was found to have been increased 5–50 times, and it was calculated that when irradiation was effective about 3 pints were equal in antirachitic value to one teaspoonful of a good cod-liver oil. So short an irradiation would not have the drawbacks alluded to above.

Heated Cow's Milk.

A question of great moment is the influence of heat upon the nutritive value of cow's milk, and in certain respects the evidence on this question is conflicting. The matter was discussed at some length by Lane-Claypon [1916] in whose book are many references to authorities who consider that there is no proof of any inferiority of heated or pasteurized, as compared with raw, milk. Since then more information upon the effect of heat upon the vitamins and other constituents of milk has accumulated.

The evidence as to any deleterious effect of *careful* pasteurization on the nutritive properties of cow's milk is not decisive. It is in any case at present advisable to pasteurize the ordinary milk supply to prevent the spread of milk-borne diseases.

Owing to the variability of the vitamin content of milk, whether heated or raw, it is wise in all cases to supplement a diet of milk with vitamin additions. Of the vitamins present in milk, vitamin C is the most sensitive to the combined effects of heat and oxidation and evidence of the connexion between the use of pasteurized or heated milk and the incidence of infantile scurvy is presented on p. 264.

Effect of Heating on the Vitamin C of Milk.

Pasteurizing. Direct comparison, by a modified form of the comparative method of Sherman, La Mer and Campbell (p. 203), of fresh milk with the same milk after aerobic pasteurization at 60° C. for 30 minutes [Schwartze, Murphy and Cox, 1931–32] showed that the degree of diminution of the vitamin C content varied with the metal with which the milk came in contact during the process, being 20–40 per cent. (i.e. hardly perceptible) with aluminium, slightly greater with tinned copper, and 80–90 per cent. with copper.

Boiling. It was found by Barnes and Hume [1919, 1, 2] that when

milk was scalded i.e. quickly heated to boiling in an enamelled pan and then allowed to cool in the air, it suffered comparatively little loss—not more than 25 per cent.—of its antiscorbutic power. A similar result was obtained by Schwartze, Murphy and Hann [1929–30] for milk boiled in glass or aluminium vessels.

Sterilizing. As regards the effect of higher degrees of heat, i.e. temperatures above 100° C., it would appear that these cause a rapid destruction of the antiscorbutic vitamin. Milk heated to 120° C., for ten minutes to one hour, has been examined by several investigators [Chick, Hume and Skelton, 1918, 1, 2; Hart, Steenbock and Smith, 1919]. No accurate estimate of its antiscorbutic power has been made, but after having been heated at 120° C. for one hour it can be used with impunity as a constituent of the basal diet of guinea-pigs for experiments with antiscorbutics (p. 202).

Effect of Heating on the Calcium of Milk.

Evidence has been forthcoming that the calcium supply in cow's milk is affected by heating. Daniels and Loughlin [1920] studied the problem on rats and found that the animals grew less well on pasteurized milk than on milk just brought to the boil, and that this was due to an insufficiency of calcium in the pasteurized milk. The calcium was found to be thrown out of solution by the more prolonged heating and the precipitate thus formed was mechanically lost.

When milk was brought to a temperature of 145–50° F., maintained at it for 30 minutes and tested 5 hours later, the loss in diffusible calcium was computed to be about 2 per cent.; at higher temperatures, up to 209° F., the loss rose to 2·5–3·6 per cent. [Mattick and Hallett, 1929].

From the Rowett Institute also comes evidence that when milk is heated there is a reduction in the diffusible calcium [Magee and Harvey, 1926, 1]. Young pigs on a diet of cereals and milk showed a lower retention of calcium, phosphorus, and nitrogen when the milk was heated than when fresh or sour [Magee and Harvey, 1926, 2]. When groups of calves were fed respectively on fresh milk, on pasteurized milk, and on pasteurized milk to which calcium lactate had been added, it was found that the increase in weight in the group on pasteurized milk, without any addition, was distinctly inferior to that in the other two groups. In one experiment the calves in the group receiving pasteurized milk actually contracted rickets [Orr *et al.*, 1926].

It was also found that heat caused the disappearance by volatilization of 20 per cent. or more, according to the severity of the heating, of the total iodine of separated milk [Magee and Glennie, 1928].

Although it cannot be disputed that the calcium supply in milk is damaged by heating, yet it might well be argued that in comparison with human milk, cow's milk contains so much calcium (38 mg. of CaO per 100 g. in human milk to 175 mg. in cow's milk [Schall and Heisler, 1927]) that a heavy loss might occur before the human infant fed on it would suffer any deprivation. If, however, it be assumed that for this reason young infants would not suffer from diminution

of available calcium in pasteurized milk, it must be remembered that milk is also one of the most important sources of calcium for children older than those who are wholly milk-fed, and any impoverishment of the calcium supply in diets which may already contain insufficient of this element is much to be deprecated.

Evidence, however, exists that heating cow's milk may render the available calcium insufficient even for babies wholly fed on it. Daniels and Stearns [1924] found that a series of infants maintained on pasteurized milk to which antiscorbutic had been added failed to grow, and in some cases actually showed a negative calcium balance, while those which received milk that had simply been brought to the boil grew better, and, except in one case, remained in positive calcium balance, though not apparently retaining the optimal amount of calcium. The faecal calcium in the periods during which pasteurized milk was given was greater than in the periods in which boiled milk was given, suggesting that the calcium was not merely lost mechanically but that a portion of it was in a form in which it was incapable of utilization. These experimental results are so important that they demand confirmation and extension.

This matter is a very important one in view of the ever increasing spread of milk pasteurization in the large cities, and cannot be lightly dismissed. The evidence indicates that heating may be detrimental to the mineral content of milk and that the duration of the heating is more important than the temperature, if this does not exceed the boiling point. There is, however, little doubt that regular addition of vitamin D as cod-liver oil would, to a large extent, correct any calcium deficiency by securing the optimum utilization of the calcium presented to the organism. Raw, sterile milk is of course the ideal to be aimed at, but as such milk is at present unobtainable, there seem to be two alternatives, either to use pasteurized milk or to bring raw milk to the boil shortly before drinking. In the latter case the heating should not be prolonged.

Dried Cow's Milk.

On general grounds, it would appear likely that much that has been said about heated milk would be applicable also to dried milk, in so far as the processes used in drying involve heating. The majority of dried milks are prepared by one or other of two processes, which may be termed respectively the drum and the 'spray' processes [Rep. L. G. Board, 1918]. The drum process involves an exposure of the milk upon a revolving drum heated internally by steam at 140° C. The exposure is, however, very short and is probably insufficient to cause any significant destruction of the vitamins. In the spray process the milk is sprayed into a chamber through which a current of dry air at a temperature of 115° C. is passing. The dried powder falls to the bottom of this chamber and is, therefore, exposed to a temperature above 100° C. until it is removed for packing. It is probable that some destruction of the vitamins might occur if the dried powder remained in the chamber for longer than an hour. Certain special advantages are claimed for a spray

process in which the milk is atomized cold and is smoothly distributed into a large stream of air, heated only to a very moderate extent. The contact with the hot air stream is brief and the dry powder is continuously removed from the influence of heat by settling into hoppers, outside the hot air stream. Any partial or general exposure of the milk to a raised temperature is thus minimized as far as possible.

The water-soluble B-vitamins do not appear to suffer damage in drying by the spray process [Johnson, 1921; Johnson and Hooper, 1921]. It is, however, probable that slow oxidation will gradually diminish the amount of the fat-soluble vitamins if the dried milks are kept for long periods before being used [see Hume, 1923, p. 140].

Investigation of the antiscorbutic value of dried milk has given conflicting results, due, doubtless, to the variation in value of the original milks before drying, to the exact methods used in drying and to the length of time the dried milk has been kept. Johnson and Hooper [1922] state that the antiscorbutic value of fresh milk is not very great and is injured by the process of drying. Barnes and Hume [1919, 1, 2], working at a time of year when the antiscorbutic potency of fresh milk was low, found that a sample of dried milk prepared at the same time, though not from the raw milk tested, was lower than the raw milk in antiscorbutic potency. They were unable to protect guinea-pigs from scurvy with any amount of dried milk which these animals were able to consume, but a monkey, which had developed scurvy on dried milk, was cured by the administration of the same amount of fresh milk, just scalded. Barnes and Hume concluded that the dried milk had about one-half the antiscorbutic value of fresh milk. They obtained indications that the vitamin C value of both fresh and dried cow's milk improved after the cows from which it was derived went out to pasture.

Hart, Steenbock and Ellis [1921] examined various milk powders and found that a considerable destruction of antiscorbutic vitamin occurred during drying, that various samples differed considerably from one another, and that less damage was suffered in the drum than in the spray process. Hess [1920], on the other hand, found that infants could sometimes be cured of scurvy by the use of dried milk. Jephcott and Bacharach [1921] found that guinea-pigs could be protected from scurvy by winter or summer milk specially dried by the drum process, but they did not compare the same milks before and after drying. A sample of milk dried by the spray process and of unknown age conferred no protection from scurvy upon guinea-pigs. Cavanaugh, Dutcher and Hall [1924], on the other hand, used milk dried by a form of the spray process in which special precautions were taken, and compared the milk thus dried with the same sample before drying. They claim that no damage was done to the antiscorbutic vitamin by this process.

It has been shown already that fresh milk is very variable in its vitamin content, and as the process of drying brings in fresh sources of variation, it can be taken for granted that the vitamin content of a dried milk is less than that of the same milk in the raw condition.

It is at once apparent that half cream and separated milks when

dried will be deficient in the fat-soluble accessory factors, nor can such fats as olive oil or lard be used to make the deficiency good.

The antirachitic power of dried milk, like that of fresh milk (p. 220), is increased by ultra-violet irradiation [Hess and Weinstock, 1925, 1; Mackay and Shaw, 1925; Eddy, 1927; György, 1925; Supplee and Dow, 1927].

Condensed Milks.

The usual process employed for the preparation of the various types of sweetened condensed milks involves a preliminary 'pasteurization', or rather heating, at 80–90° C., followed by concentration at 50° C. under reduced pressure for two or three hours. It is probable that such treatment would not effect any serious destruction of the water-soluble or the fat-soluble vitamins.

An examination of sweetened condensed milk for antiscorbutic potency was carried out by Hume [1921], who, working with monkeys, found that the protective dose was almost identical with that of its equivalent of fresh milk (150 c.c.). Hess also found that this material retained most of the antiscorbutic potency of fresh milk [1921].

Unsweetened condensed milk, on the other hand, which is subject to more drastic heating in order to ensure its preservation (preheating at 82·5–98·5° C. for 1–20 minutes; condensing at 55·7° C., and sterilizing at 107–115·5° C. for 1–20 minutes), loses more than 40 per cent. of its antiscorbutic power [Hart, Steenbock and Smith, 1919].

Condensed milks are classified as full-cream milks or machine-skimmed milks. The former are often, and the latter almost invariably, sweetened by the addition of sucrose (cane sugar). Any form of machine-skimmed milk, whether condensed or not, is unsuitable for the nutrition of the infant, owing to its low content of fat, which is further diminished when the milk is diluted before use. The dilutions necessary to reduce the effect of the added sucrose in sweetened condensed milks are so great as to result in the production of a foodstuff very deficient in fat, vitamins and salts.

CHAPTER X

VITAMINS AND HUMAN DIETS.

Introduction.

The known chemical characteristics and the biological actions of the recognized vitamins have been considered in the earlier chapters of this work. It is now proposed to review the general problem in relation to human nutrition. Many of the facts have already been discussed in some detail, but primarily in relation to experimental work rather than from their human interest.

In the last decade our knowledge of vitamins has advanced so greatly that the possibilities of its practical application to human diets have much increased. Eight years ago it was necessary to say 'it is therefore a matter for regret that our limited knowledge of the accessory substances should restrict to some extent the consideration of practical issues' [Med. Res. Coun. 1924]. Meanwhile knowledge has grown and, though still very incomplete, the limiting factor in practical application is often one of finance rather than of ignorance.

A large proportion of the facts discovered about vitamins has resulted from animal experiments and although it is certain that many of them are of the greatest importance in human nutrition, it is equally clear that their significance in some cases is either not known or imperfectly understood as far as man is concerned. Laboratory work is, in fact, a long way ahead of clinical investigation in this field at the present time.

Nutrition in man, in relation to vitamins, may be considered from two general aspects:

(1) The production and maintenance of perfect health and optimal growth;

(2) The prevention or cure of specific deficiency diseases.

One reason for this suggested differentiation is the fact that a deficient vitamin intake may not immediately result in actual disease, but may bring about abnormal development of specific structures which makes disease of these parts ultimately almost inevitable. Striking instances of this are given in Chapter IV, where evidence is adduced to show that a deficient intake of vitamin D in infancy may bring about defective formation of teeth, and subsequent increased susceptibility to dental decay, while deficient vitamin A intake may cause malformation of the gum epithelium, and may lead to subsequent development of periodontal disease, including pyorrhoea alveolaris. Undoubtedly there are many other instances where a deficient intake of vitamins is related to disease in this indirect way.

So far as Western civilization is concerned, it is no doubt true that the rareness of the occurrence of frank deficiency diseases, such as scurvy, xerophthalmia, and beri-beri indicates that an absolute deficiency of vitamins scarcely ever exists in the individual diet. On the other hand, it is now becoming generally recognized that much subnormal health and development, and even incidence of disease, are associated with a partial deficiency of one or more of these

accessory substances. The influence of such partial deficiencies, even when relatively slight, may be extremely serious when they occur in very early life, and, if we may judge from the results of experiments on animals, an adequate supply of these indispensable dietary components later in life may fail to make good the damage caused by a deficiency in youth. There is also danger that the effects of such a partial or latent deficiency may persist as a chronic condition throughout adult life. It is difficult to provide convincing evidence of this, since the well-known symptoms which characterize the disease may not be present, and the only method of clinching the diagnosis would be to make widespread therapeutic trials with the vitamin or vitamins suspected to be deficient and to observe whether general improvement in health took place. Green and Mellanby [1930] have pointed out how wide is the margin between the doses of vitamin A which will just maintain a rat in life, if it is not attacked by infection, and the dose which will wholly prevent those infections. The latter dose is about four times as great as the former. Animals receiving doses between these maximum and minimum amounts, while showing no overt symptoms of vitamin A deficiency, yet suffer from a partial or latent deficiency and are proportionately liable to infection in consequence. This example serves to show that a *latent deficiency disease* is a real thing and not an imaginary concept.

The optimal intake of vitamins depends greatly on many conditions influencing the human organism. These include the age, the rate of growth, the climate, the degree of exposure to infection, the composition of the diet as a whole, and the degree of sunshine to which the body is exposed.

It is probable that the adult, at any rate relative to his size, has smaller requirements for vitamins than the growing child, but we have little knowledge of the absolute requirements of either for any given vitamin. The diet of the adult is so varied, and different samples of the same foodstuff vary so greatly, that it would be difficult to assess the vitamin value of any particular diet. The diet of infants is simpler and it is possible to express their vitamin needs roughly in terms of cow's milk. About 500–1,000 c c. (1–2 pints) of raw cow's milk provides enough or nearly enough of the vitamins A and C and of the B-vitamins of the yeast complex, but not enough of vitamin D. Cow's milk, however, is so variable in composition that this statement may need qualifying, and does not provide more than a slender basis for calculation.

There are certain times when special demands are made on the metabolism, as, for instance, during hard physical work. Thus it has been observed that both beri-beri and scurvy are more rapidly contracted by men engaged in hard manual exercise than by others receiving the same diets who are not so engaged. This should be borne in mind when dietaries or rations are being planned for those engaged in severe manual work, such as soldiers and labourers. Pregnancy and lactation in women and growth in children also call for larger supplies of the vitamins.

The fact that outspoken deficiency diseases are almost unknown in this country has occasioned much scepticism in the minds of some

people as to the importance of vitamins in human dietaries. It is true that the average diet includes a large variety of foods, so that a deficient intake of any particular food factor appears improbable. This conclusion may, however, be misleading. In the first place it does not take into account the times and circumstances mentioned above, when the vitamin demands are enhanced. Secondly, it fails in the realization that what formerly was regarded as an excellent 'mixed diet' may, in reality, have been very defective. When the diet of the poor is considered, the general situation is even worse. Cereals are said to form 60 per cent. of the total food consumed in this country, and in the diet of the poor this percentage is higher because of the relative cheapness of this form of food. Cereals, although they provide the necessary energy, are almost devoid of vitamins A and D and have been shown to have an antagonizing action on the latter, while the high cost of milk, butter, eggs, and vegetables, which are the usual sources of these vitamins, limits their acquisition by the poor.

Vitamin Requirements in Sickness and Convalescence.

In sickness or convalescence special efforts should be made to supply a diet complete in all essentials. If the patient's condition indicates the omission from the diet of certain foodstuffs rich in vitamins or mineral salts, it is the duty of the physician to secure the provision of these from other more suitable sources, possibly from the many artificially prepared concentrates now available. It happens too often that a diet, which has been temporarily restricted through a real necessity, is continued for long periods after the disturbance has subsided. In planning restricted diets which may be taken for long periods, sight should never be lost of the composition of the diet as a whole. For example, in illness complicated by digestive disturbances, a patient may show great immediate benefit by receiving a restricted, but easily digested, diet. At the same time if this diet is continued indefinitely his chance of throwing off the morbid condition altogether may be destroyed or indeed a fresh constitutional weakness may be set up.

For example, two patients suffering from ulcerative colitis, and receiving a diet restricted to vegetable soups, cereal gruels, and meat broth, were observed by Crohn and Rosenberg [1924] to develop a corneal and conjunctival inflammation, similar to xerophthalmia. The patients recovered promptly on a changed diet. Crohn and Rosenberg emphasize strongly the importance of giving a complete diet in ulcerative colitis. Treatment of this disorder often necessitates omission of cellulose-containing items from the diet and with these will be excluded the vegetable sources of vitamin A; if fats are also reduced, the diet will become one upon which xerophthalmia is likely to develop. In such cases administration of one of the therapeutic concentrates of vitamin A would be the best safeguard.

The importance of a complete diet in sickness is much enhanced in the case of infants and young children. Infants suffering from severe digestive disturbances are often relieved by administration of a diet containing large amounts of sugar, such as sweetened condensed milk. The soluble carbohydrate is easily digested, the infant

receives adequate calories for nourishment and the digestive system is relieved. Such a diet, however, must be regarded in the light of a temporary expedient only, for, if continued for any length of time, it may lead to a condition of malnutrition caused by an insufficient intake of vitamins. This may be shown by symptoms of rickets, delayed development, or indeed, a diseased digestive system, this time due to causes other than those responsible for the original upset. Another example is afforded by 'Eiweiss-milch', the well-known preparation given to dyspeptic infants, which was found by Hess to be an excellent diet for producing rickets. It is perfectly clear that, if fat is removed from the diet of cases diagnosed as fat dyspepsia, an effort must be made to avoid the deficiency by supplying some rich sources of vitamins A and D.

The following case [cited by E. Mellanby, 1924] illustrates the cumulative effect of partial vitamin deficiencies continued over many years. A patient at the age of 9 was first treated for dyspepsia by removal of all fat from the diet. This treatment not being efficacious, all vegetables were then excluded and this was followed from time to time by other exclusions until, at the age of 19, the patient's diet consisted largely of bread and other cereals. By this time, she had developed adult rickets with considerable leg deformity, and severe scurvy with spongy gums, loose teeth, and large haemorrhages over the whole body. When the diet was corrected in the obvious way, great improvement followed, but in later life the patient still occasionally had attacks of tetany.

Schick and Wagner [1923] have described a case of xerophthalmia in a boy 17 months old in Vienna. This patient was suffering from an intestinal infection and for this reason the amount of fat in the diet had been minimal.

Fat dyspepsia is undoubtedly a reality and for such cases it is necessary that the fat intake should be greatly diminished. In view of the many vitamin concentrates at present available there is no need for the fat-soluble vitamins to be also reduced. If this is done, further ill can only result. Similarly, fruit and vegetables may be temporarily reduced for special reasons during illness, but it is usually advisable that the period of reduction should be as brief as possible. As a general rule these foods should be given in abundance in sickness and convalescence, and if necessary, may be presented in a purée form. A method has been described of preparing a vitamin-containing vegetable extract, from mixed vegetables, for hospital use. The roughage fraction, previously separated, can be restored to any amount if desired [Warren and Nissen, 1931].

In planning diabetic diets, also, the same care should be taken to secure the inclusion of adequate amounts of all the vitamins. Wohl [1926] had under his care a diabetic patient whose diet had been ill-planned and whom he considered to be suffering from both xerophthalmia and beri-beri.

Indeed far too little attention has been given in the past to the consideration of diet in disease; from whatever illness he is suffering, the patient's chances must be greatly enhanced in so far as it is possible to give him continuously everything which his body needs.

Vitamin Deficiencies and Disease.

Vitamin A.

It is noteworthy that milk, cream, butter, and, in particular, cod-liver oil (all rich sources of vitamin A or of vitamin D or of both) have long been included in the treatment of diseases of malnutrition and of tuberculosis. In this fact there is an illustration of the results of scientific investigation justifying the selections based on long practical experience.

Although the value of animal fats as sources of vitamin A is so well established, it would not appear that 'vitamin A' from plant sources is at all inferior in value from the dietetic point of view. It is probable that all over the world, except perhaps in very high latitudes, the poorer sections of the community rely chiefly upon carotene-containing vegetables as their main source of vitamin A. Such vegetable products are very widely distributed and it therefore comes about that no community is completely devoid of them except in times of drought and famine. A florid development in adults of the symptoms of absolute deprivation of vitamin A is therefore rare. Such florid symptoms as do occur in the human being are those of xerophthalmia, hemeralopia, and presumably of different infections, following upon hypoplasia and cornification of the mucous tract, as seen in the rat.

The length of time during which man can be deprived of vitamin A before developing symptoms of xerophthalmia is uncertain and by analogy with the rat must vary greatly in different individuals. It will depend upon the degree of deficiency of the diet and the extent of the reserves of vitamin A which the individual has stored up, chiefly in the liver. These reserves will be determined by the individual's previous dietary history.

Vitamin A in Food.

The provision of an adequate supply of vitamin A in the diet does not, except in certain circumstances, present such a difficult economic problem as does that of certain of the other vitamins. Animal sources of vitamin A, such as milk-fat, eggs, mammalian liver, and cod-liver oil, are expensive, but when it is remembered that this vitamin is supplied by all green vegetables and others which contain the yellow colouring matter carotene, the problem can be cheaply solved wherever vegetables can be grown. For example, green spinach and green cabbage (but not white [Coward and Drummond, 1921]) are weight for weight as good as the best New Zealand butter [Hume and Smith, 1930]; carrot also is excellent [Moore, 1929, 2]; yellow maize [Steenbock and Boutwell, 1920, 1], yellow peas [Steenbock, Sell and Boutwell [1921] are all good sources while similar articles of food, without the yellow pigment, are valueless in this particular. Red palm-oil is very rich, being equivalent to a cod-liver oil of more than ordinary strength, and if it can be made palatable may prove an excellent source of vitamin A. Some margarines can now be obtained in this country, at a very small increased cost, which have been fortified with vitamins to make them equal in this respect to butter.

Both vitamin A and carotene, as contained in natural foodstuffs,

keep well unless exposed to conditions which favour oxidation; light encourages oxidation but ordinary cooking does little damage. Even after air has been bubbled through cod-liver oil for four hours at 100° C., the oil still retains considerable activity in the cure of xerophthalmia, although similar treatment for 12 hours completely inactivates it [McCollum, Simmonds, Becker and Shipley, 1922]. Storage and transport do not therefore constitute a problem in maintaining a supply of this vitamin. Under some conditions oxidation seems to be promoted during the drying of the vegetables; thus, alfalfa hay which had been allowed to lie in the field for ten days exposed to sun and every kind of weather was found to be inferior to alfalfa hay cured in the dark [Steenbock, Nelson and Black, 1924–25]. It would not therefore be wise to rely blindly on dried vegetables as a source of vitamin A, although some such preparations are rich in this respect.

Xerophthalmia and Hemeralopia.

Xerophthalmia in young children. The xerophthalmia of rats fed on diets deficient in vitamin A was examined carefully by Knapp [1908], who appeared to appreciate the relationship it bore to certain forms of conjunctivitis in children, usually ascribed to faulty nutrition. He was, of course, unaware of the existence of the then undiscovered fat-soluble vitamins, but a study of the rations which he employed shows that they were deficient in that respect.

M. Mori [1904] described a form of external eye disease prevalent among infants in Japan, the symptoms of which bear close resemblance to those shown by rats deprived of the fat-soluble factors. In his work, also carried on prior to the discovery of vitamin A, he approached very near to the correct explanation when he attributed the condition to a deficiency of fat. He states that cod-liver oil was a useful prophylactic, whilst olive oil and sesame oil were useless.

More recently, Bloch [1917, 1, 2, 3; 1918; 1921; 1924] and also Monrad [1917] have described a form of external eye disease in young children in Denmark, which they associate with a deficiency of fat. Bloch gives a detailed account of sixty-four cases in babies treated at the State hospital, Copenhagen, in 1912–16. All the patients had been artificially fed, and the most severe and characteristic cases occurred in children about twelve months old who had been fed on separated milk. The eye disease showed every stage of severity from xerosis of the conjunctiva, in the slighter cases, to hardening of the cornea, leading to ulceration, necrosis, and ultimate blindness, in the more severe ones. Treatment of the eye condition in the ophthalmic department of the hospital gave little relief and no permanent improvement. On further study the children proved to be weak, anaemic, ill developed, apathetic and with poor appetite. In some cases, especially those of younger children, the eye lesion was associated with a state of general atrophy. In the older children the general condition was not usually so severe, although the eye condition was frequently worse; there was often distinct swelling, with oedema of the subcutaneous tissues, but no tenderness.

A careful scrutiny of the previous diets of the patients led Bloch to the conclusion that their general ill-health and the diseased state of their eyes might be associated with a lack of fat in their food. Accordingly, while in hospital, they received whole milk, cow's or human, and in addition as much cod-liver oil as could be tolerated. In these circumstances a rapid recovery took place, both of the general weakness and of the specific eye disease. In absence of the cod-liver oil, recovery was much delayed, and although it ultimately took place, it was frequently too slow to save the sight.

Bloch [1918] also described a particularly interesting outbreak of eight cases of xerophthalmia which took place in a philanthropic institute in Copenhagen in the spring of 1917. The institution, which contained eighty-six inmates, was primarily intended for healthy children up to two years, but a number of ailing children were also included; these were housed in a separate building with the infants, and in this department (section A) full milk was supplied; the children all did well and no case of xerophthalmia occurred. The second department of the institution (section B) contained thirty-two older children, who were again divided into two sub-sections, B1 and B2. The eight cases of xerophthalmia all occurred in one sub-section (B1).

The diet, which was considered liberal and satisfactory, was the same throughout section B, with the exception that at breakfast the children in B1 had rusks and gruel and those in B2 a beer-and-bread porridge served with *whole* milk. The mid-day meal consisted of soups or gruels made with milk, butter-milk, fruit syrups, barley, &c., boiled fish, minced meat, and mashed potatoes. The other meals consisted of cocoa, milk food, and various kinds of bread and margarine. The margarine was prepared from vegetable fats and the milk was all half-skimmed. No butter, eggs, or cream were used in this section of the home, and the only *whole* milk was that taken with the breakfast porridge in the one sub-section, B2. This small amount, however, seems to explain the fact that none of the eight cases of xerophthalmia occurred in sub-section B2.

These eight cases of xerosis of the conjunctiva were at first considered to be slight conjunctivitis, and local treatment with zinc salts was tried but with little result. It was then noticed that, whereas the growth and development of the children in section A had been satisfactory during the winter and spring, it had been subnormal in many instances in section B, in so far that many children had remained stationary or had lost weight during the recent months. This was true of the eight children who had developed xerophthalmia. With the exception of two cases, the eye condition was not severe. There is no mention of general oedema, and the children, who otherwise appeared to be fairly well, though weak and without appetite, were allowed to be up and about. No change was made in the diet, except that 10 g. of cod-liver oil were given twice daily. In the course of a few days the xerophthalmia improved, and within eight days was cured. At the same time the children gained weight, and improved in general health. No other cases of xerophthalmia developed. The skimmed milk in the diet was afterwards partly replaced by full milk.

The author considered this disease to be one due to lack of the specific fat-soluble growth factor, and not to the lack of fat as such, which in the above instance had been supplied as margarine. The diet was greatly deficient in fat-soluble vitamins, and cures ensued rapidly when cod-liver oil was administered.

In many of the other cases of xerophthalmia described by Bloch, which also showed marked oedema, the children had been nourished on diets consisting too largely of carbohydrates, and had been deprived of all fats. The author, therefore, makes the suggestion that lack of fat may itself be the cause of this and other oedemata arising from malnutrition (see, however, p. 122).

Bloch considers this eye disease to be identical with the 'Hikan of Japanese children described by M. Mori [1904], and successfully treated by the administration of cod-liver oil. The severer manifestations leading to blindness he ascribes to superimposed infections. Throughout the work he calls attention to the frequency with which other diseases, such as diarrhoea, bronchitis, pneumonia, pyuria, discharges from ears and nose, and catarrhs of all sorts, were found associated in these patients with xerophthalmia, showing a lowered resistance to infection resulting from their malnutrition.

A critical examination of 434 cases of keratomalacia in children and of 19 cases in adults which occurred in Denmark during the years 1909–20 has shown that it was due chiefly to lack of vitamin A in the diet and in the case of the children to the increased requirements for this vitamin involved in rapid growth [Blegvad, 1923]. This deficiency was explained by the large consumption of vegetable margarine in place of butter by the nursing mothers, as well as by the use of diets poor in vitamin A for the children (separator-skimmed milk, butter-milk, oatmeal gruel, barley gruel, &c.). During the year 1918 and part of 1919, however, no vegetable margarine was manufactured in Denmark. Butter was rationed and was eaten by every one, with the result that the cases of xerophthalmia fell to a very low level, rising again in 1920 with the re-introduction of vegetable margarine (see Table XVI).

TABLE XVI.

Incidence of Xerophthalmia in Children in Denmark.

Date	1909	1910	1911	1912	1913	1914	1915	1916	1917	1918	1919	1920
No. of cases	20	26	20	40	40	48	53	78	71	9	4	25

The greatest number of cases was observed in the winter and spring months and this was attributed to the seasonal variation in the vitamin content of the milk supply, to which attention has already been directed (p. 219).

The facts cited above must not be interpreted as an unqualified condemnation of skimmed and separated milk as an article of diet. For infants and young children it should never be used as a substitute for *full* milk, but for older children and those of school age it forms a cheap and valuable source of protein and of salts, and should be largely used in poor families which can only afford to provide small amounts of whole milk for their children after the period of infancy.

Hemeralopia, keratomalacia, and xerophthalmia in adults. In older children and adults receiving defective diet, hemeralopia or night-blindness has often been observed in endemic form and this appears to be an expression of a mild deficiency of vitamin A. The different symptoms exhibited in the varying degrees of severity of this disorder are described by Pillat [1929, 2]. It is suggested that the night-blindness is caused by a failure in the regeneration of the visual purple, after its bleaching by strong light. Fridericia and Holm [1925] were able to demonstrate this for rats starved of vitamin A. Exposure to a strong light, by accelerating the bleaching of the visual purple, should therefore precipitate an attack of night-blindness. Aykroyd [1930, 2, see below] narrates several cases where this seems certainly to have been the case.

In adults suffering from night-blindness there are often only slight physical alterations to be seen in the affected eyes, few showing typical xerosis of the conjunctiva [Meissner, 1919]. In the case of a family of undernourished children observed at the University Kinderklinik, Vienna, in May–June 1921, night-blindness was accompanied by marked xerosis of the conjunctiva in a boy and girl aged 8 and 9 years respectively (Wagner, unpublished observation).

This form of night-blindness, which has been termed deficiency-hemeralopia (Ausfallshemeralopie, Zak), was common during the late war among Austrian prisoners of war in Russia and was studied by various Austrian physicians, themselves prisoners [Zak, 1917; Meissner, 1919; Hift, 1918]. They found the disorder to be a frequent one among Russian peasants in peace time, especially in the period of religious fasting before Easter. During this period all animal foods were forbidden, including fish, eggs, milk, and butter; the diet enjoined was strictly vegetarian and included fats and oils of vegetable origin only. The curve of incidence of the disease showed a sharp peak in spring. Lightly cooked liver or cod-liver oil were the popular remedies for the disease, and their striking effect was confirmed by the Austrian observers. Hift [1920] found that the night-blindness could be cured within two or three days by administration of cod-liver oil, Zak [1920] by one or two meals each consisting of 150 g. of liver, by 3 eggs given daily for four days, or by 400 c.c. expressed juice of carrots taken daily for five or six days.

In case of prisoners of war, hemeralopia was frequently found to accompany scurvy, but many cases, on the other hand, showed no symptoms at all suggestive of the latter disease, and consideration of the type of foodstuffs found to act as specific cures, points definitely to a deficiency of vitamin A as the cause of the disease.

Night-blindness is common in certain districts of Japan, especially among coolies working in rice-fields in summer time, and among Japanese soldiers when undertaking heavy forced marches in summer. From the fact that cod-liver oil has not here proved invariably to be curative (S. Mori, private communication, 1923) it may be doubted whether in these cases the disease is always dietetic in origin. A clearer example is afforded by the small epidemics occurring since the European War among Japanese railway work-

men in Manchuria ; these have usually been localized and associated with some particular boarding-house. The incidence reaches its height in the months of February and March, and S. Mori, who has investigated these outbreaks, considers the scarcity and high price of fish in the winter months to be the direct cause. In the month of April fish at once becomes cheaper and more plentiful and the outbreaks of night-blindness promptly disappear. Cures have been observed to take place within 12–24 hours after administration of cod-liver oil. [S. Mori, private communication.]

The occurrence of night-blindness together with keratomalacia among soldiers in a military camp north of Peiping, in China, is described by Pillat [1929, 1]. Ninety-one cases of eye disease, due to deficiency of vitamin A, were seen ; of these 12 had keratomalacia, 9 in both eyes and 3 in one eye only. The three cases with keratomalacia in one eye were night-blind in the other eye. The diet was adequate in calories and none of the sick soldiers was emaciated, but they had pasty, puffy faces, dry skin, and scanty hair. No mention is made that they were subject to an increased incidence of infections of the mucous membrane, although the degree of deficiency must have been severe. The soldiers' diet was rice, corn, millet, very little flour, a small quantity of meat once a month and no green vegetables at all. In the case of two of the soldiers, it is mentioned that their eyes became much better after they had partaken of three meals of green vegetables. Pillat considers that severe cold and heavy physical labour were factors contributing to produce the state of deficiency (see p. 227).

Wille [1922] noted the frequent occurrence of xerophthalmia among inmates of institutions in the Dutch East Indies, where coco-nut oil was the chief source of fat in the dietary. He found that the giving of 100 g. of ox liver three times a week to such individuals effectively prevented the disease.

An interesting case is described from Guatemala [Macphail, 1929] where the lack of opportunity to eat bananas seems to have been the factor which differentiated a group of workers developing night-blindness from those who did not. The sufferers were members of railway gangs whose food was corn cakes, beans, rice, coffee and sugar, with meat once a week. The disease only occurred among men working on those parts of the line which did not pass through banana country. Milk was used as the cure.

Night-blindness is not unknown in England at the present time. In the ophthalmological department of an infirmary in the north of England seventeen cases of xerophthalmia and night-blindness were seen in the course of one year among a total of about 4,000 patients. Treatment with adequate doses of cod-liver oil, butter, and milk was uniformly successful. This observation [Spence 1931] may indicate a latent deficiency of vitamin A among our population which is more widespread than is usually recognized.

Aykroyd [1930, 2] has described conditions in Newfoundland in which night-blindness is developed with considerable frequency, while xerophthalmia and keratomalacia are apparently unknown, so that the deficiency is either too short in duration, or insufficiently severe in degree, to produce these symptoms. In Newfoundland,

those who become night-blind are generally men pursuing the summer fishing, but the condition can occur at other times of the year and sometimes also among pregnant women. The diet of the inhabitants of Newfoundland and Labrador seems to border on deficiency in more than one particular, since beri-beri, scurvy, and night-blindness have all been observed. The diet of fishermen when they are living on board ship or in huts far from their homes is even more restricted than that of the other inhabitants. The ship's stores usually consist of white flour, ship's biscuit, salt beef and pork, molasses, margarine, potatoes, beans, and peas. Very few carry fresh vegetables, eggs, or milk. The only sources of vitamin A therefore are the beans or peas, of which the carotene content may vary considerably.

In several of the cases which Aykroyd saw and treated, the onset of the disease was immediately preceded by an exposure to strong sunlight; the ascription of the cause of night-blindness to the failure in regeneration of the visual purple is thus supported. One patient was an old sufferer who protected himself by wearing dark glasses, but developed an attack of night-blindness after being temporarily deprived of them. Another was a boy of eighteen who connected the onset of the trouble with a day of exposure to bright sunshine. The Newfoundlanders have learnt to treat the condition themselves with fresh cod's liver or with cod-liver oil. The rapidity of the improvement is astonishing as was also noticed by Pillat; vision became normal in 12–24 hours after the beginning of treatment, and a single dose of 1–2 oz. of cod-liver oil was found sufficient to bring about a cure. The Newfoundlanders regard the liver of a gull or puffin as even more potent than that of a cod. Aykroyd discusses the antiquity of the treatment by liver. For the treatment of night-blindness Hippocrates recommended that the patient should eat, once or twice, as large an ox liver as possible, raw, and dipped in honey.

Other Pathological Conditions referred to a Deficiency in Vitamin A.

Infections of the mucous membrane. Although experimental work on animals shows that lowered immunity to infection of the mucous membrane, and possibly anaemia, are associated with a partial deficiency of vitamin A, there is little positive evidence to link up these conditions with deficiency in the human subject. Both in Newfoundland and in China the circumstances described are such that a number of individuals must be existing in a state of latent deficiency. By analogy with the rat, mucous membrane infections should be prevalent and both Aykroyd and Pillat should have noticed them. Pillat only mentions that six out of the twelve keratomalacia cases had raised temperatures and Aykroyd (verbal communication) states that such infections were not noticeably frequent. In Newfoundland liability to encounter infection might, however, not be great, owing to the sparsity of the rural population.

The official figures of the Medical Officer of Health for Bombay City in the period 1920–29 show that respiratory infections provide by far the commonest cause of death in a population where cases of xerophthalmia occur [Wills and Talpade, 1930], and where the

conditions are presumably very favourable to infection. No large-scale human tests have, however, as yet been described, in which the possible preventive action of vitamin A against respiratory infection has been tried out under properly controlled conditions. The preliminary results obtained by Green, Pindar, Davies and Mellanby in giving massive doses of vitamins A and D as a prophylactic against puerperal sepsis are described elsewhere (see p. 270).

Stone in kidney and bladder. There is no doubt that in rats stones in the kidney and bladder are produced on diets low in vitamin A but not absolutely devoid of it [Osborne and Mendel, 1917, 4; Fujimaki, 1926; McCarrison, 1926–27; 1927–28; 1929–30, 2]. An infection of the mucous membrane is, however, probably a necessary preliminary. At present there is no evidence to connect the aetiology of urinary calculi in rats with that of stone in human beings. Newcomb and Ranganathan [1929–30, 1, 2] have collected urinary calculi from patients in India, together with a medical and dietary history of each case. They have analysed the calculi and compared their composition with those of calculi from rats and find the compositions to be different in the two series. In human stones uric acid and urates are very common, the content of CaO is high and the content of MgO low, and there is much oxalate and little carbonate. In stones from rats there is no uric acid or urate, the content of MgO is high, and of CaO low, there is much carbonate and little oxalate and more P_2O_5 than in the human specimens. Unfortunately the case histories have not yet been analysed, but McCarrison [1929–30, 1] mentions that stone is commoner among wheat eaters than among rice eaters in India, and he also finds whole wheat flour much more productive of stone in rats than rice. Butter and milk [1929–30, 2; 1927–28] he finds preventive, doubtless due to their vitamin A content.

Anaemia. Mention of the possible part played by a vitamin A deficiency in promoting anaemia should not be omitted. Keefer and Yang [1929] discuss the relationship of vitamins to the production of anaemia in human beings and point out that 'all of the so-called deficiency diseases may exist without anaemia'. They emphasize the point that a diet which is sufficiently lacking in one factor to produce a deficiency disease is also liable to be lacking in other factors and the combination may be liable to promote anaemia. Berglund, Keefer and Yang [1928–29] speak of cases of anaemia in China, coupled with diarrhoea, where the diarrhoea responded dramatically to treatment with cod-liver oil. Less severe cases of a similar anaemia in pregnant women responded quickly to a generous well-balanced diet. The giving of cod-liver oil to patients with other forms of secondary anaemia had no effect on the course of the disease.

Evidence suggesting a causal relation between vitamin B deficiency and certain forms of anaemia is discussed on p. 252.

Vitamin D.

Inter-relation of Light and Diet as Sources of Vitamin D.

Vitamin D is richly present in cod- and other fish-liver oils; it is also present in the fat of egg yolk, butter fat and, to some

extent, in other animal fats and vegetable oils (for margarine enriched with vitamins, see p. 230), but not in lard. Milk is not a very rich source, even when the cows have been much exposed to sunshine. Most foodstuffs have their content of vitamin D much increased if exposed to ultra-violet irradiation.

Vitamin D, like vitamin A, in so far as it occurs in foodstuffs, is possessed of great stability during storage and transport, but is more resistant to oxidation than vitamin A (see p. 24). Oxidation is favoured by light and high temperature. The very low content of vitamin D in most natural foodstuffs has raised a doubt as to whether we depend at all on our food for our supply of this vitamin, or whether it is not exclusively obtained by the action of sunshine. The outbreak of osteomalacia (see pp. 48, 240), which occurred in Vienna as a sequel of the War shows clearly, however, that in winter we do depend on our diet for our provision of this vitamin. It is quite certain that famine was the cause of the disease in Vienna. It was truly called hunger-osteomalacia, but it was equally truly cured by summer sunlight and could also have been cured by ultra-violet irradiation from an artificial source. The immediate causative factor in this outbreak, however, was deterioration in the diet and not any reduction in the supply of sunshine.

It may also be deduced from the experience of the Vienna outbreak that the sunlight enjoyed in the summer under the ordinary conditions of life in temperate latitudes is not enough to ensure protection during the subsequent winter and that for this purpose reliance must be placed on diet. Although it is difficult to discriminate between the relative importance of light and diet as sources of vitamin D, it is often possible to decide which deficiency was the cause of any given outbreak, though the patients could be cured by a supply of vitamin D from either source. Thus, while failure in diet conditioned the outbreak in Vienna, the custom of purdah is the factor which determines the development of osteomalacia in India, since in that country men who eat the same diet but do not keep purdah are not affected. On the other hand it should be remembered that the occurrence of osteomalacia might be prevented in India by dietary alterations as well as by the abolition of the purdah. It would, however, probably be necessary to include something as potent as cod-liver oil in the diet to protect people as severely starved of ultra-violet irradiation as women living under conditions of purdah in India.

Light and diet should be regarded as factors which supplement one another in the supply of vitamin D. Where the ultra-violet irradiation is optimal less support is needed from the diet, but further North, as the sunlight becomes progressively less intense and the body more completely clothed, more and more does the function of protection pass from the light to the diet. Where adaptation is complete the diet adequately fulfils its function, as, for example, among the Eskimo who on their natural diet of meat, fish and oil are healthy and have good teeth, while their breast-fed babies have no rickets. When they change to a 'civilized' diet, while still living in the same latitudes, they are no longer adapted, the health deteriorates, the teeth decay, and the babies get rickets.

Kloster [1931] has published an interesting study of the incidence of rickets in a collection of villages and hamlets on the Varanger Fiord in Norway, situated within the Arctic circle. The population, which subsists on the fishing industry, belongs to three different races, Lapps, Norwegians, and Quains (Finlanders). Signs of rickets were present in 163 (43·4 per cent.) out of 376 children under 7 years of age and in 60 (13·3 per cent.) out of 451 attending school. The villages inhabited by Lapps showed generally the lowest incidence of rickets, in spite of greater poverty and a lower standard of housing, cleanliness and general hygiene. This is attributed partly to the habit of using reindeer skin as clothing, which enables the Lapp women and the elder children to spend more time outdoors in the cold weather, but chiefly to the fact that the Lapps received a larger supply of vitamin D in their diet. This is supplied by the very large quantities consumed of fresh fish (cod, haddock, and coal fish, up to 1 kg. per head daily) served with the liver oil (up to 70 g. per head daily) and fresh liver. The incidence of rickets was highest (present in 73 per cent. of children below school age) in a relatively well-to-do village (Skallelv) inhabited by Finlanders who enjoyed a 'better' diet including meat and milk (average amount *ca.* 500 g. daily) and consequently a better supply of calcium salts and of vitamin A, but much less fresh fish, fish-liver, and liver oil.

A study of ancient skeletons suggests that osteomalacia, which through the deformities caused in the pelvis has a crippling effect on reproduction, was the cause of the extinction, about the end of the fifteenth century, of the Norse colony founded in A.D. 985 by Eric the Red at Herjolfsnes in Greenland. If this interpretation is correct, the disappearance of this colony affords a clear case of extermination through failure to adapt the diet in such a manner as to supplement the lack of ultra-violet rays from the sun in those high latitudes [Maxwell, 1930].

In Britain, at the present day, the adaptation of diet to climate is incomplete; osteomalacia is very rare, but bad teeth are almost universal and rickets is a common disease.

A full account of the aetiology of rickets is given elsewhere (see Chapter III), together with a description of the animal experiments which played so large a part in elucidating the problem.

Osteomalacia.

The dietary experiments unfortunately provided by the late war, together with excellent surveys in India and China, have placed it beyond doubt that osteomalacia in the adult is analogous to rickets in the child. Both represent states in which there is a breakdown of calcium metabolism due to an insufficient supply of vitamin D derived either from food or from the action of light; both are curable when vitamin D is supplied in either of these ways.

The essential feature of the disease in adults is a loss of calcium from the bones, which leads to their softening and consequent deformity. McCrudden [1910] found that the total ash of a bone from a case of osteomalacia was much reduced, and the calcium was reduced to a greater extent than the phosphorus. He determined the calcium

balance in a series of eight cases and in each instance found that calcium was being lost to the body. Miles and Feng [1925] found that the calcium content of the serum was below normal in osteomalacia and in most cases which they investigated there was a constant loss of calcium from the body.

The most striking clinical feature of this crippling, painful, and even dangerous disease is a progressive deformity of the skeleton. The disease is particularly liable to appear during pregnancy, but it often also occurs in girls at the age of puberty; males are more rarely affected. The geographical distribution of the disease differs from that of rickets. It is not common in temperate climates, and is met with most frequently in India and China; numerous cases have been reported, however, from the Balkan States. Its sociological distribution throws some light on its aetiology. In India and China it is commoner among the relatively well-to-do than among the very poor; in India it affects chiefly Mohammedan and high-caste Hindu women who practise the system of seclusion known as purdah. In the Balkans it is confined almost entirely to the Mohammedan population.

In 1919–20 hunger osteomalacia, or, as it was then called, 'Knochenerweichung' or bone-softening, broke out among adults of the poorest class in Vienna [Dalyell and Chick, 1921]. The disease chiefly affected the middle-aged and old people of either sex; it was not observed in pregnant women, possibly because they were provided with better food. All the sufferers were extremely poor, though sometimes belonging to the middle class. The symptoms were pain on movement, which produced a waddling gait with special difficulty in mounting stairs, severe pain in the sacral region on pressure or movement and pain in the ribs on compression of the thorax; tetany was frequently present. Observations on dietetic therapy showed that little improvement took place on addition of sugar or cereals to the diet, even in cases where it had previously been very low in calories. Cod-liver oil was by far the most efficient therapeutic agent.

The seasonal incidence of the disorder was very striking [Hume and Nirenstein, 1921]. The disease was first noted in the late autumn of 1918 and the number of fresh cases increased steadily during the winter, reaching a maximum in April 1919. On the approach of summer there was a rapid decline to a low level which continued throughout the autumn; in January 1920, fresh cases began to appear and a maximum was again reached in April. In the late autumn of 1920, coinciding with great improvement in the food supplies of the city, the disease disappeared almost entirely. Though it was not fully realized at the time when the observations were made, it is now quite clear that the seasonal incidence of the disease was due to the seasonal variation in the amount of ultra-violet radiation from the sun.

In India and in China the conditions in which osteomalacia occurs are different from those which obtained in Vienna. In Vienna the epidemic was the result of a dietary emergency which did not recur; in India and China the disease is caused by social customs or conditions which tend to be only very slowly remedied, and

consequently is endemic. In both these countries the sufferers are chiefly women and very largely pregnant women, pregnancy supplying the extra strain on the calcium metabolism needed to precipitate the disease. In the Kangra Valley in India, however, men and boys are also affected [Wilson and Surie, 1929–30].

In India osteomalacia is associated with the custom of purdah, [Stapleton, 1925; Vaughan, 1926; 1928], which confines women to the house and prevents their exposure to ultra-violet radiation from the sun. At the same time, the diet, rich in cereals, poor in meat and genuine milk fat, and quite devoid of liver oils, is not adapted for a life without sunshine [Scott, 1916–17; Green-Armytage, 1928]. The disease is cured by administration of cod-liver oil or by exposure to sunlight [Vaughan, 1926, 1928; Green-Armytage, 1928]. Vaughan mentions the interesting point that in Kashmir there are three indigenous cures for 'trouble in the bones'—a special clay called Baramulla earth, which contains 16·2 per cent. of calcium phosphate; pills made of fish-liver; rubbing the body with mustard oil and exposing it to sunlight. In the cases from the Kangra Valley it is claimed that the sufferers were not deprived of sunshine and that the deficiency must be a dietetic one; possibly there is an insufficiency of calcium or phosphorus, since addition of fresh fruit and vegetables to the diet hastened the cures when vitamin D was given *per os* [D. C. Wilson, 1930–31, 1].

In Northern and Western China the disease is also endemic among women. The diet is very poor, consisting almost entirely of cereals, chiefly millet, a limited amount of vegetables and no milk, meat, or eggs [Miles and Feng, 1925; Maxwell and Miles, 1925]. Maxwell [1930] correlates the increase of osteomalacia in the province of Shansi, where it is most frequent, with an enormous increase in the growth of the poppy and in the use of opium, which keeps its users indoors. Binding of the feet, the customs of the people, and the extremely disturbed condition of the country also play a part in keeping the women indoors, while the disturbed political conditions also operate to diminish the live stock and in general to deteriorate the food supply. A deficiency of calcium in the diet is probably here also contributory. The disease yields to the various forms of vitamin D therapy.

It now seems probable that the rare cases of the osteomalacia of pregnancy described in England, N. Germany, and the United States, of which the aetiology was previously obscure, are a sporadic expression of the same disease. Starlinger [1927] describes the dramatic cure of a woman who had suffered for fourteen years from osteomalacia which became progressively worse after repeated pregnancies till she was almost entirely bedridden. Treated at last with vitamin D, as irradiated ergosterol, she showed marked improvement after twelve days and got rapidly better. There is nothing in the case history to show why this woman should have fallen a victim to osteomalacia, but it is easy to imagine that a woman, who combined little liking for outdoor exercise with a distaste for animal fats, might during pregnancy fall below the margin of safety. Many women tend to remain indoors during pregnancy and to enjoy little exposure to sunlight, so that with the

added drain of the foetus, it is more than probable that they are in negative calcium balance. According to the old wives' saying: 'A tooth is lost for every child which is born.' Pregnant women should therefore take special steps to combat this tendency by securing for themselves an increased supply of vitamin D, either in their diet or through sunlight or other forms of irradiation.

In some of the districts (e.g. India [D. C. Wilson, 1930-31, 2]) where osteomalacia is frequent, girls of 10–17 years are liable to develop a mild form of the disease which appears to be identical with the condition known as late rickets sometimes encountered in this country. There appears in fact to be no definite separation of the two conditions, late rickets sometimes merging into severe osteomalacia.

The conditions of puberty, pregnancy, and lactation seem to demand an increased supply of all essential factors including vitamin D, and serious disturbances in the mineral metabolism of the body may result if the necessary minimum quantity is not provided.

Vitamin D and the Supply of Calcium.

A dietary factor which cannot be omitted in considering vitamin D is the element calcium. Vitamin D is concerned with the metabolism of calcium and operates to secure the most economical use of the supply of this element in the diet. From an analysis of a number of typical American dietaries, Sherman [1920, 2] concluded that the calcium intake of one person in six fell below the calculated requirement. Mann [1926] in studying the effect of various additions to the dietary of boys living in an Institution found that growth was best promoted by the addition of one pint of whole milk a day (see p. 217). Similar results were obtained by Orr [1928], Leighton and Clark [1929], and Clark [1929], when additions of whole and of separated milk respectively were made to the diets of children attending the elementary day-schools in different towns in Scotland. The fact that the separated milk proved as beneficial as the whole milk suggests that the effect might be referred to the mineral content of the milk rather than to the vitamin D content, which in any case would be small. The extra supply of calcium salts would, however, enhance the value of any vitamin D present in the diet.

In a survey of the diets in certain Scottish towns [Orr and Clark, 1930], it was found that in 24·5 per cent. of the families the supply of calcium fell below the generally accepted standard of requirements for adults. For Dundee the values were recalculated on a higher scale, which takes account of the growth requirements of the children in the family. On this standard the necessary intake of calcium for optimum growth was only attained by nine out of thirty-seven families. It is recommended that more milk and green vegetables should be consumed in order to make up this deficiency. Sherman [1920, 2] also recommends that more milk should be consumed, and at the same time suggests that calcium carbonate or phosphate should be habitually added to human food, either separately or in the table salt. The addition

of calcium is also beneficial because it increases the calcifying action of butter and antagonizes the anticalcifying action of cereals [E. Mellanby, 1921, 1925]. Addition of vitamin D at the same time would secure the more economical utilization of any added calcium, but sunshine is the only cheap form in which vitamin D can be obtained. Oranges are particularly rich in calcium though they contain no vitamin D, and possess the advantage that they are eaten raw, whereas green vegetables boiled in water always suffer impoverishment through diffusion of the salts into the surrounding water.

Vitamin B_1 (Antineuritic).

The problems of the aetiology of beri-beri are more fully discussed elsewhere (p. 122) and it suffices here to discuss human diets on which beri-beri in the recognized sense has developed and the measures which have been found satisfactory for its prevention.

Beri-beri as the Result of a Diet consisting too exclusively of an Over-milled Cereal.

Rice is the staple diet of a great part of the poorer population of southern and eastern Asia, and it is in Japan, the Malay States, Java, and the Philippines that classical outbreaks of this disease have occurred. The researches of the investigators mentioned on p. 123 have succeeded in correlating the disease with the consumption of steam-milled, white, polished rice. Where the rice is merely husked and the outer skin and germ are retained, or where the rice is milled in domestic or native mills, so that the separation of these constituents is much less complete, beri-beri does not occur. Further, in localities where it is epidemic, beri-beri has been both prevented and cured by substituting whole rice or rice prepared in the native manner, for the fine white polished rice.

Eijkman [1897, 2] quotes an observation of Vorderman, which points forcibly to the connexion between beri-beri and highly milled rice. This worker made a survey of the prisons of Java and Madura, noting the type of rice consumed and the incidence of beri-beri. His observations extended over a population of more than 279,000 persons. The results summarized in Table XVII show how closely the incidence of beri-beri is correlated with the degree of milling which the rice has undergone.

Table XVII.

Relation between the incidence of beri-beri in Javanese prisons and the type of rice consumed (Vorderman).

Type of rice consumed.	*No. of prisons examined.*	*No. in which beri-beri occurred.*	*Per cent.*	*Proportion of cases of beri-beri among total no. of inmates.*
1. 'Half polished'; ¾ of the 'silver-skin' adherent.	37	1	2·7	1 in 10,000
2. ⅓ of the 'silver-skin' adherent.	13	6	46	1 in 416
3. 'Polished;' less than ¼ of the 'silver-skin' adherent.	51	36	71	1 in 39

Apart from these general observations, there have been a few definite human experiments, which may be cited here. Strong and Crowell [1912] obtained volunteers from the prisoners in a jail in the Philippine Islands, and these were divided into four groups placed upon strictly controlled diets. All received a certain amount of fish, bacon, lard, bananas, potatoes, and sugar, but rice was the staple article of diet and was provided in three different forms for the four groups of prisoners. The results were as follows:

TABLE XVIII.

Type of rice.	*No. of men.*	*Cases of beri-beri.*
Group 1. White polished rice and extract of rice polishings (bran).	8	2
Groups 2 and 4. White polished rice.	17	13
Group 3. Red rice, i.e., whole unmilled rice. . .	7	1 (very slight)

This experiment suggests that when the diet consists mainly of cereal food, beri-beri can generally be prevented if a whole unmilled cereal is employed, but that there is not a great margin of safety. It will be noticed that the other subsidiary articles of diet were poor in content of antiberi-beri vitamin.

In conflict with the smoothness of the foregoing experience, McCarrison [1924] points out that a century ago, long before the introduction of machine milling of rice, beri-beri was endemic in India, as it still is to-day, in the north-east coast division of the Madras Presidency (Northern Circars). He also shows that in India at the present time the incidence of beri-beri does not exactly correspond to the type of milling in vogue. He believes that the supplemental foods in a diet are most important in determining whether beri-beri will develop or not, whatever may be the kind of rice consumed. It is probable that even a diet of whole untouched rice does not provide enough vitamin B_1 for a man, though it may suffice for a bird. If this be true, it is obvious that beri-beri may occur where the diet consists almost entirely of whole rice, unsupplemented, and may not occur where the diet consists largely of highly polished rice, well supplemented with other foods, rich in vitamin B_1. This argument does not, however, upset the thesis that, since polished rice is poorer in vitamin B_1 than is whole rice, given the same but inadequate supplements, beri-beri is more likely to occur on the diet containing polished rice than on that containing whole rice. It does, however, rather shake the belief that to abolish milling would abolish beri-beri.

Experiments on pigeons [McCarrison, 1924] showed that a series of Indian rices ranged themselves in order of value for 'vitamin B' according to the accepted ideas. Raw milled unpolished rice was highest, then parboiled milled unpolished rice, and parboiled milled highly polished rice, and finally, very much lower, raw milled polished rice. In this system of grading the term 'milled' is used to include the whole process of husking and removing the germ and pericarp.

Any washing of the rice was found to deteriorate its value in

vitamin B_1. The vitamin is water-soluble and evidently diffuses easily out of the cells, when they are plunged in water, particularly when the cells have first been killed by parboiling.

The process of parboiling in the preparation of the rice affects the vitamin content in an interesting manner in two directly opposite ways. After parboiling, the whole grain may not be as rich in vitamin B_1 as before, since some of the vitamin may have diffused from the outermost layers of the grain into the surrounding water. Rice which has been parboiled and subsequently milled, compared with the same sample of grain submitted to the same degree of milling without first parboiling, is however, found to be greatly superior in antineuritic value [Aykroyd, 1932]. Presumably therefore during the process some of the vitamin from the peripheral layers of the grain diffuses inwards, into the endosperm. When parboiled and polished rice is soaked in water the vitamin diffuses out again and can be recovered from the surrounding water [Aykroyd, 1932].

In British Guiana milled parboiled rice is used, and to this fact the absence of beri-beri is attributed.

Outbreaks of beri-beri among wheat-eating populations are not common. The peoples who are accustomed to consume white wheaten bread belong to the more highly civilized and richer countries, where the diet is varied and a sufficiency of the antiberi-beri vitamin B_1 is obtained from other constituents. There are, however, a few instructive instances among which the following four cases may be mentioned.

(1) In Newfoundland and Labrador the population subsists largely on bread during the winter and spring. Formerly, when the bread was baked from 'brown' flour, beri-beri was unknown. At the present time, with the advance of civilization, the bread is made from pure white wheaten flour, and beri-beri is frequent. Little [1912] relates the following interesting occurrence. In 1910 a ship ran ashore laden with a cargo of wholemeal wheaten flour, and, in order to lighten her, a considerable portion of her load was removed and was subsequently consumed by the population in the adjacent districts. The result was that no case of beri-beri was reported in that region for a period of one year following this event.

The conditions in Labrador and Newfoundland have been reinvestigated by Aykroyd [1930, 2]. Beri-beri still occurs and a certain number of the cases show a minor degree of oedema. In winter, in the absence of meat and fresh vegetables, the diet tends to resolve itself into one of white bread and tea. The peak of incidence of the disease comes in May. Aykroyd gives an interesting analysis of the winter stores of those families in which beri-beri occurred, and of a similar set of families, a little better off, in which the disease did not occur. Both consumed an equal amount per head of white wheaten flour, but the non-beri-beri families had in addition more meat, more potatoes, and other vegetables, and on the average twice as much peas and beans added to the diet. In a few cases ignorance seemed to be the cause of the restricted diet, but in most it was sheer poverty.

(2) Beri-beri was a rare disease on Norwegian ships before the year 1894. In that year an alteration was made in the sailors' diet, in response to a popular agitation to ameliorate the hard conditions of their life at sea. The sailors had previously lived on biscuit baked from rye flour (in the milling of which there is no separation of the germ); after this date masters of ships were compelled to supply bread baked from white wheaten flour or from a mixture of wheat and rye flour, and beri-beri became a frequent disease in the Norwegian mercantile marine [Holst, 1911–12].

There is an amusing story of one old sea captain who disapproved of the new-fangled reforms and insisted upon a supply of rye flour being taken on board for his own personal consumption. He was rewarded for his independence with the satisfaction of effecting cures among his men, who, when stricken with beri-beri, were supplied with biscuit from the captain's private supplies. As, however, these gradually showed signs of depletion, he was finally compelled to husband them in order to preserve his own health.

(3) The deficiency in white wheaten bread is under ordinary conditions made good by the varied diet enjoyed by Europeans. If, however, the 'mixed' diet is derived from canned and preserved foods the case is otherwise, and beri-beri may be expected to appear. This was no doubt the cause of the beri-beri which was reported among our troops in the late war in the Dardanelles and in Mesopotamia [Wilcox, 1916, 1, 2]. In the latter campaign it is illuminating to note that the disease was confined to the British troops and was not reported among the Indian soldiers. This immunity was to be expected, for in addition to atta, a coarsely ground whole wheat flour, the native soldier received a generous daily ration of dhal or dry pulses of various kinds, which are rich in antiberi-beri vitamin (see Table XI, p. 162).

(4) The difference between the types of cereal ration issued respectively to the British and Indian troops was the basis of an unconscious 'experiment', which took place during the siege of Kut-el-Amara (Dec. 1915–April 1916). In his account of the medical arrangements during the siege, Hehir [1917] writes: 'In an early stage of the siege, a recrudescence of beri-beri among British troops gave rise to some apprehension, but it then disappeared whilst in Indian troops and followers during the latter half of the siege scurvy caused anxiety.'

At another place in this diary we learn that the British troops were receiving white wheaten flour until Feb. 5, 1916, after which date they were compelled to take part of their flour ration in the form either of barley flour or of atta. It is very significant that beri-beri should have occurred while the British troops were enjoying white wheaten bread and should have cleared up when they were compelled to share the coarsely milled, germ-containing flour of their Indian comrades. The incidence of scurvy during this siege showed an entirely opposite distribution; the British soldiers were protected by the large ration of meat, including horse flesh, which they consumed, while the Indian soldiers, largely vegetarian in habit, suffered terribly from this disease (see also below, p. 249).

The lesson to be learned from the above experience is that, for the prevention of beri-beri, it is important that the germ and bran of wheat should be included in the manufacture of bread or biscuit for any population living on a restricted diet. This is specially desirable in the case of soldiers on active service where the rest of the rations may consist largely of canned foods, which in many cases may be regarded as devoid of the less heat-stable vitamins (B_1 and C), owing to conditions under which they have been sterilized and preserved. Many people, however, will not eat brown bread when white bread is available, but in the case of soldiers such a regulation could be enforced.

Non-cereal Foods in Relation to Beri-beri.

In this connexion attention may be drawn to two articles of diet, especially rich in the vitamin B_1, and also specially suited to the needs of armies on active service, viz. yeast extract and preserved eggs.

Yeast is one of the most valuable sources of the antineuritic factor, and, in the form of an extract, gives the savoury taste to many of the soup cubes at present on the market. One commercial extract (marmite) has been found on examination to retain a considerable part of the original value of the yeast from which it has been prepared (see Table XI, p. 162). The addition of such an item to the soldier's ration affords a useful and agreeable method of increasing the supply of antiberi-beri vitamin in his diet, but no exact data are available as to the amount required for a man.

A very interesting case in which yeast functioned as the one supplementary food which stood between a community and beri-beri is afforded by the experience of the inhabitants of the island of Nauru in the Pacific Ocean [Bray, 1928–29]. The diet did not contain rice as a staple, but consisted originally of fish and coco-nut products, including a fermented drink, 'toddy', made from the flower spathe of the coco-nut palm. The island has been governed since the Peace, under mandate, by the Commonwealth of Australia, and the making of toddy was prohibited. To make up for its loss, the natives tended to use more of the imported foods, refined sugar, white flour, and canned meats. The infantile mortality was extremely high and one of the more important factors contributing to it was finally diagnosed as infantile beri-beri, which had become prevalent since the prohibition of toddy-drinking. Toddy yeast was actually used to cure the babies. Such an example suggests the question how far other native races may depend on their home-brewed fermented drinks for protection against beri-beri and pellagra as well as scurvy (p. 258).

Dried eggs. Dried eggs have been found experimentally to retain the valuable antiberi-beri properties of the fresh article. They are probably much too expensive an article to be included in the ordinary soldier's ration, but there can be no question of their suitability for inclusion in hospital stores. A large proportion of the medical casualties on foreign service is due to intestinal diseases, and both during the acute stage and the subsequent convalescence the diet is largely restricted to bread, condensed or

canned milk, and invalid foods, all of which may be regarded as either free from vitamin B_1 or very poor in it. It was noted by Willcox [1916, 1] that among twenty-six cases of beri-beri diagnosed in the autumn of 1915, eighteen had previously suffered from diseases of the digestive and alimentary system. It is not improbable that the restricted diet necessitated by these disorders may have been the predisposing cause of the beri-beri which occurred subsequently. The addition of eggs would have materially enriched these invalid diets in respect of antiberi-beri properties, and in the form of dried eggs the addition could be made with the greatest convenience.

Before leaving this subject it may prove useful to give as an illustration the details of certain diets which have been found to occasion beri-beri, and to compare them with certain others, which under similar circumstances of climate, locality, &c., were found to be satisfactory.[1]

TABLE XIX.

Showing relative amounts of foodstuffs deficient and rich respectively in antineuritic vitamin present in satisfactory diets and in those found to occasion beri-beri; the amounts are expressed as oz. per week.

Foodstuff.	*Nos. expressing approximate value in preventing beri-beri. Wheat germ = 100.*	*Diet A. (Beri-beri producing)* A 1	A 2	*Diet B (Satisfactory).*	*Diet C (Satisfactory.)*
Foods deficient in vitamin B_1					
Rice		28		21	28
Bread (white flour)		224	224	112	168
Jam		.	.	7	7
Sugar		14	14	7	
Cheese		14	14	14	
Dry fruit		.	.	14	
Salt fish		.	.	20	
Margarine or butter or oil		14	14	7	3·5
X = Total no. of oz. weekly of foods deficient in antiberi-beri factor		294	266	202	206
Foods richer in vitamin B_1					
Oatmeal	10	.	.	14	14
Fresh meat or bacon	10	42	42	30	42
Peas, beans, lentils	50		28	14	21
Potatoes (or fresh vegetables)	5	14	14	42	28
V = Total no. of oz. weekly of foods containing antiberi-beri factor		56	84	100	105
Ratio V/X		0·2	0·3	0·5	0·5

Diets A 1 and A 2, given in Table XIX, were issued during the late European War to a camp in a Mediterranean area in which beri-beri occurred. Although in many ways similar these diets differed greatly as regards the protection afforded against beri-beri. Both, however, were inferior to Diets B and C, which were issued to similar neighbouring communities, in which no beri-beri was

[1] Permission to publish the details of this investigation was originally granted by Major L. Braddon, R.A.M.C., and the Director-General of the Army Medical Service.

reported. After the diagnosis of beri-beri had been substantiated for certain men receiving Diets A 1 and A 2, a careful search among the healthy men of the same community revealed a widespread abnormality in knee-jerks and other nervous reflexes. This is an important observation, as it shows definitely that the normal health may be undermined by a deficiency in diet before any symptoms are apparent to the casual observer or to the individual himself.

In Table XIX an arrangement is adopted by which foodstuffs deficient in the antiberi-beri factor are collected in one group and those more valuable in this respect in a second. The points to be noticed are, firstly, the large preponderance of cereal food (see p. 131) in the diets A 1 (252 oz. weekly) and A 2 (224 oz.) compared with B (133 oz.) and C (196 oz.); and, secondly, the smaller proportion of vitamin-containing foodstuffs. A 1, 56 oz. weekly; A 2, 84 oz.; B, 100 oz.; C, 105 oz.

If the ratio—no. of oz. vitamin-containing food / no. of oz. vitamin-deficient food—be calculated for the four diets, we get a series of values rising from 0·2 for diet A1 to 0·5 for diets B and C. It would seem that this ratio might provide a useful indication of the value of a diet for the prevention of beri-beri.

In an ordinary mixed diet the necessary amount of vitamin B_1 is supplied chiefly in the eggs, vegetables, fruit, milk, and meat consumed, although of these only eggs and green-leaf vegetables can be considered as *rich* sources of the vitamin.

Elaborate preventive experiments on pigeons have been carried out by Jansen in Batavia [1923] with a number of foodstuffs, many of them indigenous. The express object was to ascertain which of them were suitable to supply the antineuritic vitamin B_1 to a diet of polished rice, a matter of great importance in the East. Such were found to be: eggs, meat, bran, and in general the germ and pericarp of cereals, katjang-idjo (*Phaseolus radiatus*) pea-nuts and their press cake, peté beans (*Parkia roxburghii* Don), kanari kernels (*Canarium commune* L.), and djawa nut (*Pennisetum macrochaeton* Jacq.). All these can be safely used with twice their dry weight of polished rice. The following on the other hand should be substituted for polished rice or taken with not more than an equal weight of it: maize, unmilled cereals, milk, boiled potatoes, boiled sweet potatoes, soy beans, tempe kedele (a preparation from soy beans). The remaining foodstuffs examined did not afford protection: white bread, maize meal, banana, coco-nut press cake, cassava root (gaplek) (*Manihot utilissima* Pohl.), or cassava meal, sago, dried shrimps, telor terubuk (salted dried roe of the Indian shad (*Clupea* (*Alosa*) *macrura* Blkr.), dried melindjo (fruits of *Gnetum gnemon* L.), dried fish, terassi (decayed shrimps, used as a flavouring for rice), tao-tjo (a preparation from soy beans), durian (fruit of *Durio zebethinus* Murr.), spinach (dried), French beans (dried), kangkong leaves dried (*Ipomoea aquatica*), nangka fruits (*Artocarpus integrifolia* L.), used as an ingredient of a sauce for rice dishes, kemiri nuts (*Aleurites triloba*, Forsk).

The effects of exposure to heat and of cooking on vitamin B_1 are fully described on p. 143.

Period of Development of Beri-beri.

The period of development of beri-beri has been determined with some care by Fraser and Stanton [1909] who found that the disease occurred among Japanese coolies after a period of 80–90 days upon a diet consisting mainly of polished rice. In Strong and Crowell's experiments (p. 244) the members of the groups receiving whole polished rice showed symptoms of beri-beri after 61–75 days.

Intestinal Stasis.

It has been claimed that a relatively deficient supply of the vitamin B complex is a common cause of constipation or intestinal stasis, and that this latter condition is in turn responsible for many chronic diseases and disabilities. Such claims are usually based on the contention that in experimental animals deprived of vitamin B the food progresses through the alimentary tract more slowly than in those receiving a complete diet. There is, however, evidence that any observed delay in the progress of food along the intestine is not caused simply by vitamin B deficiency. The severer grades of this deficiency may be associated with an atonic condition of the stomach which possibly explains the lack of appetite almost invariably accompanying vitamin B deficiency. No convincing clinical evidence is available to show that the vitamin B complex determines the normal functioning of the human bowel, and until such is forthcoming it is unnecessary to assume that vitamin B deficiency is concerned in the production of the constipation so commonly met with among Western peoples.

Vitamin B_1 in the Diet of Infants.

A good deal of discussion has taken place concerning the value of extra supplies of vitamin B_1 to infants who are being fed either at the breast or on cow's milk. Hoobler [1928] has claimed that a characteristic group of symptoms can be recognized in American infants as the result of a deficient supply of vitamin B_1. These include anorexia, loss of weight, fretfulness, pallor, and stiffness of the limbs and neck. The symptoms are said to respond rapidly to the administration of a rich source of vitamin B_1. Bloxsom [1929] found that a suspension of dried yeast caused an increased rate of growth, both in normal and under-nourished infants. It seems possible that a deficiency of this factor may be associated with at least minor nutritional disturbances in temperate climates where infantile beri-beri is unknown, and in view of the ease with which such a deficiency may be remedied it seems worth while bearing it in mind as a possible cause of poor development in infancy.

Vitamin B_2 (Antidermatitis).

Diet in Relation to Pellagra.

Since the publication of the former Monograph [Med. Res. Coun. 1924] pellagra has been definitely recognized as a deficiency disease, but its exact aetiology and relation to the consumption of maize as a staple are still not understood. These points are discussed elsewhere (see Chapter VII), and it suffices here, as in dealing with beri-

beri, to indicate the type of diet on which the disease has been known to develop and those foodstuffs which have been found efficacious in its cure and prevention.

The most careful dietary investigations on pellagra have been made in Egypt by W. H. Wilson and in the United States by Goldberger and his colleagues. Wilson as already explained (p. 179) believes that the disease is caused by a dietary in which the 'biological value' of the total protein, calculated on Thomas's figures [1921], falls below a certain figure. He analysed many diets from this point of view and showed how they fell into line with his hypothesis. The diet on which any deficiency disease is liable to occur is, however, generally a poor one and, because it is possible to demonstrate low biological value of the protein as a common factor, it does not necessarily follow that this is the causative factor. From Wilson's analysis of diets, it is difficult to attach a special antipellagrous value to any particular article, but experience has shown that meat, milk, and eggs, whose proteins all have a biological value of a high order, are valuable both protectively and curatively. Later [1929] Wilson slightly modified his view to include a vitamin factor in the aetiology of pellagra. He still considers, however, that, for persons living on the border-line of sufficiency in respect of their intake of that vitamin, the value of the protein intake would be the determining factor in the incidence of pellagra.

Goldberger and his colleagues attribute pellagra to a deficiency of a dietary factor contained in the yeast vitamin complex. In the United States the diet on which pellagra is liable to occur in the mill villages is a very restricted one, owing to poverty and to the small amount of ground available for cultivation, either as vegetable gardens or farms. When, for any cause, the economic conditions deteriorate, as for instance in the great Mississippi floods of 1927, the incidence of pellagra rises [Goldberger and Sydenstricker, 1927]. In that area the diet is said to consist of the three 'M's'—meat, meal, and molasses; the meat is salt pork, chiefly fat with very little lean, and the meal is maize meal. According to the people's means, this dietary is supplemented with vegetables and butter, poultry, and eggs, but only to a very small extent. A typical dietary is described as consisting of corn (maize) meal (corn bread, boiled hominy or mush), wheat flour (biscuit), white rice, dried beans, white meat (salt pork), cane molasses, and greens. It is believed that this diet will lead to the development of pellagra within three to six or eight months in fully 40–50 per cent. of those partaking of it, the time depending on the nutritional status of the individual when the diet is begun. When it is adequately supplemented with pellagra-preventing foods, such as milk, lean meat and vegetables, pellagra does not develop. With such a basic diet as is described above there would be needed daily for full prevention in the adult, of lean beef about (not over) one half pound, or of dried cowpeas fully one half pound, or of butter milk about one quart, or of canned tomatoes about one quart, or of dried pure yeast about one oz. [Goldberger and Sydenstricker, 1927]. Owing to its cheapness and usefulness canned salmon was

specially tested [Goldberger and Wheeler, 1929], and it was found that the protective amount, on the same basis of estimation, was 6 oz. daily or possibly less. Amounts as large as 1 lb. daily of cooked carrots or rutabaga (swede turnips) were found insufficient [Goldberger and Wheeler, 1927, 1].

In selecting the food or foods to be used in treating the sick, Goldberger and Sydenstricker [1927] summarize thus: 'The physician must of necessity choose such as will most satisfactorily fit the tastes and digestive capacity of the patient. Such considerations and actual experience indicate that milk, fresh meat, eggs, and dried yeast are the foods of first choice.' Thus it is seen that Goldberger and his colleagues arrive at the same conclusion as Wilson with regard to the most suitable curative substances, except as regards the addition of dried yeast to the list. Yeast was found valuable in the cure of pellagra in the Ukraine by Tcherkes (see p. 181).

That a deficiency of vitamin A plays a part in the aetiology of pellagra is maintained by Leitch [1930] who considers cod-liver oil as important as yeast for treating the disease. He has found marked improvement to follow the subcutaneous injection of vitamin A concentrates, and draws attention to a disease somewhat resembling pellagra which occurs in Sierra Leone and which was attributed by E. J. Wright [1926] to a combined A and B avitaminosis. The main features of this disease are ulcerative lesions of the skin and mucous membranes, especially at muco-cutaneous junctions, and nervous symptoms including paraesthesia, paresis, deafness, and impairment of vision. It is stated that the disease may be cured by adding cod-liver oil and yeast to the diet. In this connexion it may be mentioned that subacute combined degeneration of the spinal cord in dogs, a condition analogous to that found in human pellagra, has been shown to be associated with vitamin A deficiency (p. 31).

Although the aetiology of pellagra is not yet fully understood, it is apparent from the sketch here given that sufficient information has accumulated to make the extinction of pellagra a problem of finance rather than of exact knowledge.

Anaemia.

A severe type of macrocytic anaemia prevalent in the tropics, especially among pregnant women, with a blood picture resembling that of ordinary pernicious anaemia, has been studied by Wills [1931]. This investigator observed dramatic improvement and cure when such cases were treated with a yeast extract (marmite) rich in vitamins B_1 and B_2; the doses given were large, viz. 1 drachm (4 g.) 2–4 times a day. The condition of the patients was often complicated with oedema which also yielded rapidly to the above treatment. Similar cures were obtained with therapeutic water-soluble preparations of liver extract. Some observers have found such liver extracts tested on rats to be even richer than yeast in vitamin B_2, while relatively poor in vitamin B_1 [Guha, 1931, 2; Chick, unpublished results, quoted by Wills, 1931], a result, doubtless, due to the fact that at one stage in preparation the portion soluble

in strong alcohol is removed [Cohn *et al.*, 1927]. Gilroy [1931], on the other hand, using rather larger doses, found both B-vitamins present in 9 out of 11 specimens of liver extract.

Further observations are needed to decide which constituent or constituents of the B-vitamin complex were acting as curative agent in the cures with yeast extract by Wills described above. The fact that none of the cases showed symptoms resembling beri-beri supports the suggestion that a deficiency of vitamin B_2 may be a factor in the aetiology of this form of anaemia. This suggestion is the more likely seeing that most Indian diets are markedly deficient in foodstuffs rich in animal proteins, which are also the richest sources in nature of vitamin B_2.

Yeast and other sources of B-vitamins have been found without therapeutic value in ordinary pernicious anaemia [Cohn *et al.*, 1928; Davidson, 1931].

Vitamin C.

Scurvy has a longer clear history than the other deficiency diseases, and long before the age of vitamins it was realized that scurvy was caused by the lack of something in the diet, something elusive and incorporeal, connected with the property of freshness which disappeared from food which had once possessed it, as it became stale. It was well known that the disease could be cured by introducing fresh food into the diet.

An examination of the literature shows that before any experimental work had been done with guinea-pigs to evaluate foodstuffs for vitamin C, ordinary human experience had attached to many items a relative set of values, which subsequent experiment only confirmed. In point of fact a table of relative values, constructed upon the sifted evidence of the past, would contain the principal foodstuffs, ranged in an order not differing markedly from that constructed upon the results of experimental work as set out in Table XIII (p. 205) and described on pp. 209–212.

It is therefore useful to consider the descriptions of some notable outbreaks of scurvy under the heading of the foodstuffs which were chiefly concerned.

Fruit. The great value of fresh vegetables and fruit in the prevention and cure of human scurvy has so often been emphasized that its reiteration is a commonplace. This experience is confirmed by the figures given for the relative antiscorbutic values of foodstuffs in Table XIII. The juice of fresh citrous fruits has for centuries been regarded as antiscorbutic material *par excellence.* The 'experiment' made by Dr. Lind [1757] in 1747, quoted above, p. 187, is one of the most carefully recorded instances, but others are to be found in abundance.

Curran [1847] mentions two 'hopeless' scurvy patients who received ½ oz. lemon juice with sugar and water thrice daily, and relates that the patients sat up and took food with cheerfulness in the course of two days. He also records that in the case of a gentleman affected with scurvy of a very severe description, the eating of a single rhubarb tart produced a most decided amelioration, equally sensible to the patient and his friends'.

Lind [1757] recounts the tragic history of four ships which sailed from England to Bombay in April 1600, carrying 480 men on board, including merchants and other officials, in order to establish the East India Company. The Commodore upon his own ship had arranged for a regular issue of lemon juice, three tablespoonsful daily, to all hands, and four months later, when the flotilla reached the Cape, his men were all in good health. On the other three ships, however, the seamen were so severely attacked by scurvy that the passengers had to work as common seamen. In all, 105 men died from scurvy during the voyage, and when Bombay was finally reached after a voyage of four months, the entire work of unloading had to be performed by the crew of the Commodore's ship.

Budd [1840] relates how the voyage of the *Suffolk* to Madras in 1794, which occupied nearly six months, during which no land was touched, was accomplished with almost entire freedom from scurvy. A regular ration of 2/3 oz. lemon juice was served out daily.[1] Scorbutic symptoms were noticed in a few men, but these disappeared on increasing the lemon-juice ration. Perhaps the most impressive instance of all is to be found in the history of the Navy itself, between the latter part of the eighteenth century, when thousands of cases of scurvy were reported annually, and the early years of the nineteenth century when, after the regular issue of lemon juice had been made compulsory in 1804, scurvy became a comparatively rare disease in the Navy.

Lime juice. The results of experimental work recorded in Table XIII (p. 205) showing the inferiority of the juice of West Indian limes (*Citrus medica*, var. *acida*), both in the fresh and preserved state, compared with that of lemons (*Citrus medica*, var. *Limonum*), are difficult to reconcile with the popular esteem in which the former has generally been held as a preventive of scurvy. It is not, however, generally known that when 'lime juice' earned its laurels in the field of medicine, the term was used to signify the juice of lemons from the Mediterranean. Such was the case until the middle of the nineteenth century, when owing to political reasons and to favourable reports upon the acidity of the product, in which quality the antiscorbutic virtue was held to reside, the supply of West Indian lime juice was gradually substituted. By the date 1870 very little, if any, lemon juice was issued officially.

This point has been investigated from an historical standpoint by A. Henderson Smith [1918, 1919], and the results of her inquiry afford an important confirmation of the experimental findings given above. She was unable to find any recorded instances in which scurvy had been prevented or cured by preserved lime juice, in absence of other antiscorbutic agents. On the contrary, a careful comparison of certain Arctic expeditions in the fifties of last century equipped with *lemon* juice, with those of later date supplied with preserved *lime* juice demonstrated the inferiority of the latter. The most impressive case quoted is that of the Relief Expeditions sent in search of Sir John Franklin, 1847–59, where

[1] In 1840 the navy ration was 1 oz. lemon juice daily with 1½ oz. sugar, served after two weeks at sea [Budd, 1840].

those ships which were supplied with lemon juice of good quality enjoyed remarkable immunity from scurvy for long periods of time. One instance, that of the *Investigator* under Captain McClure, was specially noteworthy, for, in this case, there was no scurvy for twenty-seven months after leaving England, notwithstanding great privations. The event was otherwise in the *Alert* and the *Discovery*, two ships which left England in 1875 under Captain Nares, in an attempt to find the North Pole. These ships were equipped with all the improvements that the advance of twenty years had discovered and plentifully supplied with lime juice of the best quality. Notwithstanding these advantages serious scurvy broke out at the end of the first winter spent in the Arctic regions. A commission of inquiry was held by the Admiralty on the return of these ships in 1876, but no satisfactory cause was found to explain the unexpected disaster. The commission took no notice of the fact that the 'lime juice' provided in 1875 was the juice of the West Indian lime, whereas in the fifties it had been the juice of the Mediterranean lemon, but there is little doubt that in this difference lies the explanation of the different experience in the two expeditions.

Following this period a general distrust of 'lime juice' as a preventive of scurvy may be traced in the writings of those who had given careful attention to the history of later Arctic discovery, and the theory previously alluded to (p. 186) that scurvy is due to chronic ptomaine poisoning, by tainted and salted meat, found ready acceptance. For example, the experience of the Jackson-Harmsworth Expedition to Franz Josef Land in 1894–97 was accepted as being in accord with this theory [Jackson and Harley, 1900, 1, 2] on the basis of the following facts.

The land sledging party was for three years without any lime juice and without any scurvy, but large quantities of fresh bear meat were consumed. The ship party, on the *Windward*, took their daily 1 oz. of lime juice with regularity, and were well supplied with canned and salted meat, but after one winter the whole crew developed scurvy and there were three deaths. There is no record that the meat was tainted or putrid when consumed, but it was afterwards assumed that it must have been so. In earlier days 'lime juice' had for so long been regarded as the equivalent in diet of fresh vegetables and fruit, that the supporters of the 'tainted' meat theory maintained further that fresh vegetables also were not concerned in the prevention or cure of scurvy, but that the disease was connected only with the quality of the meat eaten.

Polar expeditions to-day are as liable to scurvy as ever unless proper precautions are taken. The germination of peas or beans might be resorted to on the ship, but on sledging parties a concentrate of lemon juice should be regarded as indispensable. Zilva (personal communication) has prepared concentrated lemon juice for such expeditions in the following manner. The lemon juice was concentrated in a copper vessel, tinned on the inside, heated by a steam coil at a pressure of 23–26 in. and a temperature of 140° F. A batch of about 60 litres was thus reduced to about one-third of its volume in three-quarters of an hour. At the end of this time

the concentrated juice was quickly cooled by means of an internal cooling coil. Sterilized bottles of about one litre capacity were filled with carbon dioxide and the concentrate was fed into them through a flamed nozzle from the bottom of the vat. The bottles were sealed with paraffin. It is desirable to test this concentrate for its antiscorbutic potency before issuing it, as in some instances this was found to be damaged by the manipulation. When undamaged, the potency was retained for four years when the juice was kept at 0° C. In the course of preparation, the essential oil is lost, but it can be replaced at the time of bottling, and the flavour restored. The daily dose recommended per man is an amount of the concentrate equivalent to 70 c.c. of the original juice. This may be more than is necessary, but the whole method of preparation is planned to give a margin of safety.

Lemon juice concentrated about 4½ times by the above method was used by the members of the British Arctic Air Route Expedition to Greenland in 1930–31 led by H. G. Watkins. The antiscorbutic potency of this preparation was particularly well demonstrated in the case of one member, Augustine Courtauld (age 26, weight about 10 stone). From July 20 to October 26, 1930, Courtauld's main source of vitamin C was seal and bird meat. On the latter date he set out from the base to the Central Station where he remained until May 5, 1931. Apart from a little strawberry jam, marmalade, a handful of raisins, prunes and dates, and two-fifths of a frozen ptarmigan, all consumed before December 6, Courtauld existed until May 5, 1931, on a dietetically well-balanced, but vitamin C-free, sledging ration, consisting of the following:

Pemmican	4·0 oz.
Margarine	4·0 oz.
Plasmon oats	1·4 oz.
Sugar	2·3 oz.
Chocolate	1·7 oz.
Plasmon powder	1·1 oz.
Pea flour	1·1 oz.
Cocoa	0·6 oz.
and occasionally a teaspoonful of condensed milk	—
Total	16·2 oz. = about 2,500 calories.

He also took daily one dessertspoonful of the concentrated lemon juice, one spoonful of cod-liver oil (every second day), about one gram of a salt mixture and about one gram of dried yeast. He gradually reduced his sledging ration to 9 oz. per day. From March 22 to May 5 he was snowed up in his hut, and during the last fortnight was without light or heat. He felt no craving for any particular article of food. He watched carefully for symptoms of scurvy, but none supervened and he felt normal in every way. His teeth and gums remained in good condition and he could throughout bite hard uncooked food without discomfort. He was able, when relieved, to ski about one mile unaided to the relief camp. Samples of the same batch of concentrated lemon juice tested by Zilva in London in February 1931 were found to have lost little of their antiscorbutic potency after a year's storage at 0°–1° C.

Vegetables. Among the vegetables investigated experimentally the cabbage was found to be pre-eminent, and, even after cooking, the minimum preventive ration for guinea-pigs remained small in comparison with root and other vegetables.

The following incident, related to Holst and Frölich [1912] by a political refugee from Russia, bears testimony to the value of cabbage for the prevention of human scurvy, even when taken in the form of soup, after prolonged stewing. In a Russian prison, in which the narrator had been confined with 1,400 other prisoners, the diet consisted of tea, coarse bread, and cabbage soup. The preparation of the soup was so unclean that twenty of the inmates, including himself, who were of gentle birth and upbringing, could not endure to take it. After about six months these twenty prisoners showed symptoms of scurvy, while no case occurred among those who had consumed the soup regularly.

Among herbs and 'cresses' esteemed in the past for antiscorbutic virtues it is interesting to note that those belonging to the natural order Cruciferae occupied a high place. This natural order includes also the cabbage and the swede, which were found to be the most valuable of the vegetables investigated experimentally. 'Scurvy grass' (*Cochlearia officinalis*), a small plant frequently found growing near the sea-shore, figures largely in old records of scurvy cures among mariners. Thus Bachstrom in 1734 tells the following story:

'A sailor in the Greenland ships was so over-run and disabled with scurvy, that his companions put him into a boat, and sent him on shore, leaving him there to perish without the least expectation of recovery. The poor wretch had quite lost the use of his limbs; he could only crawl about the ground. This he found covered with a plant which he, continually grazing like a beast of the field, plucked up with his teeth. In a short time he was by this means perfectly recovered, and, upon his returning home, it was found to be the herb "scurvy grass".' (Rendering given by Lind [1757, p. 395].)

Potatoes. Among roots and tubers the potato easily takes the first place in practical importance, not so much because of its intrinsic value, but because, owing to its abundance, cheapness, and general acceptability, large quantities are regularly consumed. Its consumption in fact exceeds that of all other vegetables combined [Hess, 1920].

Although a classic remedy for scurvy, the potato has only a comparatively low antiscorbutic power compared weight for weight with some other vegetables [Chick and Rhodes, 1918; Givens and Cohen, 1918; Givens and McClugage, 1920; Scheunert 1930]. Nevertheless, there is no doubt that in northern climates the potato is of the utmost value in preventing scurvy during the winter and spring, epidemics of scurvy having repeatedly followed failure of the potato harvest, e.g., in Ireland in 1847 and in Norway in 1914. The outbreaks of scurvy reported in Glasgow [1917], Manchester [Report 1917], and Newcastle [Harlan, 1917] in the spring of 1917 are doubtless to be attributed to the great scarcity of potatoes at that period.

Onions take a position between the more and the less potent

vegetables. They possess a special importance, however, owing to the ease with which they can be transported, and they are much appreciated, whether raw or cooked, by reason of their flavour. For these reasons they should always be included in rations for soldiers, sailors, or other communities of people at the end of long lines of communication cut off from fresh supplies.

Germinated pulses and cereals. There is so far little direct evidence of the antiscorbutic value of germinated pulses in human diets. The custom of eating germinated cereals and pulses obtains in some parts of China and in the Malay States and the Dutch Indies, where germinated beans, 'towgay', are a common article of native diets. There is, however, no evidence that the antiscorbutic value of these foods has been recognized.

One instance, however, in which germinated beans were found to be of great value for the cure of scurvy is recorded by Wiltshire [1918], and yields complete confirmation of the experimental work described on p. 189. This observer succeeded in curing 27 cases of mild scurvy among Serbian soldiers (selected at random from a total of 57) by the sole measure of including in their daily dietary 4 oz. (dry wt.) of germinated haricot beans. Although this ration was not very acceptable the progress made was, if anything, better than that of the remaining 30 patients who received instead a daily ration of 4 oz. fresh lemon juice. The beans were consumed after boiling for ten minutes only. Germinated peas were also found to be a satisfactory curative agent for scurvy by Stevenson in Archangel [1920].

The use of germinated pulses as an article of diet certainly deserves a prolonged trial in circumstances in which fresh food is scarce or unobtainable. Dry peas, beans, or lentils contain less than 15 per cent. of moisture, are admirably adapted for transport, and can be germinated on the spot if and when required. Pulses of various sorts, as 'dhall', form a staple article of the native Indian diet, and had this knowledge of their value after germination been applied in Mesopotamia in 1915 it is possible that the terrible wastage from scurvy in that campaign [Mesopotamia Commission Report, 1917] might have been prevented (see p. 262).

Beer and malt. Captain Cook was a great believer in the antiscorbutic virtues of a fresh infusion of malt (sweet-wort). His second voyage, 1772-75, was accomplished with an amazing record of good health among his crew, there being only four deaths during the period, of which one only was from sickness. He 'took a large quantity of "malt", of which was made sweet-wort; to such of the men as showed the least symptoms of scurvy this was given, from one to two or three pints a day each man, or in such proportion as the surgeon found necessary. This was, without doubt, one of the best antiscorbutic sea medicines then discovered, when used in time' [Captain Cook, p. 227]. According to Sir John Pringle, writing in 1776, quas, 'a small brisk acidulous liquor,' made from ground salt and rye meal, was regularly served out in Russian prisons as an antiscorbutic [1776].

Certain fermented liquors made from other germinated seeds have also been held in high esteem for the prevention of scurvy. Kafir

beer, 'leting' or 'joala', is an instance of this. It is the product of rapid fermentation of partly germinated Kafir corn (*Sorghum vulgare*) and maize, and is consumed quickly after preparation. The Kafirs are in the habit of taking large quantities when living in their own kraals in South Africa, and it is believed to be a valuable antiscorbutic. It has been the custom of the mining companies on the Rand to arrange for the brewing of this beer in the compounds of the native labourers, and large quantities are consumed. Outbreaks of scurvy were reported by Dyke (1918) among companies of Kafir labourers in France in cases where this 'joala' was replaced by a second type of beer, 'mahew', a fermented drink also made from millet and maize, but in the preparation of which *the grain is not previously germinated.*

Kafir beer has been examined by Delf [1921] who found, using monkeys as experimental animals, that fresh Kafir beer contains about as much vitamin C as fresh English dairy milk. A monkey on an average daily ration of 85 c.c. was attacked with scurvy on the 114th day, and behaved very much as a monkey in a previous experiment in England with a ration of 125 c.c. fresh milk. The individual idiosyncrasies of monkeys are, however, so great that this conclusion can only be regarded as provisional. This beer was made from Kafir corn malt and maize meal and was fermented only for a very short time. A considerable quantity (30 lb.) of the malt was added per barrel of 45 gallons just before fermentation, which only lasted 2–4 hours, after which the beer was only coarsely strained and used. 'It is drunk as a very thin watery gruel full of yeast and bubbling with carbonic acid.' As the germinated Kafir corn, from which the malt was prepared by drying, has been found by Delf to contain a certain small amount of antiscorbutic (p. 205), it seems possible that the antiscorbutic property of the beer is due to the suspended malt. Modern English beer, which is prepared from kilned malt and drunk clear, has been found to be free from demonstrable antiscorbutic properties [Harden and Zilva, 1918, 5]. Green malt contains some vitamin C, but loses it during the kilning process.

Dried vegetables. Dried vegetables have repeatedly been tried and found useless for preventing human scurvy, and in this respect the results of the experimental work find abundant confirmation in the records of failure which have been preserved. Owing to the convenience with which they can be transported, dried herbs and vegetables have been repeatedly adopted for the supply of armies and other large bodies of men separated from supplies of fresh food—and with disastrous results.

As long ago as 1720, Kramer, chief surgeon with the Austrian army in Hungary, was confronted with a serious outbreak of scurvy among the troops, and in his perplexity he wrote to Vienna for help and advice. The College of Physicians in that city arranged for a large and varied supply of dried antiscorbutic herbs to be dispatched to his aid. The consignments arrived and were given a prolonged trial, but the result was that thousands perished from scurvy. Kramer epitomized the knowledge gained by his tragic experience in the oft-quoted paragraph in his *Medicina Castrensis*, 1720:

'The scurvy is the most loathsome disease in nature: for which no cure is to be found in your medicine chest, no, not in the best furnished apothecary's shop. Pharmacy gives no relief, surgery as little. Beware of bleeding: shun mercury as a poison: you may rub the gums, you may grease the rigid tendons in the knee, to little purpose. But if you can get green vegetables; if you can prepare a sufficient quantity of fresh, noble antiscorbutic juices, if you have oranges, lemons, or citrons; or their pulp and juice preserved with whey in cask, so that you can make a lemonade, or rather give to the quantity of 3 or 4 oz. of their juice in whey, you will, without other assistance, cure this dreadful evil.' [Translation given by Lind, 1757.]

Dried vegetables were repeatedly tried in the Navy with similar results, and by the middle and end of the eighteenth century all persons who were well informed upon this subject were convinced of their uselessness for the prevention of scurvy. The following is a sample of what appears constantly in the correspondence of the Medical Board of the Admiralty at this period: *Digests of 'In' Letters*, 4806, 37. 1, August 22, 1773, Ref. M. Report from Commissioners for Sick and Hurt:

'On reference of a letter from Earl Suffolk sending a box containing specimens of a plant and herbs prepared and dried for the purpose of curing the scurvy at sea. Acquaint Lord Suffolk they cannot recommend its introduction.'

And in 1757 Lind, in describing methods suggested for preventing scurvy at sea, writes:

'The latest proposal to the Lords of the Admiralty was a magazine of dried spinach prepared in the manner of hay. This was to be moistened and boiled in their food. To which it was objected by a very ingenious physician (Dr. Cockburn), that no moisture whatever could replace the natural juices of the plant lost by evaporation, and, as he imagined, altered by a fermentation which they underwent in drying.' [Lind, 1757, p. 148.]

When scurvy ceased to be a permanent menace this knowledge was soon forgotten, and Kramer's experience was repeated again in the American Civil War. In that campaign, large rations of dried vegetables, including dried potatoes, were issued to the men, but proved powerless to prevent scurvy, of which there were many outbreaks [Medical History, 1888]. At the present day it again needs to be emphasized that *vegetables which have been dried have lost almost the whole of their antiscorbutic properties.* During the period of the late war the public were often energetically advised in the daily press to dry and preserve vegetables for dispatch to prisoners of war in Germany or to provide 'fresh' vegetables for our fleet when at sea (e.g., *Daily Chronicle*, October 19, 1917).

Dried fruits have been shown to have a distinct, though feeble, antiscurvy value in experimental trials. This result also coincides with human experience. The dried tamarind, cocum, and mango ('amchur') mentioned in Table XIII all possess a reputation in India as antiscorbutic materials. It is related that when scurvy broke out among our troops in 1833–34 at Nassirabad in Rajputana, great benefit was obtained by eating *anola*, the strongly acid dried

fruits of *Phyllanthus emblica*, which is commonly sold in the bazaars [McNab, 1837]. This difference between vegetables and acid fruits, in respect of the degree to which antiscurvy properties are preserved in the dry condition, may be due to the influence of an acid medium in checking the process of destruction which the antiscorbutic factor slowly undergoes after the cells of the plant have been disorganized by drying.

Meat. Many careful observers in the past have noted that the antiscurvy value of fresh meat, though significant, was much inferior to that of vegetables or fruit. Curran [1847] mentions three patients with scurvy at the Swift Hospital in Dublin in the epidemic of 1847, who had consumed ¾ lb. of meat on five days in the week while developing the disease.

Sir Gilbert Blayne relates an incident of the Fleet at Barbados in 1781, where a party of soldiers serving as marines were affected with scurvy and were sent to an army hospital where no fresh animal food was allowed. These men recovered much more quickly on the vegetable diet provided than the seamen on the ships who were deprived of vegetables but were fed on fresh meat.

There is no doubt, however, that scurvy can be prevented by the use of fresh meat alone if the ration is large. The history of Arctic experience is full of such cases. Dr. Rae, surgeon to the Hudson Bay Company, in his evidence to the Scurvy Commissioners of 1876, stated that among the inhabitants of that district scurvy was almost unknown. The people subsisted almost entirely on meat, but the amount consumed was upon the following scale, 8 lb. fresh venison daily per man, 4 lb. per woman, 2 lb. per child.

Nansen and Johansen, after leaving the *Fram*, spent nine months, including the winter of 1895–6, on Frederick Jackson Island in a rudely constructed hut. They remained in good health and free from scurvy, although obtaining no lemon juice and no fresh vegetables, and subsisting mainly on fresh walrus and bear meat preserved by cold.

Jackson and Harley [1900, 1, 2] describe an interesting incident at Kharborova, Yugor Straits, where six Russian priests arrived in the autumn, attended by a small Russian boy. The priests by their religious vows were prevented from eating the fresh meat available: they subsisted on salt fish and there were no vegetables. In the following May the little boy was found to be the only surviving member of the party, and had buried all his late masters in the snow. He suffered from no religious disability and had fed largely on reindeer meat through the winter. A further instance is provided in the experience of Scott's first expedition (Voyage of the *Discovery*) where an outbreak of scurvy was cured by the inclusion in the dietary of his party of large quantities of fresh seal meat. The case of the Jackson-Harmsworth Expedition, referred to above in the discussion of lime juice, is another instance of similar experience. The interpretation of these facts made by the leaders of the expedition induced them to support the theory that scurvy was a disease due to chronic poisoning by the toxins developing in canned meat.

From Hehir's report on the medical history of the siege of Kut-

el-Amara [Hehir, 1917] it is clear that in this case also, when no fresh vegetables were available, British soldiers were protected from scurvy by their regular ration of meat or horseflesh, but the amounts they consumed were considerable, 8 to 20 oz. daily. The Indian troops in Kut, on the other hand, who were vegetarian and refused meat on principle, suffered severely from scurvy.

Canned and preserved meats can be dismissed in a word as offering no possible protection from scurvy. Meat in its fresh condition contains the antiscurvy factor in comparatively low concentration, and after exposure to the temperature necessary for sterilization it is impossible that any significant antiscorbutic properties should be retained.

The value of frozen meat is probably intermediate between that of fresh meat and canned meat and is likely to be low, if it has been for a long period in the frozen condition. Wiltshire [1918], describing outbreaks of scurvy among Serbian soldiers, states that out of 132 cases occurring in 1917 all had received a ration of frozen meat nearly every day.

Stefansson [1918] met with several cases of scurvy among the members of his expedition to Melville Island in 1916–17. These were due to abstinence from fresh meat and were cured by inclusion of meat in the diet, much being eaten raw.

The comparative effect of a number of antiscorbutic materials on adult scurvy was tested by Stevenson [1920] on Russian prisoners at Archangel among whom scurvy had broken out. Germinated peas and beans (8 oz. daily), fresh meat (10 oz.), canned fruit (8 oz.), lactic acid milk (2 pints), and fresh lemon juice (4 oz.), all produced a favourable effect and ultimate cure. Lemon juice and lactic acid milk proved to be the best remedies, canned fruit and fresh meat the least effective. Germinated beans were not found as suitable as germinated peas and tended to cause digestive troubles.

Influence of Cooking upon the Antiscorbutic Value of Foods.

Seeing that the antiscorbutic accessory food factor is sensitive to high temperatures, it is clear that the value of fresh vegetables and fruit must of necessity be greatly impaired by cooking. When there is scarcity of fresh food, either by actual deficiency or by difficulty in transport or distribution, it is well to realize that raw fruit and salads have a value, weight for weight, far exceeding that of cooked fruit or cooked vegetables, and that a smaller ration will suffice to afford protection from scurvy. It is unfortunate that most fresh fruit and all salads are perishable articles of food and inconvenient for distribution. They are not available at all periods of the year nor are they universally acceptable, and the fact remains that the bulk of the population in this country takes its antiscorbutic food in the cooked condition. This being so, the method adopted for cooking becomes of great importance.

It is clear from the experimental results obtained by Delf [1918] (p. 213) that in ordinary cooking processes variation in the temperature employed has a comparatively small influence upon the rate of destruction of the antiscorbutic vitamin. For example, if in

cooking cabbage the temperature is lowered from 100° C. (boiling-point) to 80° C. the rate of destruction of the antiscorbutic factor is decreased only to about one-half: in other words the loss in anti-scurvy value caused by boiling for half an hour would be equalled by stewing for about one hour at 80° C. This time would, however, need to be greatly increased, with a consequent greater loss in anti-scurvy value, if the cabbage were to be rendered palatable. Cooking for a short period of time at a higher temperature is, therefore, much to be preferred to cooking for the much longer time necessary at a lower temperature.

In experiments made on this point by Eddy, Shelow and Pease [1921–22] it was found that the antiscorbutic potency was diminished to less than 10 per cent. of that of raw cabbage, whether the vegetable was cooked for 45–90 minutes in an open kettle at 100° C. or for about 15 minutes in a pressure boiler in absence of air at 121° C. No practical advantage is, therefore, to be gained by adopting temperatures above 100° C.

For the preparation of food in large quantities methods of slow cooking are the more convenient, and hence arises the great popularity of the stew in camps and other situations where the arrangements for cooking are perforce of a temporary character. Methods of slow cooking at temperatures below boiling-point are also economical of fuel, and it is for this reason that the hay-box and other forms of 'self cookers' were at one time widely recommended. Direct experiments have shown that the necessary long continued exposure to a comparatively low temperature is equivalent in its effect to exposure to 100° C for about three times as long as would be required for cooking in the ordinary way.

The conclusion to be drawn from these considerations is that for those articles of food upon which dependence is placed for protection from scurvy, all forms of slow cooking should if possible be avoided. In case of fresh meat of inferior quality it is often necessary to resort to slow stewing in order to provide an appetizing meal, but in such cases efforts should always be made to cook the vegetables separately and for as short a time as possible.

In this connexion it is instructive to place on record two outbreaks of scurvy in recent times which have been attributed to neglect of this principle. Scurvy broke out in a camp in Scotland in the spring of 1917 and 82 men were affected. At the time potatoes were scarce, but the ration contained a fair proportion of fresh meat and 2 oz. of swedes were available daily. These, as will be seen from Table XIII, are among the most potent antiscorbutic vegetables we possess, and, if cooked satisfactorily, should have afforded considerable protection. The cause of the outbreak was investigated by Hill, who discovered that the meat was always served as a stew, the vegetables being added and the whole cooked for about 5 hours. This circumstance was considered by Hill to be a sufficient explanation of the outbreak.

A second example is afforded by an outbreak of scurvy during the late war between May and July 1918 in a Kafir labour battalion in France in which 142 cases of pronounced scurvy were diagnosed. The daily ration contained 8 oz. of fresh vegetables, but these were

cooked with the meat and boiled for a period of at least 3 hours. In the opinion of the medical officer by whom the circumstances of the outbreak were thoroughly investigated this fact was an important contributory cause [Dyke, 1918]. An outbreak of scurvy among children in a tuberculosis sanatorium in Vienna, investigated by Chick and Dalyell [1920], was also attributed to the method of cooking employed.

Vitamin C and Infantile Scurvy.

Experimental work has shown [Frölich, 1912; Chick, Hume and Skelton, 1918, 1] that if scurvy is to be prevented by the agency of milk alone, a quantity must be consumed which practically amounts to a complete milk diet. This result is in accord with the physiological rôle played in nature by mammalian milk, as a food specially adapted for the complete nourishment of the young during the early period of life. Owing to the paucity of vitamin C in milk and the ease with which it is destroyed by heat, it would be expected that reference to the literature should bring to light accounts of outbreaks of scurvy where infants have been fed on heated milk, and this is in fact the case. Neumann [1902] attributed the many cases of infantile scurvy encountered in his private practice in 1901–2 to the practice of pasteurizing milk for infant feeding then recently introduced as a safeguard against transmission of bacterial infections. He found on inquiry that most of these infants had been receiving milk from one dairy in which it was the custom to 'pasteurize' the milk at 90-95° C. before delivery, and that in their homes the milk, as an extra precaution, had been heated for a second time in a Soxhlet or other apparatus at or near 100° C. for 10–15 minutes. Heubner [1903] has published similar experiences, and, in common with Neumann, has expressed the opinion that the marked increase of infantile scurvy which he noticed in Berlin about the same period must be attributed to the practice of pasteurizing milk before giving it to infants, which had been recently introduced and enthusiastically adopted, even to the extent of repeating the operation more than once.

The best studied instance to be found in the literature is probably that recorded by Hess and Fish [1914]. These investigators described an outbreak of mild and subacute infantile scurvy in the Hebrew Infant Asylum, New York, among infants who had been fed for several months upon a diet of cow's milk previously heated to 60° C. (145° F.) for thirty minutes. It had always been the custom in this Institution to give orange juice as an extra antiscorbutic to the babies fed upon pasteurized cow's milk, but it had been discontinued as a result of the pronouncement of the American Medical Milk Commission (1912) that, for purposes of infant feeding, heated milk might be considered the equivalent of raw milk. The result was that an outbreak of mild scurvy occurred two to four months later. The babies were not very ill, they were fretful, anaemic, had no appetite, and ceased to gain weight or grow. They were all over six months old, and it would have been reasonable to attribute the condition to teething troubles. The scorbutic nature of the illness was proved, however, by the ease and rapidity with

(From *Brit. Med. J.*, 1921, ii, 1064.)

Fig. 34 shows a photograph of twins, taken in a Vienna infants' home in 1921, and illustrates the striking effect on backward children of medication with anti-scorbutics and fat-soluble vitamins. Six months before the date of the photograph the girl, Ida P. (left), at 22 months old, weighed 7.6 kg. (35% below normal); she could neither sit nor stand and made no attempt at spontaneous movement. She had only four teeth, an open fontanelle and other signs of rickets and in her previous history two attacks of scurvy had been diagnosed and treated. During 6 months of treatment, extending from December to June, in addition to the ordinary Institution diet which contained an adequate supply of milk, she received daily, 20 g. raw swede turnip juice (occasionally substituted by raw lemon or orange juice), 10 g. butter, later increased to 20 g., and for the last three months 10 g. cod-liver oil in addition. At the end of the period she weighed 11·1 kg. (11% below normal), she had 12 teeth, the fontanelle was closed and there was striking remission in all signs of rickets. She could stand and walk a little, she tried to talk and was transformed into a merry, active little girl.

A control was provided in her twin brother Johann P. (right) who had also been in the same institution since birth, and also had suffered from severe scurvy during the first year of his life, complicated with rickets. While his sister received treatment, however, he had remained in another ward in which no extra vitamin therapy was given. At the time of the photograph, at 28 months of age, his condition exactly reflected that of his sister before treatment. He was feeble, anaemic, showed signs of severe rickets, was unable to sit without support, and gave no signs of intelligence [Chick and Dalyell, 1921].

To face p. 265]

which the symptoms cleared up when orange juice or other antiscorbutic was restored to the diet, or when raw milk was substituted for the pasteurized milk. These conclusions of Hess and Fish have received confirmation in very similar observations made by D. J. M. Miller [1917].

In Vienna for a few years after the late war scurvy was comparatively common in infants, and this has been attributed not only to the actual shortage of milk but also to the breakdown in the milk transport, with the result that the milk supply on many occasions had been repeatedly 'pasteurized' before delivery. In a large foundling institution, children fed on such repeatedly heated milk were found to be in a state of latent scurvy, similar to that described by Hess and Fish [Chick and Dalyell, 1921]. In certain children of 1–3 years old this condition continued for many months, with occasional attacks of overt scurvy. If left untreated the children became extremely dwarfed, inert, and backward. The effect of vitamin therapy in restoring such children to a normal condition is well seen in the contrast between two children, boy and girl twins, shown in Fig. 34, both of whom had suffered from attacks of severe scurvy in the first year of life. At the time of the photograph they were 28 months old. Ida P. (left) had for six months previously received treatment with vitamin C (raw swede juice, orange or lemon juice) and vitamins A and D (butter and cod-liver oil), while Johann P. (right) had, during this period, remained in a ward in which no extra vitamin therapy was given.

The above evidence indicates that an antiscorbutic supplement is absolutely indispensable for infants fed on heated milk. As regards the most suitable antiscorbutic material to be adopted, orange juice is easily the best from many points of view; it is very potent, is convenient, needs no preparation and is acceptable to the vast majority of infants. Under normal conditions oranges are cheap and plentiful in winter and spring. The fresh juice of raw swede turnips is an excellent substitute for orange juice, if for any reason this is not available; its antiscorbutic value was found by Chick and Rhodes [1918] to be about 60 per cent. of that of an equal volume of orange juice and far in excess of that of the other root vegetables examined. Swede turnips have also the merit of being abundant and cheap. The preparation of the juice is exceedingly simple. The clean cut surface of the raw swede is grated on an ordinary kitchen grater and the pulp folded in a small piece of muslin and squeezed with the fingers, when the juice readily runs out. Its slightly sweetish taste is not disliked by infants. It was used successfully in Vienna in 1919–22 when oranges were unobtainable, and it has been recommended in some infant welfare centres. The juice of fresh tomatoes is an excellent and cheap antiscorbutic for infants during summer and autumn. Canned tomatoes have also been found satisfactory by Hess and Unger [1918–19], and these are always available. Where the presence of starch is not a drawback, cooked potato is also recommended by some workers [Barlow, 1894; Hess and Fish, 1914]. The cooked potato is shaken up in water and the resulting fluid used as a diluent of cow's milk in the same manner as barley water.

For curative purposes, potato cream, orange juice, or grape juice

is usually employed, although neither the potato nor the grape is of high antiscorbutic potency. It has been shown, however [Harden, Zilva and Still, 1919; Zilva, 1923, 2], that lemon juice, from which the free citric acid has been removed by treatment with calcium carbonate and alcohol, is a valuable remedy, inasmuch as, owing to the absence of acid, a very large amount can be administered without causing digestive disturbance. This preparation can readily be concentrated, and in this form a highly potent antiscorbutic material can be administered without inconvenience. It is also possible to effect the cure of scurvy by intravenous injection of neutralized orange or lemon juice. Orenstein [1925] states that this is the most effective method of treatment in acute cases.

Incidence of Scurvy.

It might be thought that under ordinary conditions of life, adult scurvy would be extinct to-day. This is, however, unfortunately not so. In Western Europe at the present time cases are occasionally met with, chiefly among men or women in very poor circumstances who are living alone and do not bother to provide for themselves out of their very small incomes any kind of fresh vegetables, meat, or milk. A series of such cases was recently collected in Copenhagen by Meulengracht [1927]; most of the patients were unemployed bachelors or widowers living by themselves in a single room and preparing their own food.

A survey of cases of scurvy diagnosed at the Boston City Hospital and at the Massachusetts General Hospital [Shattuck, 1928] also shows that scurvy among adults under normal conditions of life is by no means extinct. In the five years 1923–27, 22 adult cases were diagnosed, in all of which restriction of the diet from some cause or other could be demonstrated. There is no doubt that scurvy is a disease to which the food crank is liable.

Aykroyd [1930, 2] in his survey of deficiency disease in Newfoundland and Labrador, found that cases of scurvy still occurred, and considered that the population was probably only protected from the disease during the long hard winter by the store of cranberries (20–40 lb. per head) which are collected in the summer and eaten raw or lightly cooked during the winter.

Frank cases of scurvy are usually easy to recognize and satisfactory to treat. Whether minor degrees of vitamin C deficiency are at all a common cause of disability or predisposition to disease in man is difficult to determine with certainty. Many observers working in warm climates where cereals form the staple article of the diet of the natives are of opinion that this is the case, even though definite scurvy may be rare. They therefore recommend that the consumption of leafy vegetables and fruit should be strongly encouraged. The diets in such instances, however, are apt to be deficient in other essential factors besides vitamin C, so that it is not a simple matter to prove how far a relative deficiency of vitamin C alone is an important contributory factor in the production of vague ill-health or of diseases other than scurvy.

Göthlin [1931] has employed a modification of the capillary

resistance test for diagnosing a possible deficiency of vitamin C. The test consists in applying a compression band above the elbow of the subject and ascertaining the number of petechiae which appear in 15 minutes in a circular area 60 mm. in diameter in the bend of the elbow, using pressures of 35, 50, and 65 mm. of mercury. The number of these petechiae increases with the deficiency of vitamin C. In this way he has shown that in Sweden many school-children in April–May are subject to a partial deficiency of vitamin C which in some cases disappears during the summer months.

Period of Development of Scurvy.

The period of development of scurvy is probably four to eight months. Scurvy is rarely seen in infants less than six months old, and according to Barlow [1883, 1884] is most common about the eighth month of life.

In the old days, when fresh food was scarce or absent during the winter, and scurvy was a common disease, it was usually in the following spring, after several months of deprivation, that symptoms occurred. Hehir has stated that four months is the minimum time in which he has observed scurvy to develop among Indian troops on active service [Hehir, 1917, p. 71]. Stevenson [1920] made similar observations on Russian prisoners at Archangel, among whom scurvy supervened usually in 4–7 months after the date of their capture (200 cases). The prison diet was low in calories and almost entirely deficient in antiscorbutic vitamin, although ½–1 oz. of preserved lime juice was administered daily. These persons before capture had all enjoyed an ample diet including fresh milk, meat, potatoes, and vegetables. Holst and Frölich [1912] quote two cases in which the period of development was observed to be longer. One is the case of a fanatical vegetarian in Christiania who wished to prove to the world that existence could be maintained on bread and water alone. During the period of his self-imposed trial he was guilty of backsliding on two occasions only, once for a pound of sugar and once for a bottle of beer. The bread was presumably rye bread and afforded adequate protection from beri-beri, but definite symptoms of scurvy in the form of haemorrhages in the legs were noticed after seven and a half months. This period of development is not very different from that in the narrative of the Russian refugee (see above, p. 257) in the Russian convict prison, where scurvy developed after six months upon a similar diet.

There is a certain amount of evidence showing that these periods of development may be shortened in case of men performing hard manual work or exposed to damp or cold climatic conditions. In both cases metabolism would be stimulated, and it would be reasonable to suppose that any reserve of accessory factors would be the sooner exhausted (see p. 227). The following are instances.

It was commonly observed that scurvy was specially prevalent among sailors exposed to cold, damp, or very rough weather. Lind [1757] places this circumstance before diet in his dissertation on the 'Causes of the scurvy'. He gives instances of beleaguered

cities where the garrison on hard duty succumbed to the malady before the inhabitants.

In the expedition of the *Alert* and the *Discovery* to seek the North Pole in 1875 the men wintered on the ships within the Arctic circle. There was no definite scurvy diagnosed during this period, although the diet was defective from the point of view of antiscorbutic substances, notably by the substitution of lime juice for lemon juice. In the spring of 1876 the sledging parties set out, and with the performance of hard manual labour scurvy at once made its appearance, the first case occurring within ten days of departure. At first the officers escaped, but, as the men fell sick and the labour of dragging the sledges devolved more and more upon the officers, they also fell victims to the disease. In due course scurvy also broke out among the crews left behind upon the ships during the spring and summer of 1876, but its onset was distinctly later [A. H. Smith, 1918, 1919].

A second instructive example is found in the outbreak of scurvy in the Scottish camp referred to on p. 263. In this instance the first cases to develop, and a large majority of those showing severe symptoms, were among a small section of the population who were engaged in hard manual work for a few hours daily.

CHAPTER XI

VITAMINS IN RELATION TO THE DIET OF THE MOTHER AND THE INFANT.

Experimental evidence and modern clinical experience are in accord in teaching that an optimal intake of vitamins is of special importance in early life and during other periods of growth. During pregnancy and lactation the problem is two-fold—the maintenance of health of the mother and of optimal conditions for the development of the foetus and infant. The diet of the mother in relation to vitamins will first be considered.

Diet of the Mother in Pregnancy.

The duty of the maternal organism during pregnancy is to supply all the substances necessary for the growth of the foetus. These substances the mother either gets ready-made in her food, or herself synthesizes from it, or supplies by depletion of her own body tissues. If the maternal diet is deficient in the special substances essential for the existence and growth of the foetus, then the necessary withdrawal from her own stores may result in varying degrees of ill-health of the mother herself and ultimately of the foetus. Ill-health of the pregnant mother, sometimes of a gross nature, is not uncommon, while disturbances of a slighter nature arising from the same cause are probably so common as to be regarded as almost normal.

Some of the commoner defects of the maternal diet during pregnancy in this country are deficiency of vitamin D, vitamin A, calcium, iron and iodine.

Deficiency of Vitamin D and Calcium.

An abundance of both vitamin D and calcium is essential for the proper development of the bony skeleton and teeth of the embryo. The maternal stores of vitamin D are small, but the bones of the mother constitute a large depot for calcium. These essentials she transfers to the developing foetus from her own tissues. If her diet is deficient the drain on her bone calcium may be so great as to result in osteomalacia (see p. 239). A more common evidence of this sacrifice is probably the increased incidence of dental caries to which pregnant women are subject (p. 242), indicating a loss of resistance in the teeth due to withdrawal of calcium.

The problem of the intake of vitamin D and calcium in pregnant women has been investigated by K. U. and G. Toverud [1929, 1, 2; 1930, 1, 2; 1931]. Among 16 women living in a home for expectant mothers and receiving an ordinary Norwegian diet they found that towards the end of pregnancy all were in negative calcium balance and 11 in negative phosphorus balance. These negative balances could be made positive either by increasing the calcium intake to 1·6–2 g., and the phosphorus to 1·8–2 g. daily, or in

some cases by increasing the vitamin D intake by giving cod-liver oil or eggs. The addition of vitamin D was not effective, however, when the calcium and phosphorus intake was very low. A similar state of negative balance was often found in lactating women also. These investigators also found that diets deficient in calcium and vitamin D predisposed the offspring to rickets and dental caries.

Deficiency of Vitamin A.

The maternal liver and the foetal liver should contain large stores of vitamin A. The exact significance of a deficient intake of vitamin A by the mother is not yet definitely known, but there is evidence that part of the great susceptibility to sepsis after parturition is related to this dietetic defect. Green, Pindar, Davies and Mellanby [1931] found that, if rats became pregnant and their diet was then made deficient in vitamin A, chronic sepsis very often developed after parturition in the uterus and fallopian tubes. The same workers made an extensive investigation on the relation of diet to puerperal sepsis. Five hundred and fifty women attending an out-patient department were divided into two groups. In one group each member received a preparation containing vitamins A and D to be taken daily during the last month of pregnancy. The second group received no such addition and acted as controls. Neither group received additional vitamins during the puerperium, and, after parturition, examination of the case sheets showed the results briefly summarized in Table XX.

In the control group the incidence of sepsis (4·7 per cent.), as defined by the B.M.A. standard of morbidity, was more than four times that occurring in the group which had received the vitamin A supplement (1·1 per cent.), while the incidence of puerperal pyrexia was 50 per cent. greater in the control group.

Table XX.

Effect of giving Vitamins A and D during Pregnancy on Incidence of Puerperal Sepsis.

Group receiving extra vitamins A and D		Controls	
No. of cases	275		275
Primiparae	147 (53.4 %)		145 (52.7 %)
Cases Morbid (B.M.A. standard).			
Cystitis (*B. coli*)	1 (0.33 %)	Cystitis—*B. coli*	4 (1.4 %)
Following manual rotation	2 (0.7 %)	,, —Staph.	1 (0.35 %)
		Bacilluria—Strep.	1 (0.35 %)
		Acute Mastitis	2 (0.7 %)
		Septic Endometritis	2 (0.7 %)
		Acute Bronchitis	1 (0.35 %)
		Septicaemia	1 (0.35 %)
Total	3 (1.09 %)	Total	13 (4.7 %)
Puerperal Pyrexia	53 (19.2 %)		85 (30.9 %)
Maternal Mortality	0		1 (0.35 %)

The results of this test indicate a certain degree of prophylaxis against puerperal sepsis, due to the addition of fat-soluble vitamins to the diet.

It is probable that other ills of pregnancy may be due to defects in diet, but the subject has been so little investigated that very few positive statements can be made. For instance, the anaemias of pregnancy and the puerperium may indicate a deficient iron intake, but there is evidence that vitamins A and B may also be implicated (p. 252).

Although, therefore, the part played by vitamins in pregnancy is not fully understood, women would be well advised to ensure that their diet at this period is rich in these substances, and especially in the fat-soluble vitamins. The inclusion, for instance, of a pint of milk, an egg (or its yolk), and a serving of green vegetables daily is to be recommended. If poverty precludes the purchase of these foods, a teaspoonful of cod-liver oil and 1 gram (15 grains) of tricalcium phosphate (bone ash) should be taken daily.

Diet of the Mother in Lactation.

Lactation, like pregnancy, involves special demands on the maternal metabolism and the indications that the maternal diet must be specially rich in vitamins apply equally to this period. A mother animal cannot put into her milk constituents which her body cannot make and which are not present in her food. On the other hand, a mother suffering from dietary deficiency may take any incoming vitamins for herself so that they are not passed on to the milk and do not reach the infant. Such a case is described in the Report on Studies of Rickets in Vienna [Med. Res. Coun., 1923] (see p. 273). The conventional views of the perfection of mother's milk and of the sacrifice of the mother's body to the growing foetus can no longer be upheld without modification. It is true that, when produced on a proper diet, the milk of a species is the ideal food for the infant of that species, and that the milks of different species are differently constituted, according to the rate of growth and special needs of the young. Doubtless there is an ideal standard composition and an ideal vitamin content for both woman's and cow's milk, when the woman or the cow is living under natural conditions to which she is fully adapted. In civilization and domestication, however, such conditions rarely occur, and, depending on the very different conditions of life or diet in which the mother is placed, the milk may vary widely in its composition and vitamin content [McCollum, Simmonds and Pitz, 1916; Kennedy and Dutcher, 1922].

Vitamin Deficiencies in Human Milk.

That breast milk may be grossly deficient in one or other of the vitamins is shown by the occurrence of diseases among breast-fed infants due to vitamin deficiency. Some instances of this are given below.

Vitamin A. There is no record of xerophthalmia amongst breast-fed babies, but since the disease has usualiy been diagnosed in the final stage by the occurrence of corneal ulcers, the deficiency would have to be severe and prolonged to produce such marked symptoms.

Vitamin D. Rickets is common among breast-fed children, as for

example in the United States, where Eliot [1925], working in New Haven, Conn., found a very slight degree of rickets almost universal. In a survey of 300 infants, 75–80 per cent. of which were breast-fed, those without rickets, past or present, numbered only about 10 per cent. Eliot states: 'If any two groups of infants show the need of early antirachitic treatment more than others, they are the large, rapidly growing, breast-fed infants and the premature babies. Our investigations have shown that a slight degree of early rickets is wellnigh universal in our climate and in our state of society.'

Hess and Weinstock [1927] state that in the month of March, in New York, all the bottle-fed, and from one-half to one-third of the breast-fed, babies show signs of rickets.

Antineuritic vitamin B_1. Infantile beri-beri occurs among breast-fed babies in the Philippines [McLaughlin and Andrews, 1910] and in the island of Nauru in the Pacific (see p. 247).

Pellagra. In the case of pellagra, it would appear [Harris, 1919] that children of pellagrous parents are often born in a very poor nutritive condition, not capable of diagnosis as infantile pellagra but very probably referable to that disease. Simonini [1905] states that the pellagrous mother should not nurse her child, but an extensive survey of pellagra among infants in Italy, quoted by Harris [1919], did not reveal a single case of pellagra in a nursing child.

Vitamin C. Scurvy occurs in breast-fed babies, but appears to be rare; instances were observed in Central Europe during and after the late war [Chick and Dalyell, 1919], but they must be regarded as isolated cases. Hess [1920] dismisses most of the cases of scurvy in breast-fed babies described in the literature as not really scurvy or not really developed on an adequate supply of breast milk.

Experimental Investigations on the Vitamin Content of Human Milk.

In addition to the above observations more complete studies have been made of the vitamin content of human milk. In America human milk has been tested by means of experiments on rats for its value in vitamin A by Macy *et al.* [1927; 1927, 1], vitamin B [1927, 2] and vitamin D [Outhouse *et al.* 1928]. The milk was obtained from the Detroit Wet Nursing Bureau; it was the pooled milk of 10–16 women receiving ordinary dietaries not specially controlled, but in no case receiving cod-liver oil. Such milk was found to provide enough vitamin A for a rat in a daily dose of 3 0 c.c. or less and is therefore described as a good source of vitamin A. Much larger daily doses of the same milk, as much as 20 c.c., were needed to supply rats with a sufficiency of the B-vitamins. The daily amount needed to prevent rickets in rats, on a rickets-producing diet, could not be determined, as the maximum amount given, 40 c.c. daily, exercised no antirachitic action whatever. Hess and Weinstock [1927] were, however, able to detect antirachitic potency for rats in a daily dose of 25–30 c.c. of human milk.

It is therefore clear that human milk from such women as attend the Detroit Wet Nursing Bureau satisfies the needs of rats for vitamin A easily, for one or both of the B-vitamins with difficulty,

and for vitamin D not at all. It must not be assumed that the needs of the human infant for these three vitamins bear the same quantitative relation to one another as do those of the rat, but these results give an indication that human milk may easily be poor in vitamin D and in the B-vitamins. In point of fact there is good evidence that infants at the breast may in some cases suffer from lack of these vitamins.

Prophylactic Treatment of the Mother by Vitamin Supplements during Lactation.

Allowing that human milk is not always perfect in its vitamin content, the question arises how best to make good any possible deficiencies. Vitamin supplements may be given to the mother or to the child, but there is no doubt that it is the best policy to give them to both.

A poor woman living in Vienna at the time of food shortage in 1922 (see p. 240), whose breast-fed baby had craniotabes, was medicated with cod-liver oil, cod-liver paste, and other foods, but her milk, tested regularly by observations on the growth of rats, showed no improvement over a period of three months, neither did the baby's craniotabes improve. The mother, however, who was in a very poor nutritive condition at the beginning of treatment, increased 40 lb. in weight before the end of the period. In the course of the same observations, the milk of another woman, who was young, healthy, and well nourished, was tested on rats before and after her treatment with cod-liver oil. In this case the improvement in the nutritive value of the milk after treatment was startling.

Macy *et al.* [1930] found that when 15 g. of cod-liver oil and 10 g. of yeast were added to the daily diet of three lactating women, they derived much benefit, and their calcium and phosphorus retention was improved. One of the women was previously in negative calcium balance and another in negative phosphorus balance, but in each case the negative was converted into a positive balance as the result of treatment. The effect on the babies was not recorded.

Hess, Weinstock and Sherman [1927] mention a case in which milk from a woman was found to have antirachitic potency after she had been irradiated with a mercury vapour quartz lamp, although none could be detected before treatment.

The cases quoted above show that nursing mothers may derive great benefit from treatment with supplementary vitamins, which should therefore on no account be withheld from them. The children also may be benefited in some instances, but in others the supplements administered to the mother may never reach them and it is therefore desirable that they too should be treated directly. Nursing mothers should be encouraged to eat a diet rich in the various accessory factors; reference to the table of foodstuffs (Appendix I) will indicate which these are. The diet chosen should also be well provided with salts, particularly with those of calcium, in which, as has already been shown, diets in this country are liable to be deficient (see p. 242). The mother should expose herself as much as possible, and without the intervention of glass, to the action of sunshine,

taking due precautions against excessive exposure. If but little sunshine is available she should take cod-liver oil or a concentrate of vitamins A and D, since, unless she is able to afford an expensive diet, the supply of vitamin D is unlikely to be sufficient without the help of sunshine.

The B-vitamins may be given in her diet in the form of eggs, green vegetables, liver or marmite, which last is, however, of variable potency. Dried yeast may be taken but is unpalatable; some commercial products, however, are valuable and not at all disagreeable. It must be borne in mind that according to Sure [1928, 2] the lactating mother dissipates at least 60 per cent. of the daily vitamin B intake in the metabolism of transfer to the milk.

The inclusion of suitable fresh fruit and green vegetables will provide a supply of vitamin C.

Vitamins in Infancy.

It is clear from what has been previously written that an adequate supply of vitamins in the food is of greater import in infancy, childhood, and adolescence than at any other time of life. On the whole, it is probable that the younger the age the greater is the importance of an adequate supply of these substances.

Most of the deficiency diseases of infants, so far as they are at present known, have been dealt with in the different chapters. These include rickets and infantile tetany (Chap. III), defective teeth (Chap. IV), scurvy and xerophthalmia (Chap. X). It only remains now to consider briefly how an abundant supply of vitamins can be guaranteed to the normal child.

Every infant should if possible be breast-fed for 8 or 9 months. If the mother's diet is good and is rich in dairy and vegetable products, accessory supplies of vitamins to the infant at this stage are probably of minor importance. Even under these conditions, however, additional supplies of fat-soluble vitamins to the infant in the form of one or two teaspoonsful daily of cod-liver oil and of antiscorbutic vitamin in the form of a teaspoonful or more of orange juice are advisable.

Supplementary additions of vitamins are even more essential (1) when the infant is partially or entirely artificially fed and (2) at the time of and following weaning. The importance of vitamins is then very great, partly because their addition helps to avoid or overcome many of the difficulties of feeding at these times, and partly because some of the foods given, as for instance cereals, make a special demand on the vitamin supply. Only too often, even in the early months of life, is some form of prepared cereal included in the diet. So long as the young infant is putting on weight and thriving on cow's milk the addition of cereals or proprietary foods containing cereal products is unjustifiable. A time comes, however, often at the age of 8 or 9 months, when increase in weight ceases and development on cow's milk is less satisfactory. At this time the addition of extra carbohydrate either as sugar or more frequently as cereal brings about rapid improvement and growth. Especially under these circumstances is it imperative to give cod-liver oil or some rich

form of vitamins A and D. Even without the addition of cod-liver oil the infant on the cereal-containing diet puts on weight rapidly and the mother is encouraged to increase the cereal food. This increase in growth is, however, often misleading, and, unless additional fat-soluble vitamins are given, the child may develop rickets and become catarrhal and susceptible to broncho-pneumonia. Even when florid rickets is avoided, it does not mean that calcification of the developing bones and teeth is perfect, for, as seen in Chapter IV, although a large proportion of children avoid this disease, there are relatively few whose teeth are properly calcified. It is probably at the weaning period, when the cusps of the molar teeth are joining up, that the risk of defective calcification of the teeth is greatest, so that it is specially important at this time to give vitamin D. The necessity for the increase of vitamin A in the diet, when artificial feeding is resorted to, is less understood, but there is reason to believe that an abundant store of this vitamin in the liver of a child is a form of insurance against certain infective conditions such as chronic catarrh and broncho-pneumonia. The mere fact that the liver stores such large quantities of vitamin A when the food is rich in this substance is an indication of its importance in the animal economy.

Egg yolk is a valuable source of both vitamins A and D and should take its place in the dietary of all artificially-fed infants, after the age of about three months. Other valuable sources of vitamin A, that should be given to infants under these conditions, include purées of vegetables such as spinach and carrots. Potato purée, although not rich in any known vitamins, is often useful for young children, especially as it enables the cereal intake to be cut down.

All children, and especially artificially-fed children, should receive a supplement of vitamin C. As this has now become a common practice, scurvy is a rare condition in infants, but it may be that a subnormal state of health due to a partial deficiency of this vitamin is more common (see pp. 103, 267). A few teaspoonsful of orange juice daily are much appreciated by the average infant and eliminate all possibility of any abnormality due to a deficiency of the antiscorbutic vitamin.

As regards the vitamin B complex, but little is known of the normal infant's requirements. Some authorities consider it useful to give infants a small amount of yeast extract such as marmite in order to provide for any possible deficiency in this respect.

To sum up, it is good to provide all infants with abundant supplies of vitamins, and this object can be best attained according to present knowledge in the following ways:

(1) By breast feeding of the infant for 8 or 9 months.

(2) By supplying the following foods to infants when partially or entirely artificially fed.

(*a*) Cow's milk to form the bulk of the diet up to 8 or 9 months and the basis of the diet for a year afterwards. At no time in the first year after weaning ought an infant to receive less than 1 to 2 pints daily, and after this not less than a pint.

(*b*) Cod-liver oil, to supply vitamins A and D (and iodine) in

the following quantities: infants 3 months—a teaspoonful daily, infants of 5 months and older—two teaspoonsful daily.

(*c*) Egg yolk, to supply vitamins D, A and B—½ to 1 egg daily.

(*d*) Orange juice or tomato juice for vitamin C—two or more teaspoonsful daily.

(*e*) Marmite for vitamin B complex—a small quantity daily.

(*f*) Purées of vegetables, e. g. spinach, carrot and turnip, cabbage, potato may also be added from about 6 months of age.

It is important that infants should not be kept for too long exclusively on a milk diet, since both human and cow's milk are low in iron; the liver of the newly-born infant contains a reserve of iron, but this tends to become depleted and the baby becomes anaemic. Some supplement rich in iron should therefore be given as early as possible, hence stress is laid on the early addition to an infant's diet of meat gravy, spinach, egg yolk, cereals, &c. The subject is discussed by Mackay [1931], who gives a table of the value of various foodstuffs for iron and recommends the administration of an iron salt to the child. Iron ammonium citrate was found satisfactory and is particularly favourable, since it may contain traces of copper and manganese, whose action is also beneficial. With dried milk this salt may be included in the milk before drying in an amount which will give 31½ grains of the salt to 1 lb. of the dried milk.

Supplementary feeding with vitamin-containing foods is particularly useful for marasmic infants and for those whose progress is retarded. To what extent marasmus is dependent upon abnormal nutrition is not known, but there is reason to believe that defective maternal feeding plays an important part in its aetiology. It is certainly true that such infants often make good improvement when fed along the lines indicated. Sometimes marasmic infants respond well to exposure to ultra-violet radiations even when they have failed to do so to cod-liver oil. A possible explanation of this may be that these infants fail to absorb the vitamin content of the oil, but can utilize the vitamin D produced in the skin by the irradiation.

Proprietary Infants' Foods.

Apart from the foods prepared from cow's milk, proprietary infants' foods (Rep. L. G. Board, 1914) usually consist largely of cereals, frequently to some extent malted. The majority of these foods, when prepared for use according to the directions given, are seriously deficient in fat, and consequently deficient in fat-soluble vitamins. It is probable that many are also inadequate with respect to the water-soluble vitamins; all are certainly deficient in the antiscorbutic vitamin and many in mineral constituents. Addition of milk to such foods will, of course, increase the amount of these factors, but the resulting preparations will still be inferior to whole milk. Clinical experience shows that both scurvy and rickets have frequently occurred in children reared on these preparations.

Numerous observers have reported a type of malnutrition in infants fed upon food mixtures rich in carbohydrate and poor in fat [Cheadle, 1906; Still, 1900; 1905]. Such infants are frequently

plump and well supplied with body-fat, and at first give the impression that they are well nourished, but they are usually flabby and show a lowered resistance to infective diseases and are particularly prone to rickets. There can now be little doubt that the ill effects of a diet deficient in fat are less a result of the insufficient supply of fat itself than of the indispensable accessory factors associated with the fat, but this must not be taken to imply that fat itself is not an important article of diet.

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APPENDIX I

Table XXI.

Distribution of Vitamins in Foodstuffs, etc.

In this table o *signifies that the vitamin has been tested for and not found,* + *that the material contains the vitamin,* + + *that it is a good source of the vitamin, and* + + + *that it is a rich source of the vitamin. The values entered under the heading vitamin B refer to the complex 'water-soluble B', entries being made under vitamins* B_1 *and* B_2 *when these have been separately estimated. References to the distribution of the vitamins will be found in the appropriate sections dealing with each vitamin separately.*

Foodstuffs.	*A*	*D*	*E*	B_1	B_2	*B*	*C*
Oils and Fats.							
Almond oil	o	. .	o				
Beef fat	+ +	+	. .	. .	. .	o	
,, ,, oil (oleo oil	+ to + +	. .	. .	. .	. .	o	o
,, kidney fat	+ +						
Butter fat (pasture)	+ +	+ +	+ +	. .	. .	. .	o
,, ,, (dry feed)	+	+	+	. .	. .	. .	o
Carrot oil	+ + +						
Cocoa butter	o	o to +	o				
Coco-nut oil	o to +	. .	+	. .	. .	o	o
Cotton-seed oil	o to +	+	+	. .	. .	o	o
,, ,, hardened	o	. .	+				
Cream	+ to + +	+	. .	. .	. .	+ +	o to +
Dog fat	+ +						
Fish-body oils.							
Coalfish	+						
Cod	+						
Eel	+ +						
Haddock	+						
Herring	+ +						
Salmon	+ to + +						
Sardine	o						
Fish-liver oils.							
Burbot	. .	+ + +					
Caplin (*Mallotus villosus*)	. .	+					
Cod	+ + +	+ + +	o to +				
Gadus Esmarkii	+ +						
Halibut	+ + +						
Herring	+ +						
Ling	+ +						
Plaice	+ +						
Pollock	+ +						
Puffer fish	. .	+ + + (very high)					
Rat fish	+ +						
Raja clavata	+ + +						
Salmon	+ + +	+ +					
Sardine							
Shark	+ +	+ + +					
Skate	+ +						
Ghee (Indian butter fat)	o to +						
Grape fruit-peel oil	o						
Hemp-seed oil	. .	. .	+ +				
Horse fat	+	. .	. .	. .	. .	o	o
Lampern, liver oil	+	+ +					
Lamprey, sea, liver oil	+	+ +					
Lard	o to +	o	o	. .	. .	. .	o
Lemon-peel oil	o						

Foodstuffs.	*A*	*D*	*E*	B_1	B_2	*B*	*C*
Oils and Fats (*cont.*)							
Lettuce-seed oil	. .	. .	+ + +				
Linseed oil	o to +	o					
,, ,, hardened	o						
Maize, yellow, oil	+ to + +	+	+ + +	. .	. .	o	o
Margarine, animal	o to +	o to +					
,, vegetable	o	. .	. .	. .	. .	o	o
,, enriched with vitamins	+ +	+ +	. .	. .	. .	o	o
Mustard-seed oil	. .	. .	o	. .	. .	o	o
Mutton fat	+	. .	+	. .	. .	o	o
Oat oil, whole oats	. .	. .	+ + +				
,, ,, husked	o						
Oleo stearin	+						
Olive oil	o to +	o to +	+	. .	. .	o	o
Orange-peel oil	+						
Palm oil	+ to + + +	o to +	+ +				
,, kernel oil	. .	. .	o				
Peach-kernel oil	. .	. .	+ +				
Pea-nut oil (Arachis oil)	o to +	o	+	. .	. .	o	o
Petrel, Australasian, stomach oil	. .	+ +					
Petrel, fulmar, stomach oil	+ +	+ +					
Pig fat	+	. .	+	. .	. .	o	o
,, kidney fat	+ +						
Poultry fat	+						
Rape oil	+						
Seagull (*Larus marinus*) liver oil	+ + +						
Seagull (*Larus marinus*) fat	+ +						
Sesame oil	o	o to +	o				
Soy-bean oil	o to +						
Walnut oil	. .	. .	+				
Whale oil	+ to + +						
Wheat oil	. .	. .	+ + +				
Wheat germ oil	+	. .	+ + + (very rich)				
Yeast fat	o	. .	o				
Cereals and Cereal Products.							
Barley, husked	. .	. .	. .	+ +	. .	. .	o
,, sprouted	+	. .	. .	. .	. .	. .	+
,, whole grain	+	. .	. .	+ +	. .	+	o
Bread, rye	. .	. .	. .	. .	. .	+ +	
,, wheaten, white, milk	+	. .	. .	. .	. .	. .	o to +
Bread, wheaten, white, water	o	. .	. .	+	+	. .	o
Bread, wheaten, wholemeal, milk	+	. .	. .	. .	. .	. .	o to +
Bread, wheaten, wholemeal, water	+	. .	. .	+ +	+ +	. .	o
Buckwheat	. .	. .	. .	+			
Custard powders and egg substitutes	o	. .	. .	. .	. .	o	
Dari	. .	. .	. .	+ +			
Hegari (Sorghum grain)	o						
Kafir corn, sprouted	. .	. .	. .	. .	. .	. .	+
Kaoliang, red	+	. .	. .	. .	. .	+ +	
,, white	+	. .	. .	. .	. .	+ +	
Maize, white, embryo	o	. .	. .	+ + +	+	. .	o

Foodstuffs.	*A*	*D*	*E*	B_1	B_2	*B*	*C*
Cereals and Cereal Products (*cont.*)							
Maize, white, endosperm	o	. .	. .	o	o	. .	o
,, ,, wholegrain	o	. .	. .	+ +	+	. .	o
,, yellow, embryo	o to +	. .	+ + +	+ + +	+	. .	o
,, ,, endosperm	+ +	. .	. .	o	o	. .	o
,, ,, gluten	+ +	. .	. .	o	. .	. .	o
,, ,, whole grain	+ +	o to +	+	+ +	+	. .	o
Maize, young cobs .	. .	. .	. .	. .	. .	. .	+
Malt, extract, commercial	. .	. .	. .	+	. .	+	o
Malt, green . . .	+	. .	. .	. .	. .	. .	+ +
,, kilned . . .	. .	. .	. .	+	. .	+	o
Millet	+	. .	. .	+ +	+	. .	o
Milo (Sorghum grain)	o						
Oats, husked . . .	o						
,, whole grain .	o to +	o	+	+	. .	+	o
,, ,, sprouted	. .	. .	. .	. .	. .	. .	+
Oatmeal	o to +	. .	. .	. .	. .	+	o
Rice, bran. . . .	. .	. .	. .	+ +	. .	. .	o
,, embryo. . .	. .	. .	. .	+ + +	. .	. .	o
,, parboiled, whole	. .	. .	. .	+ +			
,, ,, polished	. .	. .	. .	+ +			
,, polished . .	o	. .	o to +	o	o	. .	o
,, polishings . .	+	. .	. .	+ + +			
,, ,, ether extract	. .	. .	+ + +				
Rice, polishings, alcohol extract	. .	. .	. .	+ + +	+		
Rice, starch . . .	. .	. .	. .	o	o		
,, whole grain .	+	o	. .	+ +	+		
,, wild. . . .	+	. .	. .	. .	. .	+ +	o
Rye, embryo . . .	+ +	+	. .	. .	. .	. .	o
,, whole grain .	+	. .	. .	+ +	. .	. .	o
Wheat, bran . . .	+	. .	. .	+ +	+ +	. .	o
,, embryo . .	+ +	. .	+ + +	+ + +	+ +	. .	o
,, ,, extract (80 % alc.)	. .	. .	. .	+ + +	o		
,, endosperm .	o	. .	. .	o	o	. .	o
,, flour, white.	o	. .	. .	o	o	. .	o
,, ,, wholemeal	o to +	. .	. .	+	+	. .	o
,, middlings .	. .	. .	. .	+ +	+	. .	o
,, whole grain.	+	. .	+	+ +	+	. .	o
Legumes.							
Beans, green, canned	. .	. .	. .	. .	o to +	. .	doubtful
,, haricot . .	. .	. .	. .	+ +	. .	. .	o
,, katjang idjo (*Phaseolus radiatus*) .	. .	. .	. .	+ + +			
Beans, kidney . .	+	. .	. .	. .	. .	+ +	
,, mung . . .	+	. .	. .	. .	. .	+ +	
,, ,, sprouted	+	. .	. .	. .	. .	. .	+ +
,, navy, canned	+						
,, runner, green	. .	. .	. .	. .	. .	. .	+ to + +
,, string, green.	+ +	. .	. .	. .	. .	+	+ to + +
,, velvet. . .	. .	. .	. .	+ +	+		
,, ,, pod meal	. .	. .	+ +				
Cowpeas, dried (*Vignum sinense*) . . .	+ +	. .	. .	+ +	+		
Cowpeas, sprouted .	. .	. .	. .	. .	. .	. .	+ + +
Lentils.	+	. .	. .	+ +	. .	+ +	just +
,, sprouted . .	. .	. .	. .	. .	. .	. .	+ +
Peas, dried . . .	+	. .	. .	+ +	+ +	. .	+
,, green . . .	+ +	. .	. .	. .	. .	+ +	+ + +

Foodstuffs.	*A*	*D*	*E*	B_1	B_2	*B*	*C*
Legumes (*cont.*)							
Peas, green, canned	+ +	. .	. .	. .	. .	+ +	+ +
,, ,, sprouted	o to +	. .	. .	. .	. .	. .	+ +
,, yellow	+ +						
Soy beans	+	. .	+	+	+	+	o
,, sprouted	. .	. .	. .	. .	. .	. .	doubtful
Nuts							
Almond	+	. .	. .	+	. .	+	
,, sprouted	. .	. .	. .	. .	. .	. .	+
Brazil-nut	+	. .	. .	. .	. .	+	
Butter-nut	+	. .	. .	. .	. .	+	
Candle-nut	low						
Chestnut	. .	. .	. .	+	. .	+	
Coco-nut	+	. .	. .	. .	. .	+	
,, press-cake	+	. .	+	. .	. .	o	
Copra	o						
Filbert	. .	. .	. .	+ +	. .	+	
Hazel-nut	. .	. .	. .	+ +	. .	+	
Hickory-nut	. .	. .	. .	. .	. .	+ +	
Litchi-nut	o	. .	. .	. .	. .	o	
Pea-nut (Arachis)	+	. .	+	+ +	. .	+	
Pecan	+	. .	. .	. .	. .	+ +	
Pine-nut	+	. .	. .	. .	. .	+ +	
Walnut, black	. .	. .	. .	. .	. .	+	
,, English	+	. .	+	. .	. .	+	
Seeds.							
Babassu seed	very low						
Cacao	very low						
Carrot seed	+						
Citician ,,	o						
Coffee bean	. .	. .	. .	+			
Cohune seed	o						
Cotton ,,	+	. .	. .	. .	. .	+	
,, ,, meal	+						
Djave ,,	o						
Fennel ,,	o						
Kapok ,,	low						
Lettuce ,,	. .	. .	+ + +				
Linseed	+ +	. .	. .	. .	. .	+	
,, cake	+						
Lucerne seed	. .	. .	+ + +				
Rape ,,	low						
Sesame ,,	low						
Sunflower ,,	low						
Vegetables.							
Artichoke, globe	+ +	. .	. .	. .	. .	+ +	
Asparagustips, bleached	+						
,, ,, green	+ +	. .	. .	. .	. .	+ +	+ + +
,, ,, boiled	. .	. .	. .	. .	. .	. .	very low
Bamboo shoots	. .	. .	. .	. .	. .	o	
Beet, leaf and stem	+ +	. .	. .	. .	. .	+	
,, root	o to +	. .	. .	. .	. .	+	+
,, ,, juice	. .	. .	. .	. .	. .	. .	+
,, ,, sugar	. .	. .	. .	. .	. .	o	
Begonia leaf, fresh	. .	. .	+ + +				
Bilimbi (*Averrhoa carambla*)	. .	. .	. .	. .	. .	+	
Brussels sprouts	+ +						
Cabbage, canned	. .	. .	. .	. .	. .	. .	+ +
,, cooked	+	. .	. .	. .	. .	. .	+
,, etiolated	o to +	. .	. .	+ +	+		
,, green, fresh	+ +	. .	+	+ +	+ +	. .	+ + +
,, ,, dried	+	. .	. .	+ +	+ +	. .	+

Foodstuffs.	*A*	*D*	*E*	B_1	B_2	*B*	*C*
Vegetables (*cont.*)							
Cabbage, pickled	. .	. .	. .	. .	. .	. .	o
,, whole head	+						
Carrot, dried	. .	. .	. .	+ +	+	. .	+
,, juice	. .	. .	. .	. .	. .	. .	+
,, old, raw	+ +	. .	. .	+ +	+	. .	+
,, ,, cooked	. .	. .	. .	. .	. .	. .	+
,, young, canned	. .	. .	. .	. .	. .	. .	o
,, ,, cooked	. .	. .	. .	. .	. .	. .	+
,, ,, raw	+	. .	. .	+ +	+	. .	+ +
Cauliflower, cooked	. .	. .	. .	. .	. .	+	+ +
,, raw	+	. .	. .	. .	. .	+	+ + +
Celery, leaf, green	+ +						
,, ,, etiolated	+						
,, stalk	o to +	. .	. .	. .	. .	+	+ +
Chard	+ +	. .	. .	. .	. .	+	
Chayote	+ +						
Clover	+ +	. .	. .	. .	. .	+ +	
Collards	+ +	. .	. .	. .	. .	+ +	+ +
Cucumber	o to +	. .	. .	. .	. .	. .	+ +
Dandelion	+ +	. .	. .	. .	. .	+ +	+
Dasheen (*Caladium colocasia*)	+	. .	. .	. .	. .	+	+
Duhat (*Eugenia jambolana*)	. .	. .	. .	. .	. .	+	
Eggplant	+	. .	. .	. .	. .	+	+
Endive	+	. .	. .	. .	. .	. .	+
Escarole	+ +						
Hay	. .	+	. .	. .	. .	. .	o to +
Kale	+ +	. .	+ +				
Kohl-rabi	. .	. .	. .	. .	. .	. .	+ (low)
Lettuce, etiolated	+	. .	. .	+ +	+	. .	+ + +
,, green	+ +	. .	+ + +	+ +	+	. .	+ + +
,, ,, dried	. .	. .	+ + +	+ +	+	. .	o
,, ,, ether extract	+	. .	+ + +				
Lucerne (Alfalfa), dry	+ +	. .	+ + +				
,, fresh	. .	. .	+ + +	. .	. .	+ +	
Mangold, leaf	. .	. .	. .	. .	. .	. .	+ + +
,, root	o to +	. .	. .	. .	. .	. .	+
Mushroom, (*Agaricus campestris*)	. .	o	. .	. .	. .	+	o
Mushroom, Morchel	o	+	. .	. .	o to +		
,, Pfifferling	+ +	+	. .	. .	o to +		
,, Steinpilz	o	+	. .	. .	o to +		
Okra	. .	. .	. .	. .	. .	+	
Onion	o to +	. .	. .	+	+	. .	+ to + +
Parsley	. .	. .	. .	. .	. .	+ +	
Parsnip	o to +	. .	. .	. .	. .	+ +	
Pepper, green	+ +	. .	. .	. .	. .	+	+ + +
Pea seedlings, etiolated	. .	. .	+ + +				
,, ,, green	. .	. .	+ + +				
Pollen	. .	. .	. .	+ +			
Potato, cooked	+	. .	. .	. .	. .	. .	+ to + +
,, raw	+	. .	. .	+ +	+	. .	+ + to + + +
,, peel	. .	. .	. .	+ +			
Pumpkin	+ +	. .	. .	. .	. .	+	+
Radish	o to +	. .	. .	. .	. .	+	+ +
Rape leaf	. .	. .	. .	+	+ +		
Rhubarb	. .	. .	. .	. .	. .	. .	+ +
Romaine	+ +	. .	. .	. .	. .	+ +	
Sauerkraut	+	+	. .	. .	. .	+	+ to + +
Straw	. .	+					
Sorrel	. .	. .	. .	. .	. .	. .	+
Spinach, canned	+ +	. .	. .	. .	. .	. .	+ to + +

Foodstuffs.	*A*	*D*	*E*	B_1	B_2	*B*	*C*
Vegetables (*cont.*)							
Spinach, cooked	+ +	. .	. .	+	+ +	.	+ to + +
,, dried	+ +	. .	. .	+	+ +		
,, raw	+ +	o to +	. .	+	+ +	.	+ + +
Squash, Hubbard	+ +						
Sugar cane	. .	. .	. .	. .	. .	. .	o
Swede	+	. .	. .	. .	. .	+	
,, juice	. .	. .	. .	. .	. .	. .	+ + +
Sweet potato	+ to + +	. .	. .	. .	. .	+	
,, ,, juice	. .	. .	. .	. .	. .	. .	+ +
,, ,, leaf	. .	. .	. .	. .	. .	+	
Tea infusion, black	. .	. .	. .	. .	. .	o	o
,, ,, green	. .	. .	. .	. .	. .	. .	+ +
,, leaf, dried	. .	. .	+ +				
Timothy grass	. .	. .	. .	. .	. .	+	
Turnip, leaf	+ +	. .	. .	. .	+ +	+ +	+ +
,, root	o to +	. .	. .	+	+	. .	+ +
Vegetable-marrow juice	. .	. .	. .	. .	. .	. .	+ +
Velvet-bean leaf	. .	. .	. .	+	+ +		
Watercress, dried	+ +	. .	. .	+	+ +		
,, fresh	. .	. .	. .	+	+ +	. .	+ + +
Yautia, white	+	. .	. .	. .	. .	+	
,, yellow	+ +	. .	. .	. .	. .	+ +	o to +
Fruits.							
Alligator pear	+ +	. .	. .	. .	. .	+	
Apple	+	. .	. .	+	+	+	+ to + +
,, canned	. .	. .	. .	. .	. .	. .	+ to + +
,, dried	. .	. .	. .	+	+	. .	+
,, juice, conc.	. .	. .	. .	. .	. .	. .	+ to + +
Apricot	. .	. .	. .	. .	. .	. .	o to +
,, dried	. .	. .	. .	. .	. .	. .	o
,, sulphured and dried	. .	. .	. .	. .	. .	. .	+
Banana	+ to + +	low	+ +	+	+	. .	+ +
,, baked	. .	. .	. .	. .	. .	. .	+ to + +
,, dried	. .	. .	. .	+	+	. .	o to +
Bilberry (*Vaccinium Myrtillus*)	. .	. .	. .	. .	. .	. .	o to +
Blackberry	. .	. .	. .	. .	. .	. .	+ +
Breadfruit	+ +	. .	. .	. .	. .	+	
Canteloup	+ +	. .	. .	. .	. .	+	+ +
Cashew apple	+						
Cherry	+ +	. .	. .	. .	. .	+	+ to + +
Chico (Sapodilla)	. .	. .	. .	. .	. .	. .	+ +
Cloudberry (*Rubus Chamaemorus*)	. .	. .	. .	. .	. .	. .	+
Cocum	. .	. .	. .	. .	. .	. .	+
Cranberry juice	. .	. .	. .	. .	. .	. .	very low
Currant	. .	. .	. .	. .	. .	. .	+ +
,, dried	. .	. .	. .	o			
Custard apple	o						
Date	+	. .	. .	+			
Durian, white	o						
,, yellow	+						
Fig	+						
Grape	+	. .	. .	. .	. .	. .	very low
,, juice, conc.	+	. .	. .	. .	. .	+	o
Grape fruit	+	. .	. .	. .	. .	+	+ + +
,, juice	+	. .	. .	. .	. .	. .	+ + +
,, ,, dried	. .	. .	. .	. .	. .	. .	+ +
Lemon	+	. .	. .	. .	. .	+	+ + +
,, juice	+	. .	. .	. .	. .	+	+ + +
,, ,, dried	. .	. .	. .	. .	. .	. .	+ + +
,, ,, preserved	. .	. .	. .	. .	. .	. .	o to +
,, ,, tablets	. .	. .	. .	. .	. .	. .	+ +

Foodstuffs.	*A*	*D*	*E*	B_1	B_2	*B*	*C*
Fruits (*cont.*)							
Lemon peel	+	. .	. .	. .	. .	. .	very low
Lime	o	. .	. .	. .	. .	. .	+ +
,, juice	o	. .	. .	+	. .	. .	+ +
,, ,, concentrated	. .	. .	. .	. .	. .	. .	+
,, ,, preserved	. .	. .	. .	. .	. .	. .	o to +
Mango, dried	. .	. .	. .	. .	. .	. .	low
,, ripe	+ +	. .	. .	. .	. .	. .	+ + to + + +
,, unripe	+						
Mangosteen	o						
Mulberry	. .	. .	. .	. .	. .	. .	+
Naartje (*Citrus nobilis* Loureiro)	. .	. .	. .	. .	. .	. .	+ + +
Orange, pulp	. .	. .	. .	+ +	+	+	
,, dried and minced	. .	. .	. .	. .	. .	. .	o to +
Orange juice	+	. .	+	. .	. .	+	+ + +
,, ,, concentrated	+	. .	. .	. .	. .	. .	+ + +
Orange juice, dried	+	. .	. .	. .	. .	. .	+ + +
,, peel	. .	. .	. .	. .	. .	. .	+ +
,, ,, dried	. .	. .	. .	. .	. .	. .	+
,, ,, outer	+ +	. .	. .	. .	. .	. .	+ +
Paw-paw (papaya)	+ +	. .	. .	. .	. .	+	+ + +
Peach	+	. .	. .	. .	. .	+	+ to + +
,, canned	+	. .	. .	. .	. .	. .	+ to + +
,, dried	. .	. .	. .	. .	. .	. .	+
,, juice	. .	. .	. .	. .	. .	. .	+ to + +
Pear	. .	. .	. .	. .	. .	+	+
,, canned	. .	. .	. .	. .	. .	. .	+
Persimmon, Chinese	. .	. .	. .	. .	. .	. .	+
Pineapple	+ +	. .	. .	. .	. .	. .	+ +
,, canned	+ +	. .	. .	. .	. .	. .	+ +
,, juice	. .	. .	. .	. .	. .	. .	+ +
Plum	. .	. .	. .	. .	. .	. .	+ to + +
Prickly pear (*Opuntia decumana*)	. .	. .	. .	. .	. .	. .	+ +
Prune	. .	. .	. .	. .	. .	. .	+
,, dried	+ +	. .	. .	. .	. .	+	o
,, sulphured and dried	. .	. .	. .	. .	. .	. .	+
Raisin	o	. .	. .	. .	. .	+	o
Raspberry	. .	. .	. .	. .	. .	. .	+ +
,, juice dried	. .	. .	. .	. .	. .	. .	o
Strawberry	+	. .	. .	. .	. .	+	+ to + + +
,, canned	+	. .	. .	. .	. .	. .	+ + to + + +
Tamarind, dried	. .	. .	. .	. .	. .	. .	+
Tomato, green	+	. .	. .	. .	. .	+ +	
,, ripe	+ +	. .	. .	+	+	+ +	+ + +
,, canned	+ +	. .	. .	. .	. .	+ +	+ + to + + +
,, dried	. .	. .	. .	. .	. .	+ +	+ +
Meat and Fish.							
Bacon	o to +						
Beef	. .	. .	+ +	+ +	+ +	. .	o to +
,, juice	. .	. .	. .	. .	. .	+	o to +
Blood	. .	. .	. .	• o	+		
,, meal	o	o					
Chicken liver	. .	+ +	. .	. .	. .	. .	+
Clams	+	+	. .	. .	. .	o	
Eels, dried	+ +						
Fish, fat, muscle	+	+					

Foodstuffs.	*A*	*D*	*E*	B_1	B_2	*B*	*C*
Meat and Fish (*cont.*)							
Fish, white, muscle.	o to +	. .	. .	+			
,, liver . . .	+ + +	+ + +					
,, meal . . .	+ +	+ +					
,, roe	+ +	. .	. .	+			
Ham	o to +	. .	. .	+	+		
Liver extract, commercial	. .	. .	. .	+ +	+ + +		
Meat juice. . . .	. .	. .	. .	. .	. .	+	very low
,, meal. . . .	o	o					
Mutton	. .	. .	. .	+ +	+ +	. .	o to +
Ox brain	+	. .	. .	+ +			
,, heart	. .	. .	. .	+ +			
,, hypophysis . .	. .	. .	+ +				
,, kidney . . .	. .	. .	. .	. .	+ + +		
,, liver	+ + +	. .	+ +	+ +	+ + +		
,, testes	. .	. .	. .	+			
Oysters	+ +	+ +	. .	. .	. .	+ +	+
Pig brain	+	. .	. .	. .	. .	+	
,, choroid . . .	o						
,, heart	. .	. .	+ +	. .	. .	+	
,, kidney . . .	. .	. .	. .	. .	. .	+	
,, liver	+ + to + + +	+	+ +	. .	. .	+ +	+ +
,, muscle . . .	. .	. .	+ +				
,, pancreas . . .	. .	. .	+ +				
,, retina	+ + +						
,, spleen. . . .	. .	. .	+ +				
Placenta, human .	. .	. .	+ +				
Pork	o to +	. .	. .	+ +	+ +	. .	o to +
Poultry	o to +						
Rabbit liver . . .	. .	. .	. .	. .	. .	. .	+ +
Rat heart	. .	. .	+ +				
,, kidney . . .	+ +	. .	+ +				
,, liver	+ + +	. .	+ +				
,, lung	+ +						
,, muscle . . .	+	. .	+ +				
,, testes	. .	. .	+ +				
,, blood	+						
Salmon, canned . .	+						
Sheep brain . . .	. .	. .	. .	+ +			
,, liver . . .	+ + +	o	. .	. .	. .	+ +	
Shrimps	+						
Sweetbreads . . .	+	. .	. .	. .	. .	+ +	
Toheroa	+ +						
Veal	o to +						
Whale meal . . .	o	o	. .	. .	. .	+	
Eggs.							
Duck, fresh . . .	+ +						
,, salted . . .	+ +	. .	. .	. .	. .	o	
Hen, fresh . . .	+ +	+ +	. .	+	+ to + +		
,, dried . . .	+ +	+	. .	. .	. .	. .	o
,, preserved . .	+ +						
,, yolk. . . .	+ +	+ + +	+ +	+ +	+	. .	o
,, white . . .	o	o	. .	. .	+ +	. .	o
Milk and Milk Products.							
Buttermilk . . .	+	. .	. .	. .	. .	+	o to +
Caseinogen . . .	. .	. .	+	o	o		
Cheese, American cheddar	+ +	. .	. .	o	+		
Cheese, skim milk .	+						
,, Limburger .	+ +						
,, Swiss . .	+ to + +						

Foodstuffs.	*A*	*D*	*E*	B_1	B_2	*B*	*C*
Milk and Milk Products (*cont.*)							
Cow's milk, whole, fresh	+ (varies)	o to + (varies)	+ (varies)	+	+ +	. .	+
Cow's milk, whole, condensed	+ + (varies)	. .	. .	+	. .	+	+
Cow's milk, whole, dried	+ + (varies)	o to + + (varies)	+ (varies)	. .	. .	+ +	o to +
Cow's milk, whole, pasteurized	+ (varies)	. .	. .	. .	. .	+	+
Cow's milk, whole, scalded	+ (varies)	. .	. .	. .	. .	+	+
Cow's milk, whole, sterilized	+ (varies)	. .	. .	. .	. .	+	o to +
Cow's milk, skim. .	o to +	. .	. .	. .	. .	. .	o to +
,, ,, ,, dried	o to +	. .	o	. .	. .	+	o to +
Goat's milk . . .	+	. .	. .	. .	. .	+	+
Human milk . . .	+	o to +	. .	+	+	. .	o to +
Lactic acid milk . .	. .	. .	. .	. .	. .	. .	+
Lactose, pure . . .	. .	. .	. .	. .	. .	o	o
Miscellaneous.	. .	. .	. .	. .	. .	o to +	o
Ale, pale	. .	. .	. .	. .	. .	. .	+
Beer, Kafir . . .	. .	. .	. .	. .	. .	. .	+
,, spruce . . .	. .	. .	. .	o	+		
,, stout . . .	o	o					
Cocoa	+	+ to + +					
Milk chocolate . .	(varies) + +	o					
Copepods							
Diatoms (*Nitzschia closterium*) . . .	+ + + . .	o + +					
Ergot of rye . . .	o	o	. .	o	o	. .	o
Honey	. .	. .	. .	. .	. .	. .	very low
Marmalade, orange .	o	. .	. .	. .	. .	+	
Molasses, beet . .	o	. .	. .	. .	. .	+ +	
,, cane . .	+ +						
Seaweed, *Cladophora*							
,, *Chondrus crispus*	o + +						
Seaweed, *Polysiphonia*	+ +						
,, *Ulva* . .	o	. .	. .	. .	. .	o	o
Sugar, cane, pure .	o	. .	. .	. .	. .	o	o
,, glucose ,, .	o to +	o	o to +	+ + +	+ + +	. .	o
Yeast, brewer's . .	. .	. .	. .	o	+ +		
,, ,, autoclaved							
,, ,, autolysed	. .	. .	. .	+ + +			
Yeast, brewer's, dried	. .	. .	. .	+ + +	+ + +	. .	o
,, ,, extract (marmite) . . .	. .	. .	. .	+ + +	+ + +	. .	o

APPENDIX II

Official No. : C. H. 1055(1).
Geneva, 1931.

LEAGUE OF NATIONS.

HEALTH ORGANIZATION. PERMANENT COMMISSION ON BIOLOGICAL STANDARDIZATION.

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REPORT OF THE CONFERENCE ON VITAMIN STANDARDS, HELD AT LONDON FROM JUNE 17th TO 20th, 1931.

List of Participants.

Foodstuffs.	*A*	*D*	*E*	B_1	B_2	*B*	*C*
Milk and Milk Products (*cont.*)							
Cow's milk, whole, fresh	+ (varies)	o to + (varies)	+ (varies)	+	+ +	. .	+
Cow's milk, whole, condensed	+ + (varies)	. .	. .	+	. .	+	+
Cow's milk, whole, dried	+ + (varies)	o to + + (varies)	+ (varies)	. .	. .	+ +	o to +
Cow's milk, whole, pasteurized	+ (varies)	. .	. .	. .	. .	+	+
Cow's milk, whole, scalded	+ (varies)	. .	. .	. .	. .	+	+
Cow's milk, whole, sterilized	+ (varies)	. .	. .	. .	. .	+	o to +
Cow's milk, skim. .	o to +	. .	. .	. .	. .	. .	o to +
,, ,, ,, dried	o to +	. .	o	. .	. .	+	o to +
Goat's milk . . .	+	. .	. .	. .	. .	+	+
Human milk . . .	+	o to +	. .	+	+	. .	o to +
Lactic acid milk . .	. .	. .	. .	. .	. .	. .	+
Lactose, pure . . .	. .	. .	. .	. .	. .	o	o
Miscellaneous.	. .						

ERRATUM

SPECIAL REPORT SERIES NO. 167 'VITAMINS'

The 'Miscellaneous' section on p. 312 (Appendix I) should read thus:

Foodstuffs.	*A*	*D*	*E*	B_1	B_2	*B*	*C*
Miscellaneous.							
Ale, pale	. .	. .	. .	. .	. .	o to +	o
Beer, Kafir . . .	. .	. .	. .	. .	. .	. .	+
,, spruce . . .	. .	. .	. .	. .	. .	. .	+
,, stout . . .	. .	. .	. .	o	+		
Cocoa	o	o					
Milk chocolate . .	+ (varies)	+ to + +					
Copepods	+ +	o					
Diatoms (*Nitzschia closterium*) . . .	+ + +	o					
Ergot of rye . . .	. .	+ +					
Honey	o	o	. .	o	o	. .	o
Marmalade, orange .	. .	. .	. .	. .	. .	. .	very low
Molasses, beet . .	o	. .	. .	. .	. .	+	
,, cane . .	o	. .	. .	. .	. .	+ +	
Seaweed, *Cladophora*	+ +						
,, *Chondrus crispus*	o						
Seaweed, *Polysiphonia*	+ +						
,, *Ulva* . .	+ +						
Sugar, cane, pure .	o	. .	. .	. .	. .	o	o
,, glucose ,, .	o	. .	. .	. .	. .	o	o
Yeast, brewer's . .	o to +	o	o to +	+ + +	+ + +	. .	o
,, ,, autoclaved	. .	. .	. .	o	+ +		
,, ,, autolysed	. .	. .	. .	+ + +			
Yeast, brewer's, dried	. .	. .	. .	+ + +	+ + +	. .	o
,, ,, extract (marmite) . . .	. .	. .	. .	+ + +	+ + +	. .	o

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REPORT OF THE CONFERENCE ON VITAMIN STANDARDS, HELD AT LONDON FROM JUNE 17th TO 20th, 1931.

List of Participants.

Chairman:

Professor E. Mellanby, Professor of Pharmacology, University of Sheffield, Sheffield.

Participants:

Professor J. C. Drummond, Professor of Biochemistry, University College, University of London.

Professor H. von Euler, Professor of Biochemistry, University of Stockholm, Stockholm.

Professor L. S. Fridericia, Director, Institute of Hygiene of the University, Blegdamsvej 21, Copenhagen.

Professor B. C. P. Jansen, Professor of Physiological Chemistry, University of Amsterdam, Amsterdam.

Professor E. V. McCollum, Professor of Chemical Hygiene, School of Hygiene and Public Health, Johns Hopkins University, Baltimore.

Professor E. Poulsson, Director of the State Vitamin Institute, Skoyen-Oslo.

Mme G. L. Randoin, 'Directeur du Laboratoire de physiologie au Centre de recherches sur l'alimentation (Institut de recherches agronomiques), Directeur du laboratoire de physiologie de la nutrition a l'École des Hautes-Études', Paris.

Professor A. C. Scheunert, 'Direktor des Tierphysiologischen Instituts der Universität', Leipzig.

Dr. A. Seidell, Chemist, National Institute of Health, United States Public Health Service, Washington, D.C.

Professor H. Steenbock, Professor of Agricultural Chemistry, University of Wisconsin, Madison, U.S.A.

Professor A. Windaus, 'Direktor, Allgemeines Chemisches Laboratorium der Universität Göttingen', Germany.

Technical Secretaries:

Dr. H. Chick, Lister Institute of Preventive Medicine, London.

Dr. W. R. Aykroyd, Lister Institute of Preventive Medicine, London.

The following were also present:

Dr. C. E. Bills, Director of Research, Mead, Johnson & Co., Evansville, Indiana, U.S.A.

Dr. R. Bourdillon, National Institute for Medical Research, London.

Dr. Katharine-Coward, Pharmaceutical Society's Laboratories, London.

Professor R. A. Peters, School of Biochemistry, Oxford.

Dr. O. ROSENHEIM, National Institute for Medical Research, London.
Dr. S. S. ZILVA, Lister Institute of Preventive Medicine, London.

In the following recommendations, the question of standards and units for the four following vitamins only is considered:

I. The fat soluble Vitamin A.
II. The antirachitic Vitamin D.
III. The antineuritic Vitamin B (also known as Vitamin B_1).
IV. The antiscorbutic Vitamin C.

It is the general opinion that, in the present state of our knowledge, only these vitamins can be profitably discussed in this connexion.

I. THE FAT SOLUBLE VITAMIN A.

(a) *International Standard.*

The Conference recommends that carotene be accepted as an international provisional standard of reference for Vitamin A and that a selected sample of cod-liver oil be held in view as a possible secondary standard.

(b) *Mode of Preparation.*

It was decided that the information available does not yet justify the selection of one isomer of carotene as a standard. The similar biological activity of the two isomers that have recently been described is further justification for adopting as the provisional international standard a mixture prepared in an approved manner.

It was decided to employ a preparation of carotene made from carrots by Willstätter's method and purified by recrystallization by the method described in the memorandum issued by the Department of Biological Standards, National Institute for Medical Research, London (see Appendix II a, p. 319), until the melting-point determined is above 179° C. It was suggested that preparations should be made in various countries and dispatched immediately and with all necessary precautions against decomposition to the National Institute for Medical Research, London, where they will be mixed to form a uniform preparation by the most suitable method. The details of this final purification is to be left to the discretion of the authorities of the National Institute. It was suggested that original preparations of, say, 4 to 5 grammes might be made in the following institutions:

Department of Physiological Chemistry, University of Amsterdam.
'Laboratoire de physiologie de la nutrition, École des Hautes-Études', Paris.
'Tierphysiologisches Institut', Leipzig.
National Institute for Medical Research, Hampstead, London.
Department of Agricultural Chemistry, University of Wisconsin, U.S.A.
Biochemical Department, University of Stockholm, and 'Institut für organische Chemie der Universität', Zürich.
School of Hygiene and Public Health, Johns Hopkins University, Baltimore, U.S.A.

(c) *Place of Preparation.*

It was decided that the National Institute for Medical Research, London, acting for this purpose as the *central laboratory on behalf of the Health Organization of the League of Nations*, should be asked to undertake the final preparation of the sample of carotene to be used as the international standard for Vitamin A.

(d) *Mode of Distribution.*

It was considered desirable that, as far as possible, the standard preparation should be distributed to workers through the appropriate official institution in each country, preferably that now responsible for the distribution of similar biological standards. The material should be sent out in tubes of 10 mg. as described in Appendix IIa, p. 319.

(e) *Definition of Unit.*

The unit of Vitamin A recommended for adoption is the Vitamin A activity of 1 γ (0·001 mg.) of the international standard.

Note.—Daily doses of about 3 γ to 5 γ of the international standard, when administered to young rats suitably prepared on a Vitamin A-deficient diet, have been found adequate to restore growth and to cure xerophthalmia.

(f) *Permanence of the Standard.*

The Conference recommends that this international standard and unit be accepted provisionally for two years.

(g) *Subjects recommended for Future Investigations.*

It is highly desirable that, during the provisional period, further investigations of the standard should be made regarding the stability of the carotene preparation both when sealed in the original tubes and after it has been removed and dissolved for biological testing. In the latter connexion, emphasis is laid on the importance of minimizing contact with air or oxygen of the solutions used for animal feeding. It is recommended that they be always stored in an inert gas and at low temperature.

It will be of great value if investigators submit to the League of Nations Health Organization observations on the stability of the preparation, its behaviour in various solvents and under different conditions of storage, and any other information bearing on its use as a biological standard. The use of suitable 'antioxidants' ('*antioxygènes*' in French) should, in particular, be studied.

(h) *Biological Methods for Estimation of Vitamin A.*

It was decided not to recommend any one particular method of conducting the biological assay, but to invite members of the Conference to submit to the League of Nations Health Organization their observations on the value of the methods they have been using.

It is recommended that further attention be given to the methods based on the curative action of carotene for xerophthalmia and other lesions characteristic of Vitamin A deficiency, as well as to those based on increase in weight.

(i) *Selected Sample of Cod-Liver Oil for Use as a possible Secondary Standard.*

It is recommended that a supply of an approved sample of cod-liver oil be obtained with the object of making a series of comparative tests to determine its suitability as an alternative standard. The Conference is informed that the United States Department of Agriculture is making arrangements for such a standard substance to be available in the United States during the coming year.

It was decided to ask the United States Department of Agriculture to obtain sufficient supplies of the oil for distribution in order to enable investigators of other countries to assay this oil in terms of the international unit of standard carotene. It is hoped thus to obtain evidence regarding the stability of Vitamin A in cod-liver oil as affected by conditions and time of storage.

II. The Antirachitic Vitamin D.

(a) *International Standard to be adopted and Arrangements for Control.*

The Conference recommends that the standard solution of irradiated ergosterol at present issued from the National Institute of Medical Research, London, be adopted as international Vitamin D standard for the next two years.

If within this period it should become necessary, owing to threatened exhaustion of the present supply, to replace this solution by a fresh standard, the equivalence shall be determined by experts of different countries who have had the opportunity of comparing the proposed new standard with the one at present issued. It is suggested that the following Institutions, among others, be invited to co-operate in those tests:

'Allgemeines Chemisches Laboratorium', Göttingen.
'Tierphysiologisches Institut', Leipzig.
Food and Drugs Administration Laboratory, Department of Agriculture, Washington, D.C.
Biochemical Department, University of Stockholm.
Department of Agricultural Chemistry, University of Wisconsin.

School of Hygiene, Johns Hopkins University, Baltimore.
'Laboratoire de physiologie de la nutrition, École des Hautes-Études', Paris.
Pharmaceutical Society, London.

(b) *Method of Preparation.*

1. It is recommended that, in the preparation of the solutions of irradiated ergosterol, used as standards of reference for Vitamin D (or as sub-standards), irradiation with ultraviolet light shall be done in ethereal solution in the absence of any appreciable traces of oxygen, and the solution should be kept in rapid motion. The conditions of exposure should be such as to transform between 30 per cent. and 80 per cent. of the ergosterol. The solution of the product and of further dilutions shall be made in a stable unsaturated natural vegetable oil, which has given a negative test for Vitamin D.

2. The standard solution of irradiated ergosterol at present issued from the National Institute for Medical Research, London (Standard Solution III), was, however, prepared as follows, in January 1929:

A 0·1 per cent. solution of the ergosterol in absolute alcohol was exposed for half an hour in a silica cell, 1 cm. thick, to the unfiltered radiation from a K.B.B. (Kelvin, Bottomley and Baird) mercury vapour lamp, taking 2·5 ampères and 125 volts at atmospheric pressure, at 15 cm. distance from cell to lamp. The resulting solution was mixed with a little olive oil, and then evaporated at 45° C. at a low pressure to remove the alcohol. The concentrated oily solution thus obtained was diluted with pure olive oil to give a concentration corresponding to 1 mg. of the original ergosterol in 10 c.c. of olive oil at 18° C. The olive oil used was tested for stability and gave a negative test for Vitamin D.

The additional larger quantity of standard (prepared January 1931), which is available for distribution later if required, was prepared with observance of the general conditions indicated under 1.

(c) *Mode of Distribution.*

The National Institute for Medical Research, London, *acting for this purpose as the central laboratory on behalf of the League of Nations Health Organization*, shall distribute to each country wishing to use the standard a sufficient quantity of the solution to enable the standard to be effectively applied according to the conditions in the particular country. Such quantity should be supplied only to a central institution nominated for the purpose by the country concerned, which will be responsible for the distribution either of the portion of international standard solution received or, wherever possible, of an equivalent sub-standard prepared by comparison therewith.

(d) *Stability of the International Standard.*

The stability of the standard solution at present issued from the National Institute for Medical Research, London, has proved satisfactory on the results of tests over a period of two years, when preserved at or below 0° C. with exclusion of air.

(e) *Definition of Unit.*

The unit of Vitamin D recommended for adoption is defined as the Vitamin D activity of 1 mg. of the international standard solution of irradiated ergosterol.

Note.—The international standard solution has been prepared to have such potency that approximately 1 mg. thereof given daily to a rachitic rat for eight successive days will produce a wide line of calcium deposits in the metaphysis of the proximal ends of the tibiae and of the distal ends of the radii.

(f) *Permanence of the Standard.*

The international standard at present recommended shall be regarded as provisional for the next two years, in the hope that a more stable crystalline substance may in the meantime become available.

(g) *Subjects recommended for Further Investigation.*

1. The influence of various oils as solvents upon the stability of solutions of irradiated ergosterol.

2. Further investigation of the crystalline antirachitic substances recently isolated from irradiated ergosterol. It was decided that Professor Windaus

and Dr. Bourdillon shall be asked to investigate the constancy of the physical properties of the crystalline products recently isolated by them respectively, in order to determine whether these may be regarded as pure substances. If so, the potency of these products should be accurately compared with that of the (present) international standard at intervals of three months during the next two years, in order to compare the stability in each case. If the results are satisfactory, it is hoped that one of these crystalline substances may eventually replace the solution of irradiated ergosterol as international Vitamin D standard.

3. The toxicity of the present standard and of the crystalline products should be investigated (see also p. 319).

(h) *Biological Methods for Estimation of Vitamin D.*

In using the international standard solution for the determination of the antirachitic potency of unknown preparations, it is recommended that not fewer than twenty rats (preferably more) be used for a determination, half of these to receive the standard and the remaining litter-mates the unknown substance. Provided this precaution is observed, it is considered permissible to use various biological methods of estimation, either prophylactic or therapeutic. For instance, the 'line' test, X-ray examination or determination of the bone ash, are all considered reliable methods.

III. The Antineuritic Vitamin B.

(a) *International Standard.*

The Conference recommends the adoption, as international standard, of the adsorption product of the antineuritic Vitamin B prepared in the Medical Laboratory, Batavia (Java), by the method of Seidell, as described by Jansen and Donath.

(b) *Terminology.*

The international standard preparation should be known as the 'standard adsorption product of the antineuritic Vitamin B'.

(c) *Method of Preparation.*

The international standard is prepared by extracting rice polishings with water, sufficient sulphuric acid being added to make the *p*H 4·5. Salicylic acid to a concentration of 0·2 per cent. and toluene are then added to prevent bacterial decomposition. The process of extraction is continued for two days, after which the solution is filtered. For each 100 kilogrammes of the original rice polishings, 3 kilogrammes of fuller's earth (specially selected for its adsorptive powers) are added to the solution, which is then stirred for twenty-four hours. Subsequently, the solution is filtered off and the fuller's earth, after being washed with water and alcohol, is dried; 3 kilogrammes of the fuller's earth adsorbate represents the antineuritic Vitamin B from 100 kilogrammes of rice polishings.

(d) *Place of Preparation.*

It is recommended that the Medical Laboratory, Batavia, Java, should be asked, through Professor Jansen, of Amsterdam, to prepare a batch of 25 kilogrammes of the standard preparation. This should provide an adequate supply for many years.

(e) *Place of Distribution.*

It is suggested that this batch of standard adsorption product of antineuritic Vitamin B should be kept at the National Institute for Medical Research, London, *acting for this purpose as central laboratory on behalf of the Health Organization of the League of Nations.*

One hundred grammes would be an amount suitable for distribution to individual laboratories. No special precautions are necessary in keeping this preparation, except that it should be stored in a dry place. In the presence of moisture, bacterial decomposition readily takes place.

(f) *Definition of Unit.*

The unit recommended for adoption is the antineuritic activity of 10 milligrammes of the international standard adsorption product.

Note.—A daily dose of 10 to 20 mg. of this preparation is required to maintain normal growth in a young rat on a diet deficient in the antineuritic Vitamin B but complete in all other respects, including the antidermatitis Vitamin (B_2); the curative 'day dose' for a pigeon (300 grammes weight) suffering from polyneuritis on a diet of polished rice is about 20 to 30 mg. (method of Kinnersley and Peters).

(g) *Permanence of the International Standard Recommended.*

This standard adsorption product should serve as a provisional international standard for five years, or until advances in the knowledge of this vitamin make a revision desirable.

(h) *Recommendations for Further Investigations.*

1. The standard adsorption product should be investigated for its content of other B-vitamins.

2. Although there is no evidence that loss of potency is liable to occur in the standard adsorption product, the Conference suggests that the following laboratories be asked to undertake a further investigation of its stability:

Department of Physiological Chemistry, University of Amsterdam.
National Institute of Health, United States Public Health Service, Washington, D.C.
Institute of Hygiene, University of Copenhagen.
School of Biochemistry, University of Oxford.
'Tierphysiologisches Institut', Leipzig.
Biochemical Institute, University of Stockholm.
'Laboratoire de physiologie au Centre de recherches sur l'alimentation (Institut des recherches agronomiques)', Paris.
Lister Institute of Preventive Medicine, London.

(i) *Biological Methods for Estimation of the Antineuritic Vitamin B.*

The Conference expresses no opinion on the relative merits of current biological methods for estimation of the antineuritic Vitamin B (as recorded in the report on this vitamin, presented to this Conference and in the literature generally). It considers that good evidence is provided by that report that the different methods described, either prophylactic or curative in type, and employing either the rat or the pigeon as experimental animal, may yield equally valid results.

IV. The Antiscorbutic Vitamin C.

(a) *International Standard.*

The Conference recommends the adoption as international standard of the fresh juice of the lemon, Citrus limonum.

(b) *Definition of Unit.*

The unit of the antiscorbutic Vitamin C recommended for adoption is the Vitamin C activity of 0·1 c.c. of fresh juice of the lemon, *Citrus limonum*,

Note.—This is about 1/10th of the daily dose necessary to prevent development of macroscopic scorbutic lesions in a young guinea-pig maintained on a scurvy-producing diet.

(c) *Method of Use.*

The fresh lemon juice used as standard may be decitrated as follows: to the expressed juice after filtration through muslin an excess of calcium carbonate is added until effervescence stops. After standing for one hour, the mixture is filtered through a Buchner funnel. The decitrated juice should have a reaction of *p*H about 6 and should be administered to the experimental animal within two hours of filtration.

(d) *Permanence of Standard.*

This international standard shall be regarded as provisional for the next two years.

* * *

Note on the Toxicity of Irradiated Ergosterol.

In view of the toxic effects which have been reported after administration of certain specimens of irradiated ergosterol, this Conference suggests the advisability of testing all preparations of irradiated ergosterol, destined for medicinal use, for toxicity as well as for antirachitic potency.

APPENDIX II a.

Memorandum on Carotene supplied for testing its suitability as a possible Standard for Vitamin 'A'.

Preparation.

The material provided has been prepared as follows: Commercial carotene (B.D.H.) was dissolved in benzene and filtered, and the clear solution poured into a large volume of warm absolute alcohol. The crystallization was allowed to proceed at 37° C., and the crystals filtered off at the same temperature. All these operations were carried out in an atmosphere of CO_2. The crystalline material was dried *in vacuo*. Melting point 179° to 180° C. (taken in electrically heated 'Berl block').

For distribution into tubes the material was dissolved in benzene at 37° C. to make a 2 per cent. solution; 0·5 c.c. of this solution was run into each of the brown glass tubes in which the material is distributed, the whole process of solution and filling out being again carried out in an atmosphere of CO_2. The filled tubes were transferred to a desiccator containing paraffin shavings and $CaCl_2$. The desiccator was evacuated and left attached to a pump until the benzene had completely evaporated and the carotene had been deposited, mainly as a crystalline residue, at the bottom of each tube. When drying was complete, the desiccator and contained tubes were again filled with CO_2, evacuated, and refilled with CO_2. The tubes, before filling, had been drawn out into narrow constrictions to facilitate sealing, which was thus rapidly effected with minimal contamination of the CO_2 by air.

Suggestions for Use.

It is assumed that less than the whole contents of one tube (10 mg.) will be required for a test. The tube having been opened, the necessary quantity of carotene can be removed with the aid of a fine glass rod or narrow platinum spatula, and immediately dissolved in the chosen solvent. The partly used tube should be enclosed in a test tube of suitable size, which should then be drawn out in preparation for sealing. The test tube and contained specimen tube can then be refilled with CO_2 in the vacuum desiccator, as above described, removed, quickly sealed, and preserved in a cold, dark place until again required. The prepared solution, if it is to be used for several tests, should be preserved from light and oxygen by similar or equivalent precautions.

January 6th, 1931.

Department of Biological Standards,
National Institute for Medical Research, London.

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See also Surgery (No. 138).

Blood Physiology:

No. 72. The Acid-base Equilibrium of the Blood. By the Haemoglobin Committee. [1923.] 2*s.* (2*s.* 1*d.*).

See also Shock, Surgical.

Borna Disease: *see* Animals, Diseases of.

Brain Surgery: *see* Surgery.

Bright's Disease: *see* Nephritis.

* For overseas agencies see p. ix.

Burns:

No. 141. The Tannic Acid Treatment of Burns. By W. C. Wilson. [1929.] 1*s.* (1*s.* 1*d.*).

Cancer:

No. 99. An Investigation into the Statistics of Cancer in Different Trades and Professions. By Matthew Young and W. T. Russell. [1926.] 1*s.* 6*d.* (1*s.* 7*d.*).

See also RADIUM.

Catgut: *see* SURGERY (No. 138).

Cerebro-spinal Fever:

No. 2. Report of the Special Advisory Committee upon Bacteriological Studies of Cerebro-spinal Fever during the Epidemic of 1915. [1916.] *Out of print.*

No. 3. Bacteriological Studies in the Pathology and Preventive Control of Cerebro-spinal Fever among the Forces during 1915 and 1916. By M. H. Gordon, Martin Flack, P. W. Bassett-Smith, T. G. M. Hine, and W. J. Tulloch. [1917.] *Out of print.*

No. 17. (I.) A Report upon the Seasonal Outbreak of Cerebro-spinal Fever in the Navy at Portsmouth, 1916–17. By Paul Fildes and S. L. Baker. (II.) The Treatment of Cerebro-spinal Meningitis by Antimeningococcus Serum at the Royal Naval Hospital, Haslar, 1915–16–17. By G. P. Adshead. [1918.] 2*s.* 6*d.* (2*s.* 8½*d.*).

No. 50. Cerebro-spinal Fever. Studies in the Bacteriology, Preventive Control, and Specific Treatment of Cerebro-spinal Fever among the Military Forces, 1915–19. By M. H. Gordon and others. [1920.] 4*s.* (4*s.* 3*d.*).

No. 124. The Meningococcus. By E. G. D. Murray. [1929.] 3*s.* 6*d.* (3*s.* 8½*d.*).

Chemotherapy: *see* STREPTOCOCCAL INFECTIONS.

Child Life (ANTENATAL and POSTNATAL INVESTIGATIONS):

No. 10. The Mortalities of Birth, Infancy, and Childhood. By A. K. Chalmers, W. A. Brend, L. Findlay, and J. Brownlee. [1918.] 1*s.* 6*d.* (1*s.* 7½*d.*).

No. 74. The Relation between Home Conditions and the Intelligence of School Children. By L. Isserlis. [1923.] 1*s.* (1*s.* 1*d.*).

No. 81. The Effect of Maternal Social Conditions and Nutrition upon Birth-weight and Birth-length. By M. Bruce Murray. [1924.] 1*s.* (1*s.* 1*d.*).

No. 82. Maternal Syphilis as a cause of Death of the Foetus and of the New-born Child. By J. N. Cruickshank. [1924.] 1*s.* 6*d.* (1*s.* 7½*d.*).

No. 86. The Estimation of Foetal Age, the Weight and Length of Normal Foetuses, and the Weights of Foetal Organs. By J. N. Cruickshank, M. J. Miller, and F. J. Browne. [1924.] 2*s.* 6*d.* (2*s.* 7½*d.*).

No. 101. Poverty, Nutrition, and Growth: Studies of Child Life in Cities and Rural Districts of Scotland. By D. Noël Paton, Leonard Findlay, and others. [1926.] 10*s.* (10*s.* 4½*d.*).

No. 109. A Clinical and Pathological Study of 1,673 Cases of Dead-Births and Neo-natal Deaths. Compiled by E. L. Holland and J. E. Lane-Claypon. [1926.] 3*s.* 6*d.* (3*s.* 7½*d.*).

No. 114. Social Conditions and Acute Rheumatism. [1927.] 2*s.* 6*d.* (2*s.* 8*d.*).

No. 117. The Toxaemias of Pregnancy: A Clinical and Biochemical Study. By J. N. Cruickshank, J. Hewitt, and K. L. Couper. [1927.] 4*s.* (4*s.* 2*d.*).

No. 118. The Cause of Foetal Death in 144 Cases. By A. C. Palmer. [1928.] 3*s.* (3*s.* 2*d.*).

No. 145. The Causes of Neo-natal Death. By J. N. Cruickshank. [1930.] 1*s.* 6*d.* (1*s.* 7½*d.*).

No. 157. Nutritional Anaemia in Infancy: The Influence of Iron Deficiency on Infant Health. By H. M. M. Mackay, L. Goodfellow, and A. Bradford Hill. [1931.] 2*s.* (2*s.* 2*d.*).

No. 162. Intelligence and Disease. By Shepherd Dawson assisted by J. C. M. Conn. [1931.] 1*s.* (1*s.* 1½*d.*).

See also NUTRITION; RICKETS.

Dental Disease:

No. 70. The Structure of Teeth in relation to Dental Disease. By J. Howard Mummery. [1922.] 2*s.* (2*s.* 1*d.*).

No. 97. The Incidence of Dental Disease in Children. By the Committee for the Investigation of Dental Disease. [1925.] 1*s.* 6*d.* (1*s.* 7½*d.*).

No. 140. Diet and the Teeth: An Experimental Study. Part I. Dental Structure in Dogs. By May Mellanby. [1929.] 17*s.* 6*d.* (18*s.*).

No. 153. Diet and the Teeth: An Experimental Study. Part II. A. Diet and Dental Disease. B. Diet and Dental Structure in Mammals other than the Dog. By May Mellanby. [1930.] 2*s.* 6*d.* (2*s.* 8½*d.*).

No. 159. The Influence of Diet on Caries in Children's Teeth (Interim Report). By the Dental Committee. [1931.] 6*d.* (7*d.*).

Diphtheria:

No. 115. The Prevention of Diphtheria. By J. Graham Forbes. [1927.] 2*s.* (2*s.* 1½*d.*).

(Book). Diphtheria: its Bacteriology, Pathology, and Immunology. By the Bacteriological Committee. [1923.] 12*s.* 6*d.* (13*s.* 3*d.*).

See also EPIDEMIOLOGY (No. 75), STATISTICS (No. 137).

Dysentery:

Reports upon Investigations in the United Kingdom of Dysentery Cases received from the Eastern Mediterranean:—

No. 4. I. Amoebic Dysentery and the Protozoological Investigation of Cases and Carriers. By Clifford Dobell. [1917.] *Out of print.*

No. 5. II. Report upon 878 Cases of Bacillary Enteritis. By L. Rajchman and G. T. Western. [1917.] *Out of print.*

No. 6. III. Report upon recovered Cases of Intestinal Disease in the Royal Naval Hospital, Haslar, 1915–16. By Paul Fildes and others. IV. Report upon combined Clinical and Bacteriological Studies of Dysentery Cases from the Mediterranean. By S. R. Douglas and L. Colebrook. [1917.] 4*s.* 6*d.* (4*s.* 7½*d.*).

No. 7. V. Report upon 2,360 Enteritis 'Convalescents received at Liverpool from various Expeditionary Forces'. By E. Glynn and others. [1918.] 2*s.* (2*s.* 2*d.*).

No. 15. A Study of 1,300 Convalescent Cases of Dysentery from Home Hospitals: with special reference to the Incidence and Treatment of Amoebic Dysentery Carriers. By Clifford Dobell, H. S. Gettings, Margaret W. Jepps, and J. B. Stephens. [1918.] 1*s.* 3*d.* (1*s.* 4*d.*).

No. 29. A Contribution to the Study of Chronicity in Dysentery Carriers. By W. Fletcher and Doris L. Mackinnon. [1919.] 9*d.* (10*d.*).

No. 30. An Investigation of the Flexner-Y Group of Dysentery Bacilli. By H. S. Gettings. [1919.] 1*s.* (1*s.* 1*d.*).

No. 40. Studies of Bacillary Dysentery occurring in the British Forces in Macedonia. By L. S. Dudgeon and others. [1919.] 3*s.* (3*s.* 1½*d.*).

No. 42. A Study of the Serological Races of the Flexner Group of Dysentery Bacilli. By F. W. Andrewes and A. C. Inman. [1919.] 2*s.* (2*s.* 1½*d.*).

See also FOOD POISONING.

Encephalitis:

No. 108. The Sheffield Outbreak of Epidemic Encephalitis in 1924. [1926.] 1*s.* 9*d.* (1*s.* 10½*d.*).

Enteric Infections:

No. 9. A Report upon the Use of Atropine as a Diagnostic Agent in Typhoid Infections. By H. F. Marris. [1917.] 1*s.* (1*s.* 1*d.*).

No. 48. A Report on the probable Proportion of Enteric Infections among Undiagnosed Febrile Cases invalided from the Western Front since October 1916. By W. W. C. Topley, S. G. Platts, and C. G. Imrie. [1920.] 3*s.* (3*s.* 1½*d.*).

See also BACTERIOLOGY; FOOD POISONING.

Epidemiology:

No. 75. The Schick Test, Diphtheria and Scarlet Fever. By S. F. Dudley. [1923.] 1*s.* (1*s.* 1½*d.*).

No. 111. The Spread of Droplet Infection in Semi-isolated Communities. By S. F. Dudley. [1926.] 1*s.* 6*d.* (1*s.* 7½*d.*).

No. 120. An Inquiry into the Relationship between Housing Conditions and the Incidence and Fatality of Measles. By J. L. Halliday. [1928.] 1*s.* (1*s.* 1*d.*).

See also SMALL-POX; STATISTICS; TUBERCULOSIS; etc.

Flying, Medical Problems of:

Reports of the Air Medical Investigation Committee:—

No. 28. The Sense of Balance and Stability in the Air. By Henry Head. [1919]. 9*d.* (10*d.*). (Included in No. 53.)

No. 37. The Effects of Diminished Tension of Oxygen, with especial reference to the Activity of the Adrenal Glands. By C. H. Kellaway. The Ear in relation to certain Disabilities in Flying. By S. Scott. [1919.] 1*s.* (1*s.* 1*d.*).

No. 53. The Medical Problems of Flying (including reports on oxygen want, selection of candidates for flying, sense of balance, and flying strain). [1920.] 6*s.* (6*s.* 4*d.*).

No. 84. The Application of the Air Force Physical Efficiency Tests to Men and Women. By L. D. Cripps. [1924.] 1*s.* 6*d.* (1*s.* 7½*d.*).

Food Poisoning:

No. 24. A Report on the Investigation of an Epidemic caused by Bacillus aertrycke. By H. Marrian Perry and H. L. Tidy. [1919.] 9*d.* (10*d.*).

No. 91. An Investigation of the Salmonella Group, with Special Reference to Food Poisoning. By W. G. Savage and P. Bruce White. [1925.] 3*s.* 6*d.* (3*s.* 8*d.*).

No. 92. Food Poisoning: a Study of 100 Recent Outbreaks. By W. G. Savage and P. Bruce White. [1925.] 2*s.* 6*d.* (2*s.* 8*d.*).

No. 103. Further Studies of the Salmonella Group. By P. Bruce White. [1926.] 5*s.* (5*s.* 2½*d.*).

Goitre:

No. 154. Iodine Supply and the Incidence of Endemic Goitre. By J. B. Orr. [1931. 4*d.* (5*d.*) *See also* NUTRITION (No. 123).

Haemoglobin: *see* BLOOD.

Hearing:

No. 166. Reports of the Committee upon the Physiology of Hearing. I. The Localization of Sounds in the Median Plane. By J. H. Shaxby, and F. H. Gage. II. Some Factors in Auditory Localization. By H. E. O. James, and Marion E. Massey. [1932.] 1*s.*

Heart:

No. 8. Report upon Soldiers returned as Cases of 'Disordered Action of the Heart' (D.A.H.), or Valvular Disease of the Heart. By Sir Thomas Lewis. [1917.] 1*s.* (1*s.* 1*d.*).

No. 147. The Electrocardiogram. By W. H. Craib. [1930.] 1*s.* 3*d.* (1*s.* 4½*d.*).

Industrial Health:

The Annual Reports of the Industrial Health (formerly Fatigue) Research Board, and special reports on particular subjects, are published for the Council in separate series. The subjects dealt with include accident causation, rest pauses, spells of work, movement study, vocational selection, and problems of particular industries. A list can be supplied on application to the Secretary of the Board, 38 Old Queen Street, Westminster, S.W. 1.

Influenza:

No. 36. Studies of Influenza in Hospitals of the British Armies in France, 1918. [1919.] 3*s.* 6*d.* (3*s.* 8*d.*).

No. 63. Studies in the Aetiology of Epidemic Influenza. By J. McIntosh. [1922.] 2*s.* 6*d.* (2*s.* 7*d.*).

Jaundice:

No. 113. Spirochaetal Jaundice. By G. Buchanan. [1927.] 4*s.* (4*s.* 2*d.*).

Light Treatment:

No. 131. Irradiation and Health: Two Experimental Studies. By Dora Colebrook. [1929.] 1*s.* 6*d.* (1*s.* 7*d.*).

Malaria : *see* QUININE.

Maternal Mortality : *see* CHILD LIFE and STREPTOCOCCAL INFECTIONS.

Measles : *see* EPIDEMIOLOGY (No. 120).

Miners' Dietaries : *see* NUTRITION.

Miners' Diseases, etc. :

No. 89. Report on Miners' 'Beat Knee', 'Beat Hand', and 'Beat Elbow'. By E. L. Collis and T. L. Llewellyn. [1924.] 1*s.* 6*d.* (1*s.* 7*d.*).

See also JAUNDICE (No. 113).

Miners' Nystagmus : *see* VISION.

Nephritis :

No. 43. Albuminuria and War Nephritis among British Troops in France. By H. MacLean. [1919.] 2*s.* 6*d.* (2*s.* 8*d.*).

No. 142. A Classification of Bright's Disease. By Dorothy S. Russell. [1929.] 8*s.* 6*d.* (8*s.* 10*d.*).

Nerve Injuries :

Reports of the Committee upon Injuries to the Nervous System :—

No. 54. The Diagnosis and Treatment of Peripheral Nerve Injuries. [1920.] 2*s.* (2*s.* 1½*d.*).

No. 88. Injuries of the Spinal Cord and Cauda Equina. [1924.] 1*s.* 6*d.* (1*s.* 7½*d.*).

Nutrition :

No. 13. An Enquiry into the Composition of Dietaries, with special reference to the Dietaries of Munition Workers. By Viscount Dunluce and Major Greenwood.
Out of print.

No. 87. Report on the Nutrition of Miners and their Families. By the Committee upon Quantitative Problems in Human Nutrition. [1924.] 1*s.* 3*d.* (1*s.* 4*d.*).

No. 105. Diets for Boys during the School Age. By H. C. Corry Mann. [1926.] 2*s.* 6*d.* (2*s.* 7½*d.*).

No. 123. Iodine in Nutrition : a Review of Existing Information. By J. B. Orr and I. Leitch. [1929.] 2*s.* 6*d.* (2*s.* 8*d.*). *See also* GOITRE (No. 154).

No. 135. The Carbohydrate Content of Foods. By R. A. McCance and R. D. Lawrence. [1929.] 2*s.* (2*s.* 1½*d.*).

No. 146. The Antiscurvy Vitamin in Apples. By Mary F. Bracewell, E. Hoyle, and S. S. Zilva. [1930.] 9*d.* (10*d.*).

No. 151. A Study in Nutrition. An Inquiry into the Diet of 154 Families of St. Andrews. By E. P. Cathcart and A. M. T. Murray, assisted by M. Shanks. [1931.] 1*s.* (1*s.* 1½*d.*).

No. 155. Studies of Nutrition : The Physique and Health of Two African Tribes. By J. B. Orr and J. L. Gilks. [1931.] 2*s.* (2*s.* 2*d.*).

No. 158. The Quantitative Estimation of Vitamin D by Radiography. By R. B. Bourdillon, H. M. Bruce, C. Fischmann, and T. A. Webster. [1931.] 1*s.* (1*s.* 1½*d.*).

No. 165. Studies in Nutrition. An Inquiry into the Diet of Families in Cardiff and Reading. By E. P. Cathcart and A. M. T. Murray, assisted by M. Shanks. [1932.] 6*d.* (7*d.*).

No. 167. Vitamins : A Survey of Present Knowledge. By a Committee appointed jointly by the Lister Institute and Medical Research Council. [1932.] 6*s.* 6*d.*

See also CHILD LIFE ; RICKETS ; DENTAL DISEASE.

Pituitary Extract : *see* STANDARDS.

Pneumonia :

No. 79. Bacteriological and Clinical Observations on Pneumonia and Empyemata, with special reference to the Pneumococcus and to Serum Treatment. By E. E. Glynn and Lettice Digby. [1923.] 5*s.* (5*s.* 3*d.*).

Pneumothorax, Artificial : *see* TUBERCULOSIS.

Print, Legibility of : *see* VISION.

Protozoan Infections :

No. 59. A Report on the Occurrence of Intestinal Protozoa in the inhabitants of Britain. By Clifford Dobell. [1921.] 2*s.* (2*s.* 1½*d.*).

Quinine :

No. 96. Clinical Comparisons of Quinine and Quinidine. By the Committee upon Cinchona Derivatives and Malaria. [1925.] 1*s.* (1*s.* 1*d.*).

Radium :

No. 62. Medical Uses of Radium : Studies of the Effects of Gamma Rays from a large Quantity of Radium. By various authors. [1922.] 5*s.* (5*s.* 3*d.*).

No. 90. Medical Uses of Radium : Summary of Reports from Research Centres for 1923. [1924.] 1*s.* (1*s.* 1*d.*).

No. 102. Ditto for 1924. [1926.] 1*s.* 6*d.* (1*s.* 7*d.*).

No. 112. Ditto for 1925. [1926.] 1*s.* 3*d.* (1*s.* 4*d.*).

No. 116. Ditto for 1926. [1927.] 1*s.* (1*s.* 1½*d.*).

No. 126. Ditto for 1927. [1928.] 1*s.* (1*s.* 1½*d.*).

No. 144. Ditto for 1928. [1929.] 1*s.* (1*s.* 1*d.*).

No. 150. Ditto for 1929. [1930.] 9*d.* (10*d.*).

No. 160. Ditto for 1930. [1931.] 1*s.* (1*s.* 1*d.*)

Rheumatism : *see* CHILD LIFE (No. 114).

Rickets :

No. 20. A Study of Social and Economic Factors in the Causation of Rickets, with an Introductory Historical Survey. By L. Findlay and Margaret Ferguson. [1918.] *Out of print.*

No. 61. Experimental Rickets. By E. Mellanby. [1921.] 4*s.* (4*s.* 2*d.*).

No. 68. Rickets : the Relative Importance of Environment and Diet as Factors in Causation. By H. Corry Mann. [1922.] 2*s.* 6*d.* (2*s.* 7½*d.*).

No. 71. The Aetiology and Pathology of Rickets from an experimental point of view. By V. Korenchevsky. [1922.] 4*s.* (4*s.* 3*d.*).

No. 77. Studies of Rickets in Vienna, 1919–22. [1923.] 7*s.* 6*d.* (7*s.* 10½*d.*).

No. 93. Experimental Rickets : The Effect of Cereals and their Interaction with other factors of Diet and Environment in producing Rickets. By E. Mellanby. [1925]. 3*s.* 6*d.* (3*s.* 8*d.*).

Salvarsan : *see* VENEREAL DISEASES ; STREPTOCOCCAL INFECTIONS ; STANDARDS, BIOLOGICAL (No. 128).

Scarlet Fever : *see* STATISTICS (No. 137).

Scurvy : *see* NUTRITION (No. 146).

Shock, Surgical :

Reports of the Committee on Surgical Shock and Allied Conditions :—

No. 25. Wound-Shock and Haemorrhage. [1919.] 4*s.* (4*s.* 5½*d.*).

No. 26. Traumatic Toxaemia as a Factor in Shock. [1919.] 1*s.* (1*s.* 1*d.*).

No. 27. Blood Volume Changes in Wound-Shock and Primary Haemorrhage. By N. M. Keith. [1919.] 9*d.* (10*d.*).

Small-pox :

No. 98. Studies of the Viruses of Vaccinia and Variola. By M. H. Gordon. [1925.] 3*s.* 6*d.* (3*s.* 8½*d.*).

No. 106. Small-pox and Climate in India : Forecasting of Epidemics. By Sir Leonard Rogers. [1926.] 2*s.* (2*s.* 1½*d.*).

No. 143. Diagnostic Value of the 'Vaccinia Variola' Flocculation Test. By W. L. Burgess, James Craigie, and W. J. Tulloch. [1929.] 1*s.* 3*d.* (1*s.* 4*d.*).

No. 156. Further Investigations on the Variola-Vaccinia Flocculation Reaction. By James Craigie and W. J. Tulloch. [1931.] 3*s.* (3*s.* 2½*d.*).

Spinal Deformities : *see* SURGERY (No. 161).

Standards, Biological :

No. 69. I. Pituitary Extracts. By J. H. Burn and H. H. Dale. [1922.] 1*s.* 6*d.* (1*s.* 7*d.*).

No. 128. II. Toxicity Tests for Novarsenobenzene (Neosalvarsan). By F. M. Durham, J. H. Gaddum, and J. E. Marchal. [1929.] 1*s.* 9*d.* (1*s.* 10*d.*).

See also VENEREAL DISEASES (No. 44) and NUTRITION (No. 158).

Statistics (Miscellaneous).

No. 16. A Report on the Causes of Wastage of Labour in Munition Factories. By Major Greenwood. [1918.] 1*s.* 6*d.* (1*s.* 7*d.*).

No. 60. The Use of Death-rates as a Measure of Hygienic Conditions. By John Brownlee. [1922.] 3*s.* (3*s.* 1½*d.*).

No. 95. Internal Migration and its Effects upon the Death-rates: with Special Reference to the County of Essex. By A. B. Hill. [1925.] 3*s.* 6*d.* (3*s.* 8*d.*).

No. 137. Scarlet Fever, Diphtheria, and Enteric Fever, 1895–1914: A Clinical-Statistical Study. By E. W. Goodall, M. Greenwood, and W. T. Russell. [1929.] 2*s.* (2*s.* 1½*d.*).

Streptococcal Infections:

No. 119. A Study of some Organic Arsenical Compounds with a view to their Use in certain Streptococcal Infections. By L. Colebrook. [1928.] Price 1*s.* 3*d.* (1*s.* 4*d.*).

Surgery.

No. 125. A Study of Intracranial Surgery. By H. Cairns. [1929.] 3*s.* (3*s.* 2½*d.*).

No. 138. The Preparation of Catgut for Surgical Use. By W. Bulloch, L. H. Lampitt, and J. H. Bushill. [1929.] 4*s.* (4*s.* 3*d.*).

No. 161. The Intervertebral Discs. Observations on their Normal and Morbid Anatomy in relation to certain Spinal Deformities. By O. A. Beadle. [1931.] 2*s.* (2*s.* 2*d.*).

See also Burns; Shock, Surgical.

T.N.T. Poisoning:

No. 11. The Causation and Prevention of Tri-nitro-toluene (T.N.T.) Poisoning. By Benjamin Moore. [1917.] 1*s.* (1*s.* 1½*d.*).

No. 58. T.N.T. Poisoning and the Fate of T.N.T. in the Animal Body. By W. J. O'Donovan and others. [1921.] 3*s.* (3*s.* 1½*d.*).

Tuberculosis:

No. 1. First Report of the Special Investigation Committee upon the Incidence of Phthisis in relation to Occupations.—The Boot and Shoe Trade. [1915.] 3*d.* (3½*d.*).

No. 18. An Investigation into the Epidemiology of Phthisis Pulmonalis in Great Britain and Ireland. Parts I and II. By John Brownlee. [1918.] Price 1*s.* 3*d.* (1*s.* 4½*d.*).

No. 22. An Inquiry into the Prevalence and Aetiology of Tuberculosis among Industrial Workers, with special reference to Female Munition Workers. By Major Greenwood and A. E. Tebb. [1919.] 1*s.* 6*d.* (1*s.* 7*d.*).

No. 33. Pulmonary Tuberculosis: Mortality after Sanatorium Treatment. By Noel D. Bardswell and J. H. R. Thompson. [1919.] 2*s.* (2*s.* 2*d.*).

No. 46. An Investigation into the Epidemiology of Phthisis in Great Britain and Ireland: Part III. By John Brownlee. [1920.] 2*s.* 6*d.* (2*s.* 7½*d.*).

No. 67. Report on Artificial Pneumothorax. By L. S. T. Burrell and A. S. MacNalty. [1922.] 2*s.* 6*d.* (2*s.* 8*d.*).

No. 76. Tuberculosis in Insured Persons accepted for Treatment by the City of Bradford Health Committee. By H. Vallow. [1923.] 6*d.* (7*d.*).

No. 83. Tuberculosis of the Larynx. By Sir St. Clair Thomson. [1924.] 2*s.* 6*d.* (2*s.* 8*d.*).

No. 85. An Inquiry into the After-Histories of Patients treated at the Brompton Hospital Sanatorium at Frimley, during the years 1905–14. By Sir P. H.-S. Hartley, R. C. Wingfield, and J. H. R. Thompson. [1924.] 1*s.* 6*d.* (1*s.* 7*d.*).

No. 94. Tuberculin Tests in Cattle, with special reference to the Intradermal Test. By the Tuberculin Committee. [1925.] 3*s.* (3*s.* 3*d.*).

No. 122. The Intradermal Tuberculin Test in Cattle: Collected Results of Experience. By J. B. Buxton and A. S. MacNalty. [1928.] 1*s.* 6*d.* (1*s.* 7½*d.*).

No. 149. Tuberculosis in Man and Lower Animals. By H. H. Scott. [1930.] 4*s.* (4*s.* 4*d.*).

No. 152. Studies of Protection against Tuberculosis: Results with B. C. G. Vaccine in Monkeys. By A. Stanley Griffith. [1931.] 9*d.* (10½*d.*).

No. 164. The Value of Tuberculin Tests in Man with special reference to the Intracutaneous Test. By P. D'Arcy Hart. [1932.] 2*s.* (2*s.* 2*d.*).

Venereal Diseases:

No. 14. The Wassermann Test. By the Committee upon Pathological Methods. *New Edition.* [1921.] 1*s.* (1*s.* 1*d.*).

No. 19. The Laboratory Diagnosis of Gonococcal Infections. Methods for the Detection of *Spironema pallidum*. By the Bacteriological Committee. *New Edition.* [1923.] 1*s.* 6*d.* (1*s.* 7½*d.*).

No. 21. The Diagnostic Value of the Wassermann Test. By the Committee upon Pathological Methods. [1918.] *Out of print.*

No. 23. An Analysis of the Results of Wassermann Reactions in 1,435 Cases of Syphilis or Suspected Syphilis. By Paul Fildes and R. J. G. Parnell. [1919.] 2*s.* (2*s.* 1*d.*).

No. 41. (I.) An Investigation into the Ultimate Results of the Treatment of Syphilis with Arsenical Compounds. By Paul Fildes and R. J. G. Parnell. (II.) A Clinical Study of the Toxic Reactions which follow the Intravenous Administration of '914'. By R. J. G. Parnell and Paul Fildes. [1919.] 2*s.* (2*s.* 1*d.*).

No. 44. Reports of the Special Committee upon the Manufacture, Biological Testing, and Clinical Administration of Salvarsan and of its Substitutes. I. [1919.] 1*s.* (1*s.* 1*d.*).

No. 45. Unsuspected Involvement of the Central Nervous System in Syphilis. By Paul Fildes, R. J. G. Parnell, and H. B. Maitland. [1920.] 1*s.* (1*s.* 1*d.*).

No. 47. The Accuracy of Wassermann Tests, applied before and after death, estimated by Necropsies. I. The Wassermann Test applied before death. By H. M. Turnbull. [1920.] 2*s.* 6*d.* (2*s.* 7½*d.*).

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ALCOHOL AND INHERITANCE: AN EXPERIMENTAL STUDY

By F. M. DURHAM and H. M. WOODS

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INTRODUCTION

In 1913 and 1914 Professor C. R. Stockard, of Cornell University, published in the United States the results of a long experiment he had conducted to test the effects produced by alcohol on the fecundity of guinea-pigs and on the quality of their offspring. His records seemed to prove that the daily administration of alcohol, in quantities enough to produce visible intoxication, but not otherwise to impair the vitality and health of the parents, caused a fall in the number of births, an increased liability to still-births, and the production of weakly and defective offspring. Of even more serious import was the evidence that these tendencies were inherited, so that, without further exposure of the stock to alcohol, the proportion of animals showing weakness and deformity continued to be high in later generations. The results clearly suggested that alcohol in the circulation can injure the germ-plasm and produce a permanent deterioration of the race, even without obvious injury to the generation directly exposed. In later publications Professor Stockard suggested, indeed, that a process of natural selection ultimately corrected this tendency and established a stronger stock from the survivors.

It was obvious that Professor Stockard's findings, if confirmed, would have implications of the gravest importance for humanity. There could be no reason for assuming that alcohol alone, of all the poisons distributed by the circulation, could thus injuriously affect the germ-plasm and so injure the descendants through many generations. Any circulating poisons, such as those which occur in certain acute infections, might, on this evidence, be expected to cause an injury revealing itself only in subsequent generations. It was thus clearly desirable that Professor Stockard's experiments should be repeated with critical care, and with the application of more recent knowledge of the constituents of a diet necessary for full health and normal reproduction.

Miss Durham accordingly undertook, in the course of her work in the National Institute for Medical Research at Hampstead, an experiment carefully devised so as to repeat, if possible, Professor Stockard's results by means of the same methods, but with adequate numbers of control experiments under identical conditions except for the absence of treatment with alcohol. In the statistical treatment of the data obtained she has had the collaboration of Miss H. M. Woods of the London School of Hygiene and Tropical Medicine.

It will be seen from the conclusions now presented that on no essential point did they find it possible to obtain any confirmation of Professor Stockard's results. The litters of young obtained from alcoholic parents, or from their descendants, have been as numerous and as heavy as those from the control matings, and have shown no excess of still-births or of deformities. Their offspring for several generations have exhibited no transmitted defects of the kinds described by Professor Stockard.

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The conduct of an experiment of this kind on an adequate scale involves many years and much labour, and its repetition with successive variations of particular conditions would be impracticable. It is, therefore, unlikely that further experiment will be made in order to reveal the nature of the factors that contributed to the production of Professor Stockard's results. It is possible that the difference between the results obtained by Miss Durham and those obtained by Professor Stockard may be dependent upon some undetected defect in the diets of his animals under experiment. It is clear that her supervision of the diets of the successive generations under observation was more complete than his. The sizes of the litters of young throughout her experiment, and their average weights at birth, give clear indication of this. In other words, Professor Stockard's results might mean, for instance, that the effects of alcohol combined with deficiency of vitamins might produce a permanent racial deterioration. His claim, however, was that alcohol *per se* is capable of inflicting a transmissible injury upon the germ-cells. Miss Durham's own suggestion of inadequacy in Professor Stockard's experiment is in quite another direction, namely, that he had not adequately controlled the genetic behaviour of his stock, and that the results which he attributed to alcohol were due to the introduction of one or more parents having within them the unseen but heritable qualities able to yield the observed effects.

It is justifiable to conclude only that alcohol by itself cannot produce the results assigned to it by Professor Stockard, and that the opinions which have been based upon them as to the effects of parental alcoholism on the genetic qualities of a race, cannot be upheld. It is hardly necessary to point out that these negative findings have no relation whatever to the effects of alcoholic parentage on the upbringing, as distinct from the inherited qualities, of children in an organized human society.

MEDICAL RESEARCH COUNCIL,
38 OLD QUEEN STREET,
WESTMINSTER, S.W.1.

15th June, 1932.

ALCOHOL AND INHERITANCE: AN EXPERIMENTAL STUDY

BY

F. M. DURHAM AND H. M. WOODS

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INTRODUCTION

AMONG the items of evidence which have, from time to time, been put forward in support of the inheritance by offspring of a character induced by some external influence on the parent, those concerned with the effects of drugs hold a special position. There is a fundamental difficulty in the conception of a mechanism whereby a change, induced by some influence restricted to the somatic part of the organism, can influence the germ-cells in such a way that the induced somatic modification is inherited. This difficulty does not apply to the suggestion of an effect on the offspring, produced by the treatment of the parents with a drug. The effect on inheritance in such

a case would not be in the ordinary sense the transmission of an acquired characteristic, the defects in the offspring, though attributed to the administration of the drug to the parents, having no relation to any effects which it might directly produce on the somatic parental organs. The drug being generally distributed in the parental body, there would be no difficulty of mechanism involved in the suggestion of its directly modifying the germ-plasm.

A demonstration of the production of heritable defects or modifications in the offspring, by exposure of the parents to the prolonged or repeated action of a drug would have an obvious and direct sociological importance. Its indirect importance, by analogy, might well be even greater. If artificial exposure to the action of a drug could be shown to produce such germinal inferiority, the question would arise whether natural exposure of the germ-cells to the toxins produced by an infection would not result in a similar genetic impairment.

The importance of the conclusions which might be based on its results makes it necessary to frame a practical test of such possibilities with great care. The genetic qualities of the race of animals used in the experiment must, in particular, be thoroughly known, lest an hereditary defect, emerging through the chance mating of individuals carrying a latent recessive character be attributed too hastily to the effect of a drug administered to the parents.

PREVIOUS EXPERIMENTS WITH ALCOHOL

Alcohol has been given by many observers to different kinds of animals in the endeavour to produce a modification of the germ-cells.

The results obtained from these experiments show, with one notable exception, that alcohol has very little, if any, action on the germ-cells. Stockard (1910, 1912, 1913, 1914, 1916, 1918, 1923), using guinea-pigs, claims to have produced in the treated animals profound changes, which are inherited by their offspring and transmitted to later generations.

In his paper with Papanicolau (1916) he states 'that this injury to the male germ-cells is of such a nature that an alcoholized male guinea-pig almost invariably begets defective offspring, even when mated with a vigorous normal female, and the F_1 animals, the offspring of treated parents, though themselves not treated, had the power to transmit the defective condition to their young and such F_2 young were equally, if not more defective than the immediate offspring of the treated animals'. The fullest account of his experiments is given in the *Further Studies on the Modification of the Germ-cells in Mammals,* 1918, where he states: 'The germ-cells in either the male or female mammal may be changed or affected by a chemical treatment administered to the body of the individual. The progeny derived from such chemically treated animals showed more or less marked deviations from the normal in many definitely measurable qualities such as their mortality records, structural appearance, nervous reactions, and ability to reproduce. The treatment also

affected in the male, the crucial germ-cell test for mammals, their ability to beget offspring when mated with normal females.

'In general, it may be stated that the offspring produced when treated males were paired with normal females were inferior in several respects as compared with other offspring from the same normal mothers bred to control males of exactly the same original stock. Further, when the male offspring from treated fathers were mated with normal females, the individuals resulting from such matings were as a group decidedly inferior to the young produced by normal females when mated with control males. This group inferiority was not only present in the grandchildren or F_2 generation, but also in the F_3 generation descended from alcoholized great-grandparents.' On page 130 of the same paper, however, he states, 'the entire care of the animals has been much improved during the last two years. Our records for monsters and other weakened conditions are, therefore, somewhat reduced; yet the same marked contrast between alcoholic and control is present, even though the weakened alcoholic lines have no doubt profited more by the improved methods of care and feeding than have the healthier controls. The defects are also the same in type as those formerly observed, though not so marked in degree.'

The defects observed by Stockard were of various kinds: gross changes such as paralysis, nervous symptoms, various affections of the eyes, including complete blindness and absence of the eyes, &c., are recorded. Even the otherwise normal young of alcoholic parentage are stated to have been inferior in weight and power of growth to offspring of control, untreated animals. Further, the treated animals showed loss of fertility, as evidenced by smaller and fewer litters, and often there was complete sterility. The mortality records among the newly born were also higher for the progeny of the alcoholized animals and their descendants than for the offspring of the control, untreated animals.

These results have not been confirmed by other workers. Pearl (1917) was unable to show any such profound changes as the result of exposing fowls to alcohol fumes. The findings of MacDowell (1922) on rats, of MacDowell and Lord on mice (1926), and rats (1927), of Hanson (1923) on rats, and of Pictet (1924) on guinea-pigs, are all in accord with those of Pearl. Bluhm (1921) and (1930) using mice, claims to have shown an effect on sex-ratio as a result of using alcohol, and also on the early mortality, which she believes to be inherited. Otherwise, all these other observers are in agreement that there is no evidence of any lasting effect on the organism, as the result of alcoholic treatment.

Apart from the data presented by Stockard the evidence of any change occurring, which can be shown to be inherited, is very slight, if, indeed, it can be said to exist at all. It seemed worth while, therefore, to attempt a repetition of the experiments on alcoholized guinea-pigs and their descendants, as nearly as possible on the same lines as those laid down by Stockard.

PLAN OF THE PRESENT EXPERIMENT

Mature guinea-pigs were taken from the breeding stock at the National Institute for Medical Research, Hampstead. They were chosen as being good healthy breeders and unrelated to one another. From these a stock of guinea-pigs was raised. Three years later, fresh blood was introduced. Young immature animals were purchased from Miss Rhodes' guinea-pig farm. They were guaranteed to be of healthy origin and unrelated to one another.

In accordance with Stockard's scheme, two litters were taken from each pair of guinea-pigs before any animal was treated with alcohol. This was done in order to obtain some evidence concerning the genetic behaviour of the stock before the administration of the drug. Stockard mated the offspring of alcoholized parents with control animals. In our experiment the Mendelian rules were followed. The offspring of alcoholized parents were mated either *inter se*, or with animals of like origin. If alcohol injures the germ-cells to such an extent that the genetic behaviour is upset and the injury is inherited, the Mendelian system of breeding would show the result more quickly than the other system of breeding, and by inbreeding the controls, if the abnormality were not due to the alcoholic treatment but inherent in the race of guinea-pigs, its presence would be demonstrated in the control stock.

The guinea-pigs were alcoholized by inhalation of alcohol as in Stockard's experiments, and not by direct administration by the mouth. Stockard tried giving them alcohol by the mouth and found that their digestion became upset, and moreover, that there was difficulty in making them take it.

Tanks were made according to Stockard's plan (1912). These tanks are 36 inches long by 18×12 inches deep, lined inside with galvanized iron. The floor slopes to the centre for drainage. On the floor cotton wool soaked in 95 per cent. alcohol is placed and over this is a wire screen on which the animals sit. The whole tank is practically air-tight, but no such lack of oxygen is produced during the exposure of the animals as to have an adverse effect on them. One side of the tank is of glass, so that the animals can be observed without opening the box.

For convenience, as the males are apt to become quarrelsome, especially when intoxicated, small wire cages were put inside each tank, each large enough to hold one guinea-pig. In this way several guinea-pigs could be put in the one tank without harm. A determination kindly made by Dr. King showed that the air in the tanks was about half saturated with alcohol.

The times of exposure to alcohol were the same as those used by Stockard, the bucks remaining in the tanks for 2 hours on each occasion and the does for 3 hours. Stockard does not say why he selected these times for the experiment. The time is, however, amply sufficient to intoxicate the animals, which do not appear to be otherwise injured by this treatment. At first, until they get used to it, the irritant effect of the alcohol vapour causes some lachrymation and

slight clouding of the cornea; but this effect passes off after a few exposures, and the general health of the animals is excellent. It is to be noticed that in the early stages of pregnancy the alcohol seems to affect the does more than in the later. They become much more easily intoxicated, as shown by inability to stand at this period. They are also more affected just after the birth of the young. Early pregnancy and early lactation would, therefore, appear to make them more sensitive to alcohol.

Stockard used a device to avoid handling the does when pregnant. Special small alcohol boxes were arranged to hook on to the breeding-cages, so that the animals could run into the alcohol box without being lifted. We found this arrangement unnecessary. A control experiment was made by lifting normal pregnant does from their cages and shutting them up in boxes, while the alcoholized females were in the tanks. Neither set appeared to suffer any ill effects from the lifting.

Care of the animals. In the early stages of the experiment, the animals went through some vicissitudes. There was difficulty in getting sufficient green food for them, and it was some time before a reliable assistant was found to help in the cleaning of the cages and the feeding and general care of the animals. The cages were cleaned once a week and examined twice daily for births and miscarriages. Every day the animals received fresh food: bran, oats, hay, and cabbage, and also fresh water. It is very important to see that guinea-pigs have sufficient food, and this is often neglected. A guinea-pig can eat its own weight in food in a day. If the food pots are emptied early in the day, it is a sign that insufficient has been given: there should always be a slight excess, if guinea-pigs are to be kept in good condition.

Stockard gave his guinea-pigs carrots every day and green food twice a week. Carrots cannot replace green food, and it may be that the difference in his fertility records from ours (to which reference will be made later) may be due in some degree to this difference in feeding. During one winter there was difficulty in getting sufficient cabbage for the animals. The cabbages had been grown at the Medical Research Council's experimental farm, Mill Hill; they had all bolted, so that the supply consisted chiefly of stalks. This supply of cabbage was rejected, as having insufficient vitamin value, and a special supply of properly grown cabbage was procured. The result was of interest, because the laboratory stock of guinea-pigs at the breeding establishment at Mill Hill were fed on stalks and during this winter refused to breed, and no young or very few were produced, while in our breeding-room there was no cessation, and the litters were as many and as large as usual, including many of 4 and 5 each.

There can be no doubt as to the value of fresh green food to the animal, and sufficient emphasis cannot be laid on the importance of fresh green stuff for the alcoholized animals if they are to breed freely; before diminished fertility or sterility can be attributed to

any other cause, there must be proof that the supply of cabbage has been ample.

It might be legitimate to put the matter in another way and state that the differences in fertility between our experiments and those of Stockard may mean that if the animals have plenty of cabbage, alcohol has no effect on their breeding powers, but that the exposure to alcohol may cause a diminution in breeding power if the supply of green food is restricted. Special experiments would be necessary to settle this point.

The following is a short summary of the similarities and differences between our experiments and those of Stockard.

	Stockard.	*Durham and Woods.*
1.	Two litters from each pair before alcohol used.	
2.	Offspring of alcoholics mated with controls.	Offspring of alcoholics mated with offspring of alcoholics.
3.	Alcoholized by inhalation.	
4.	Does not lifted.	Does lifted.
5.	Green food twice a week.	Green food every day.

The accommodation available, particularly in the early part of the experiment, did not make it possible to deal with such large numbers of animals as Stockard used, and the experiments had to be limited accordingly. It was, for example, not possible to keep all the young guinea-pigs until they were 3 months old, and the records for this age are accordingly small. Seventy-nine ♀'s were used in the control series and the total young numbered 674. Eighty-two ♀'s and 49 ♂'s in all were alcoholized and the total number of young born from alcoholized parents and their descendants was 6,309.

At the beginning of the experiment there was considerable trouble with pseudo-tuberculosis, a disease which is always prevalent among guinea-pigs in England. Proper care as regards food and conditions generally suffices to control it. Petrie (1930) has described a disease due to a pneumococcus which particularly affects the does, causing a metritis, which brings about still-births and may cause the death of the mother. It also causes pleuro-pneumonia. The incidence is less among the bucks. Unfortunately these results were published after our experiment was finished, and the guinea-pigs were not examined for this trouble. There are a number of cases where the doe died after a miscarriage or still-births at full term, and the post-mortem examination showed an inflamed uterus; possibly some of the animals were infected with the pneumococcus. Loss of weight is one of the signs of the animal being affected. Accordingly, every male guinea-pig in the breeding-room was weighed every week. It was, of course, useless to weigh the does, but they were examined every week in order to estimate the probable pregnancies. In this way it was possible to pick out and discard any animal showing signs of ill-health. Thus, every animal was under observation and its condition known.

RESULTS

Summary of Questions Discussed

In the account which now follows the questions considered are:

1. Does exposure to alcohol fumes injure the germ-cells so that abnormal young result?
2. Is the power to produce abnormal young alleged to result from the administration of alcohol inherited, and do abnormalities occur in succeeding generations from alcoholized stock, even though the immediate parents are not so treated?
3. Is the fertility of the alcoholized stock and their descendants affected by the drug, and is this effect more noticeable in the male than in the female?
4. Are the offspring from alcoholized stock and their descendants smaller, weaker, as well as fewer in number, than those of the control, untreated stock?
5. Are there a greater early mortality, more still-births, and more deaths in the first three weeks after birth, among the offspring of the alcoholics and their descendants than in the control stock?
6. Is the sex-ratio of the offspring affected by the administration of alcohol to their parents, and is this effect inherited?

These are the main points which will be discussed, but the records were also analysed to see if there was any effect on the offspring from long exposure of the parent to alcohol. It was not realized until too late in the experiment, when the data were submitted to statistical examination, that age of the parent must be taken into account as well as length of time of treatment. It was then found that the age distributions of the treated and control groups were entirely different and no proper comparison could be made as to the effect of age.

Abnormalities

Before one can attribute the appearance of abnormalities among the offspring of animals treated with alcohol to the action of this drug, one must attempt to find out whether the animals used are naturally liable to produce defective offspring. The best way is to inbreed, taking into consideration the fact that such abnormalities are likely to be recessive and not dominant characters. They will, of course, occur among outbred families, when both parents happen to carry the factors for the observed abnormality. In our experiments, intensive inbreeding has been carried out.

In his paper, 'Transmission of Degeneracy', Stockard (1916) describes the types of abnormality that he found. 'Many of the young animals show gross tremors, paralysis agitans. . . . Eye defects are very common, such as opaque cornea, opaque lens, various degrees of monophthalmicum asymmetricum, and finally several cases of complete anophthalmia have occurred, the entire eyeballs, optic nerves, and optic chiasma being absent.' Stockard (1918) said: 'It has been suggested on certain occasions, that the defects and degenerate

conditions, which have been reported in our alcoholic lines were probably present in the original stock on which the experiment was conducted. Such a remark in the face of the experimental control, which has been fully described, scarcely warrants discussion, yet we should like to state in the beginning, for the benefit of the casual critic who may not wander through the following pages, the real nature of the original control.' This may be briefly summarized as follows: After test matings, a certain number of guinea-pigs both male and female were put on alcohol treatment. They were mated both with alcoholics and with normals, and the normals used in these matings were also mated together, the offspring of the normals being considered as controls.

Later on he states that the alcoholic lines analysed in the report are practically devoid of inbreeding. 'In our former report ('16) there were shown to be more injurious effects in the alcoholic inbred lines, than in the non-inbred.' He concluded that the difference between the earlier and present results indicated that inbreeding in these alcoholic lines may be easily carried to a degree which will make the injurious effects more marked. While he avoided even the slightest approach to such a degree of inbreeding in the alcoholic lines, the control animals were, in almost all cases, either brothers, parents, or other blood relations of the treated animals.

It would appear from these statements that strict inbreeding was not carried out, and the fact that Stockard obtained no abnormalities among his normals, and that all the defective offspring occurred among the alcoholic families or their descendants, was taken as a sufficient proof that their appearance was due to the effect of alcohol, and not to an inherent defect in the stock.

But the question cannot be answered so simply. Sewall Wright (1922) has put on record a very valuable account of the effects of inbreeding in guinea-pigs extending over a long period from 1906–19, and his results are of far-reaching importance. He gives an account of various kinds of abnormalities which had appeared in his breeding farm; these included cyclopean monsters, deformed or missing legs, brain protruding through the skull, absence of one or both eyeballs, and so on. These abnormalities appeared not only among the inbred animals, but in the control (or outbred) stock.

He concluded that 'the tendency to produce a given type of monstrosity has been characteristic of certain families. Such a tendency has had no connexion with the vigour of the family in other respects. . . . There was evidence of heredity within the families, of the tendency to produce these abnormalities. There was no evidence that inbreeding has any specific causal connexion with the origin of the monsters. Inbreeding seems merely to have brought to light genetic traits in the original stock.'

From Sewall Wright's paper it will be seen that in the stock of guinea-pigs not subjected to any treatment by alcohol or any other drug, all forms of abnormalities described by Stockard, and others which he has not observed, are to be found.

Pictet (1924) repeated Stockard's experiments and found that his results did not agree with Stockard's. Among his control guinea-pigs he found much variation. When conditions were bad (for instance, heat, drought, and insufficient food), there was an increase of miscarriages, a higher death rate, still-births and early deaths, a lowering of birth-weight, slow development, and the occurrence of many abnormalities. The abnormalities occurred generally among descendants of the same stock, and he questions whether Stockard was not using a similar stock, so that the abnormalities in his experiments were due, not to alcohol, but to inheritance.

In the literature on guinea-pigs there are many cases cited of monsters and other aberrations, and it would appear that these occur frequently in the now domesticated guinea-pig lines.

In our experiments, as already stated, more inbreeding has been practised than in Stockard's experiments, and abnormalities have also appeared in the breeding room, but not to the same extent as Stockard obtained. Stockard (1918, p. 209) gives a table in which he sums up the differences between the normal and alcoholic lines.

Abnormalities:	Normal stock	0 per cent.
	Alcoholic stock	2·52 per cent.

This alcoholic stock we take to mean all alcoholics and their descendants, since he does not include in his table a separate entry for descendants of alcoholics. If we compare our results in the same way, contrasting the control animals with all alcoholics and descendants, we find 1 abnormality among 674 control animals and 10 abnormalities among 6,309 alcoholic stock.

If we regard the proportion of abnormalities, which occurred among over 6,000 offspring of the alcoholic stock, as representing not more than the normal chance of such occurrences in the stock under observation, it will be obvious that not more than one abnormality was to be expected among 674 control offspring. Indeed, the chance of even one occurring in this number of controls would not be a very high one. If our controls had happened to show no abnormality, which is statistically not an improbable event, the apparent contrast between our alcoholic offspring and our normal controls would have been similar to, though less striking than, that shown in Stockard's figures. In such circumstances it is obviously desirable to consider, whether there is any other factor than alcohol affecting both stocks alike, to which the occurrence of such abnormalities can be traced. In particular, it is important to enquire, whether the appearance of abnormalities can be traced back in the pedigrees to any particular ancestor or ancestors as in Sewall Wright's experiments.

Chart I (*a*, *b*, and *c*) enables the pedigree of each of the abnormalities observed by us to be traced back, and it will be seen that there is a highly significant degree of community in their inheritance. In the ancestry of any abnormal birth we find either one particular doe (♀ 2) or one particular buck (♂ 2). Six of the recorded abnormalities can trace their descent from ♀ 2 (Chart I *a*), 4 from ♂ 2 (Chart I *b*),

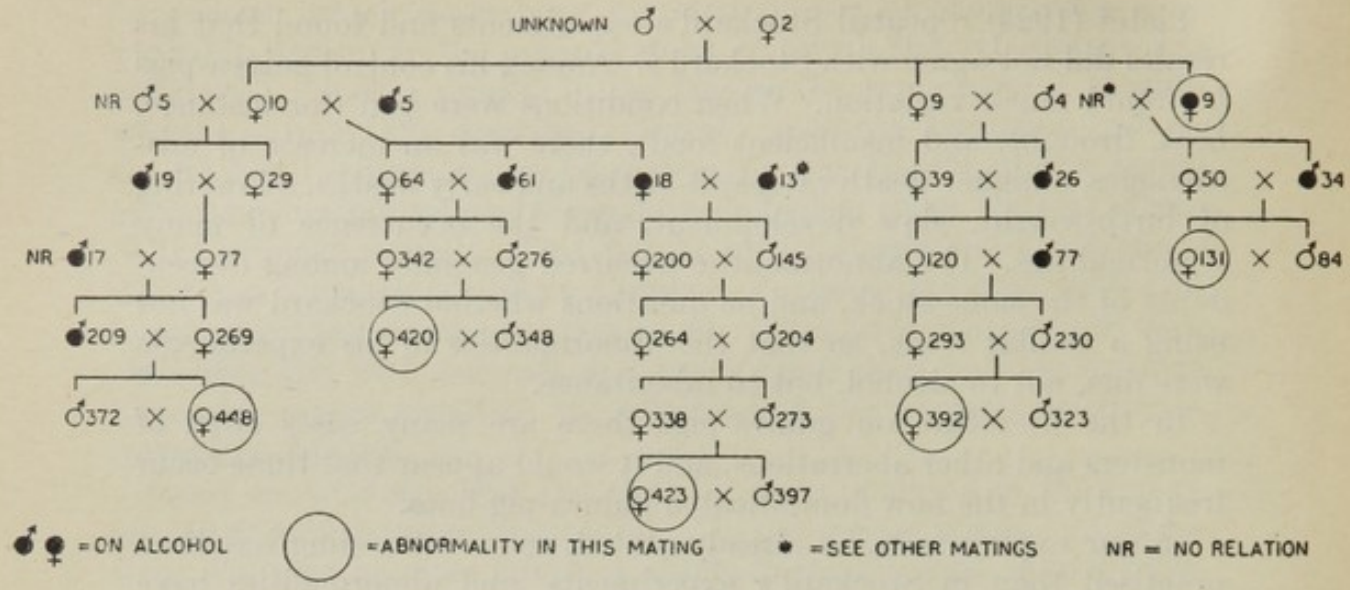

CHART I *a*

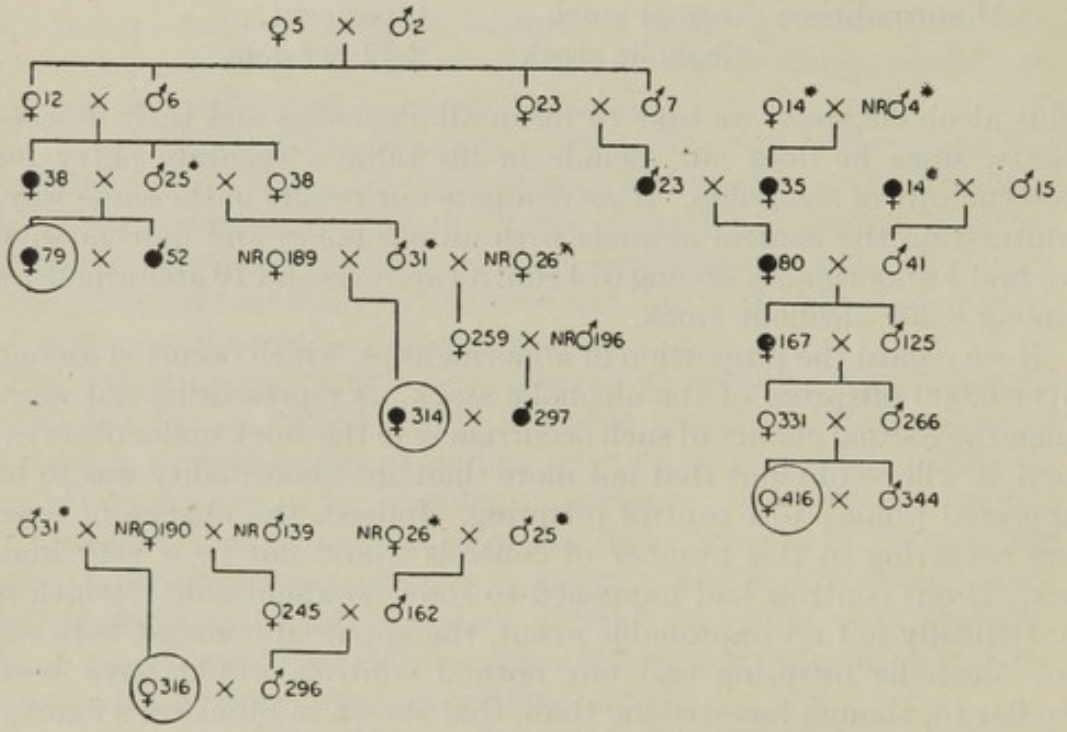

CHART I *b*

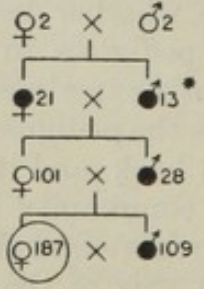

CHART I *c*

and one in a line which started in a mating between ♀ 2 and ♂ 2 (Chart I *c*). It is impossible to say what contribution to the tendency to produce abnormal offspring may have come from the other partner in the original mating of either ♂ 2 or ♀ 2 or in any of the later matings. We may note, for example, that 6 abnormalities are recorded among the descendants of ♀ 2 with an unknown male, ♀ 2 having been already pregnant when she was introduced into the experiment. Four abnormalities again, attributed to ♂ 2, occur in a line which starts with a mating of this animal with ♀ 5; the only other abnormality occurring among the descendants of ♂ 2 being traceable to the mating with ♀ 2, it is accordingly quite uncertain whether the heritable tendency to produce abnormalities should not be attributed rather to ♀ 5 than to ♂ 2. In any case, it is quite clear that every abnormality observed by us, the 10 among alcoholic and the one from the control stock, can be traced back to one or other of two common ancestors, one of these being ♀ 2 and the other either ♀ 5 or ♂ 2.

It would require a great deal of experimental breeding to determine, exactly, the probable frequency of the appearance of these abnormalities, and it is therefore not possible from our figures to make an exact computation as to how far they agree with a Mendelian expectation. All that can be said is, that here is a case of probable inheritance of a recessive character or characters, causing abnormalities of more than one kind among the offspring. There are nervous symptoms, tremors, head on one side, deformed eyes, and incompletely developed foetuses. And it is significant that none of these defects have appeared in the descendants of any of the rest of our stock, when not mated to any of the descendants of ♀ 2, or ♂ 2, or ♀ 5. In all, 345 ♀'s and 274 ♂'s descended from ♀ 2 × ♂ 2 or ♀ 2 × unknown ♂ or ♀ 5 × ♂ 2 have been used for stud purposes. 210 ♀'s and 159 ♂'s from other parentage have also been used and from these no abnormalities were obtained.

Stockard found when he ceased to inbreed among his alcoholic stocks that the percentage of abnormalities among the offspring was lowered. The objection may be raised that as we only obtained one defective animal from a normal mating, and all the rest of the abnormalities appeared after the animal had been exposed to alcohol, or the progenitors of the immediate parents had been alcoholized, perhaps the effect of alcohol was to increase the number of abnormalities. But one must remember that it is impossible to breed from all the offspring which a pair of guinea-pigs may produce, and that the chance of selecting, for stud purposes, just the pair which will give an abnormality in the first, or even the second generation, is very remote, since the abnormality will occur very rarely. Since the later generations consisted of more matings than the earlier ones the chance of encountering an abnormality in the later generations was somewhat greater, but all the guinea-pigs in these later generations were from stock which had, at one stage or another, been exposed to alcohol. We must therefore guard against the conclusion that these abnormalities were due to alcohol because they might have occurred in any case.

There is another factor which must also be considered, and that is the effect of time on the alcoholization of the germ-cells. It seems obvious that if alcohol be responsible for deterioration of germ-cells, then the longer the exposure, the greater the injury; unless one is to suppose that eventually a new condition of affairs is set up, and the germ-cells become resistant to the drug.

Stockard makes no reference to the influence of time. He considers that it takes a little while for alcohol to produce an effect, but he does not mention how long this latent period lasts, nor does he show whether more abnormalities were produced at the end of daily exposure for several years, than in the earlier months. We have made tables showing the length of exposure to the fumes of alcohol in the case of each doe at the birth, and of each buck at the conception, of each litter. These tables will be referred to later.

It will suffice, at present, to give a short account of the conditions before the production of an abnormality. Examination of the charts in which the mothers of abnormal offspring are specially marked shows that in 2 cases abnormalities were produced by alcoholized parents with no alcohol in their ancestral history, i.e. 1 generation of alcohol.

(1) (Chart I *a*) ex ♀ 9 × ♂ 4: here the ♀ only was treated and had been alcoholized for 20 months, when the abnormality appeared. An abnormality was produced by the grandchildren of this pair (see below). (2) (Chart I *b*) ex ♀ 314 × ♂ 297: here both animals were treated; the ♂ for 2 months before conception and the ♀ for 6 months before the abnormality was born.

One case occurred when 2 generations had been alcoholized (Chart I *b*) ex ♀ 79 × ♂ 52; the parents of the abnormality were treated for 2 months and 4 months respectively, and were descended from an alcoholized mother treated for 9 months. One case occurred, when 3 generations had been alcoholized: (Chart I *c*) ♀ 21 had been treated for 22 months before ♀ 101 was born and for 4 months before ♂ 28 was born. ♂ 13 had been treated for 20 months, and 1 month at the times of conception for ♀ 101 and ♂ 28 respectively. ♂ 28 was treated for 16 months and 12 months before the conception of ♀ 187 and ♂ 109 respectively; finally, ♂ 109 had been treated for 3 months, when the abnormality produced by its mating with ♀ 187 was conceived.

Among the descendants of 2 generations of alcohol, there was 1 case occurring in the F_4 generation (Chart I *a*) ex ♀ 423 × ♂ 397. The great-great-grandmother of this pair was alcoholized for 38 months and the great-great-grandfather for 36 months.

Among the descendants of 2 generations of alcohol there are 3 other cases. One occurs in the F_1 for 2 generations of alcohol (ex ♀ 131 × ♂ 84), the grandchildren of ♀ 9 × ♂ 4. ♀ 9 was alcoholized for 3 months when ♀ 50 and ♂ 34 were born. ♂ 34 was alcoholized for 16 months when ♀ 131 and ♂ 84 were conceived.

There are 2 cases in the F_2 for 2 generations of alcohol: (1) ex ♀ 420 × ♂ 348: these are descended from alcoholized males only; the grandfather was alcoholized for 29 months, the great-grandfather

for 8 months. (2) ex ♀ 392 × ♂ 323: these were also derived from alcoholized males only; the grandfather was treated for 9 months and the great-grandfather for 5 months before the respective conceptions.

In the F_1 for 3 generations of alcohol, there was 1 case (ex. ♀ 448 × ♂ 372). The father had been alcoholized for 9 months, grandfather for 41 months, and great-grandfather for 6 months before the respective conceptions.

In the F_2 for 3 generations of alcohol there was 1 case (Chart I *b*) ex. ♀ 416 × ♂ 344. This pair had two great-great-grandmothers on alcohol for 8 months and 6 months respectively; one great-great-grandfather was on alcohol for 7 months before the conception, one great-grandmother was treated for 8 months and one grandmother was on alcohol for 7 months.

The main points brought out by this discussion are briefly as follows:

1. Abnormalities similar to those described by Stockard occurred in the breeding experiments of Sewall Wright who showed that these defects were inherited in his stock.

2. Stockard found that the number of abnormalities in his alcoholized animals diminished when he ceased to inbreed and when the conditions for the animals were improved.

3. Of the 11 abnormalities occurring in our stock, all were descended from 1 or 2 of 3 original ancestors. No abnormalities occurred among the descendants of any of the other animals used to build up the stock. One abnormality occurred among the untreated stock.

4. Abnormalities were produced from directly treated animals or from the descendants of alcoholized animals. Periods of exposure to alcohol varied from 2 months to over 3 years. (It will be shown later, pp. 37 and 53, that animals with long exposure to alcohol did not have worse offspring than those with short exposure.)

5. The fact that alcohol has been used either for the parents or for the ancestry of degenerates, cannot be regarded as evidence of the influence of the drug. Abnormalities may be due to congenital defects in the ancestry.

Sterility

The question of sterility or of apparent sterility is a difficult matter to deal with, since so little is known of the causes of sterility. In guinea-pigs, as well as in many other mammals, it is not uncommon to find that absorption of the foetus or foetuses may take place *in utero*. This fact may have an important bearing on the question of apparent sterility, for absorption of the fertilized ova can take place at any time, both in the early and later stages of development. In the very early stages this would be impossible to prove without examination of the corpora lutea. In the later stages it can be shown to have occurred by external examination, but even then the estimation can only be a rough one.

Corner (1923) has shown that in pigs absorption may occur at any

stage during embryonic development, leading to total disappearance of the embryo, or to degenerate and abnormal foetuses, very similar in appearance to those which occur in guinea-pigs.

The method adopted by Stockard (1918, p. 157) was to examine the does a month after they had been mated. 'The female to be examined is allowed to stand on a flat surface, and the investigator with both hands presses the ventral abdominal wall so as to feel with the fingers the horns of the uterus against the dorsal abdominal wall. With considerable practice the small embryos and placentae may be definitely counted within one or both horns of the uterus. . . . The number of young finally born helps to show how nearly correct the examinations have been.'

Stockard (1918) states that he has considerable data to show that absorption of embryos is more frequent in the alcoholic than in the normal lines. He has not given these data in his tables, for he includes the number absorbed with the premature and still-born.

MacDowell (1924) has produced a technique by which he is able to count the number of corpora lutea in pregnant mice. He shows, by this means, that alcohol has no effect upon the reproduction of mice. The length of the oestrus cycle, the number of corpora lutea per ovulation, the prenatal and natal mortality, and the number of living young per litter in no way differ from the normal. If it be true that the influence of alcohol in guinea-pigs is to cause absorption to occur more frequently in the treated does than in the normals, and that this absorption occurs more often in the early than in the late stages of pregnancy, there would be apparent failure to breed, partial or complete sterility as a result. But the possibility of heredity must be considered. In pigs absorption is a well-known phenomenon, and it has been suggested that in these animals the phenomenon is inherited.

As we have already stated, the does were examined every week in order to estimate the probable pregnancies. The method of ascertaining the probable size of the litter to be born is of practical value for the discovery of miscarriages, which in the early stages would be missed, if there were not records of fertilization having occurred. The guinea-pig always cleans up everything after a miscarriage, in the same way that it eats the placenta, &c., after birth, and unless a miscarriage occurs at a suitable moment for observation it is very easily overlooked. We have omitted the absorption of foetuses from our tables altogether, since the estimation of the number of foetuses could not be complete. The only satisfactory way of finding out how many ripe ova have been fertilized is to count corpora lutea. This is obviously impossible if the animal is to be used for further breeding.

Since Stockard lays stress on absorption, and considers that the action of alcohol is to increase the percentage of times that it occurs, we cannot omit all reference to it. We may state at the outset that we had no certain case of absorption among the controls mated to controls. Among the alcoholics and their descendants there were 26 cases in all. It is probable that absorption really occurred more

often in our experiments and our failure to detect it can be attributed to various causes. For instance, if a miscarriage occurred, the diagnosis of the number of foetuses present could not always be confirmed, for the guinea-pig has a habit of eating the aborted young; unless, therefore, the miscarriage took place at a time when observation of the event was possible, no correct conclusion could be arrived at and also before we were sufficiently expert we probably overlooked several. This applies to the control group equally with the alcoholic group.

The 26 cases of absorption among the alcohol stock were distributed as follows:

F_1	1 generation of alcohol	4 cases
F_2	,, ,,	2 ,,
F_3	,, ,,	5 ,,
F_4	,, ,,	1 case
F_5	,, ,,	2 cases
F_1	2 generations of alcohol	1 case
F_2	,, ,,	5 cases
F_1	3 generations of alcohol	1 case
F_2	,, ,,	2 cases
Control ♀ × F_1	2 generations of alcohol	1 case
Control ♀ × F_1	3 ,, ,,	1 ,,
	3 generations of alcohol	1 case

As regards the effect of alcohol in the histories of the animals showing this characteristic, the times on alcohol range from 1 month to over 3 years, but there does not appear to be an increased amount of absorption after long exposure to alcohol. It is recorded once only for each doe in every case, and it may occur in the first family, or in the last (after 11 previous good-sized litters, for instance), or halfway through her pregnancies. There appears to be no rule about it.

Absorption as far as we were able to tell occurred only once among the offspring of directly alcoholized animals and all the other cases were among the descendants of alcoholized animals. The total number of young born from alcoholic parents and their descendants was 6,309. As in the case of abnormalities, the chances of finding cases of absorption among the controls were correspondingly not very great, since among the controls there were only 674 births. We may, therefore, consider whether some influence other than that of the effect of alcohol may not have been at work. On examining the pedigrees of the animals concerned, we find that 9 out of the 26 cases all occurred in the direct line from 2 guinea-pigs of the original stock ♀ 6 and ♂ 3. Two families were taken from this pair to serve as stock and the young, 5 in all, were used for stud. Unfortunately, one pair of these which should have served for the formation of a control line died early before the control line could be established, and the remaining animals passed into the alcoholic line. Strict inbreeding was practised in this family. In no other family where strict inbreeding was carried out, were there so many cases of absorption. One of the F_1 ♀'s (1 generation of alcohol), ♀ 127, which belonged to this family produced

a daughter, ♀ 204 (F_2, 1 generation of alcohol), and in both cases of ♀ 127 and ♀ 204 absorption occurred.

Among the remaining cases (17 in all) there was an ancestor, ♂ 2, common to them all. There is not the same close relationship among the parents of these 17 cases that obtains among the descendants of ♀ 6 and ♂ 3. Our cases of absorption can all be traced back to 3 common ancestors; they occur either among the descendants of ♀ 6 × ♂ 3 or among the descendants of ♂ 2. It appears, therefore, that heredity and the effect of alcohol might equally probably be the determining factor in the production of this phenomenon, though the non-appearance of cases of absorption in the controls might well be due to sampling fluctuations.

Closely related to the phenomenon of absorption of the fertilized and implanted ovum in the earlier stages of its development, are the cases in which particular pairs of guinea-pigs appear to show a relative or absolute sterility. It happens, sometimes, that a pair mated together will produce no offspring, although perfectly fertile with other mates. It may be, in these cases, that the fertilized ova were absorbed very young, or that early abortion may have occurred. When a pair of guinea-pigs has been mated without success, each of them has been tried with other mates, in order to find out to which the failure may be due. There were 2 cases among the controls in which the does were found to be bad breeders, ceasing early to breed. The 1st case, ♀ 366, mated with her brother ♂ 297, produced first one only, and then a miscarriage. She was then mated with an unrelated buck, on alcohol 1 year and 9 months, and produced nothing. This buck was mated with 2 other does and was perfectly fertile. ♀ 366 was mated with another control buck, by which she had a litter of two, and then with another control buck, by which she had nothing. She was not tried further. She was obviously a bad breeder, and may have been a case in which absorption took place. ♂ 297 was perfectly fertile, and bred with many other ♀'s. ♀ 30 was also a bad breeder. She had 1 and then 3 and then 1 by her brother, and was F_2 inbred; she never had other offspring, although tried with another, unrelated, control buck which was fertile with other ♀'s subsequently. Her brother also ceased to breed, though tried with 2 other does, both of which proved fertile with other mates later.

There were 15 cases of *partial* sterility among the alcoholics and their descendants. Alcohol would not appear to have had any effect, as here, again, there is no correlation between the time of alcoholization and the failure to mate. But among the offspring of one particular family there were more cases of apparent or partial sterility than in the rest of our stock. ♀ 26 × ♂ 17, brother and sister from original stock, ♀ 6 × ♂ 3, produced 12 litters in all; many of the young were used for stud, and their descendants also. ♀ 26 had litters by other bucks as well, and ♂ 17 by other does. ♂ 17 was on alcohol 4 years before he was got rid of, and he lived not quite 5 years. During all that time, right up to the end, he was fertile. ♀ 26 was normal and

lived to a similar age. Her last litter was born when she was 57 months old. There was, therefore, no sign of any failure on the part of either mate, but among 8 pairs of their descendants there were cases of failure, in the F_1, F_2, up to F_5 generations. Three of the bucks were probably sterile. None of the does were completely sterile, but two of them had only miscarriages and not full-time births.

This family is one which might be selected as showing that alcohol had a deleterious effect on the breeding powers of these animals. It is the family in which failure to breed and absorption appeared most often. Also, and this is most important, it is the family in which inbreeding has been carried on to 6 generations. It would be possible to give the histories of other families which have been much alcoholized and bred from, but none of them show this failure, even in the case of descendants of males on alcohol for 5 years.

To sum up, the evidence of apparent failure to breed or of diminished fertility in animals which have been exposed to alcohol, can be equally well explained by the effect of heredity or the effect of inbreeding, and there does not seem to be sufficient reason to attribute these phenomena to alcohol, either by itself, or taken in conjunction with congenital conditions.

Controls Compared with one Generation of Alcohol

Table I gives a summary of the results from the control untreated and the alcoholic lines. The control series includes all generations from untreated animals; the alcoholic series relates only to the initial stock, where only one generation has been on alcohol. Seventy-nine does were used in the control series, and 82 in the treated series. Animals in the treated group were first used in the control group, and later in the alcoholic group.

Table I

	Controls.	*One generation alcohol.*
Number of ♀'s used	79	82
Number of litters	214	252
Total births	674	813
Average size of litter	3·15	3·23
Dead births and post-natal deaths	118	153
Mortality per 100 births	17·51	18·82
	Diff. 1·31	
Number of miscarriages	5	2
Number of malformations	1	2
Sex-ratio	98	102
Mean birth-weight of guinea-pig	83·1 ± 0·75 g.	86·1 ± 0·56 g.
	Diff. 3·0 ± 0·94	
(Per cent. weighed of total young)	(53 per cent.)	(76 per cent.)
Mean birth-weight of male	84·0 g.	88·5 g.
Mean birth-weight of female	82·3 g.	83·7 g.

It might be suggested that only does which showed good breeding capacities in the control group were selected for use in the experimental groups. As a test of this, the fertility and mortality records,

previous to treatment, were examined of the stock coming into both groups. There is no evidence of selection as regards fertility, because the average fertility of the does, before appearing in the treated group, was actually slightly lower than that of does only appearing in the control group, 3·12 against 3·18, but mortality was much lower among the young of does who afterwards came in the treated group, 12·6 per cent. against 22·3 per cent. for all untreated does, so that there may have been some unconscious selection as regards mortality of young. This possibility will be examined later. There was no evidence of selection as regards weight of offspring, the average birth-weight was 82·7 g. in the control and 82·4 g. for offspring of does which afterwards came into the treated groups.

The average fertility in the treated and untreated groups was approximately the same, 3·23 against 3·15. Mortality is measured as the percentage of known dead births, and deaths in the first 3 weeks of life, to total births. The mortality of young from treated animals was slightly higher than that from untreated animals, 18·8 per cent. against 17·5 per cent., but the difference is not significant with regard to its probable error, though it must be noted that if one compares mortality experience in the same animals before and after treatment there was an increase after treatment.

Miscarriages were rather more numerous in the control groups. The total number of defective offspring was only 3, 1 from the controls and 2 from treated animals.

The sex-ratios (or births of males per cent. of birth of females) of the two groups are not significantly different, 98 and 102.

Only live-born young were weighed, but unfortunately not all the weights were recorded in the early part of the experiment. The weights are based on 53 per cent. of the young in the control group, and 76 per cent. of the treated groups. The mean weights of our guinea-pigs are so much higher than those of other records that one might suspect selection.

Mean birth-weight.	*Controls.*	*Alcohols.*
Stockard (1918) . . .	77·2 g.	70·4 g.
Sewall Wright (1922) . .	80·0 „	
Russell and Hartley (1930). .	81·2 „	
Durham and Woods . . .	83·1 „	86·1 „

We think it fairly safe to regard the numbers weighed as a random sample of the whole, and the two groups as comparable, because, although only live guinea-pigs were weighed, mortality in the two groups is not very different. The higher mean weight in our experiment may be due to better feeding.

There is no evidence, from the animals weighed, that the young from alcoholic parents are physically inferior to those from untreated parents—the mean birth-weight is actually higher in the treated series $86{\cdot}1 \pm 0{\cdot}56$ g. against $83{\cdot}1 \pm 0{\cdot}75$ g., and significantly higher, when one takes into account the size and variability of the sample, but there was a greater proportion of males in the treated series, and since males are heavier than females, this may explain the difference

in weight. When one compares the sex weights the male birth-weight is higher than the female on the average. This is in agreement with human experience.

Effects of Inbreeding.

It was formerly generally believed that intensive inbreeding produced progressive degeneration, i.e. reduction in size, vigour, and fertility. Nowadays, most of the modern special breeds of cattle originated from close inbreeding of selected stock, though there appears to be much difference of opinion amongst practical breeders about the ultimate effects. The low fertility of shorthorn cattle has been attributed to intensive inbreeding.

Sewall Wright (1922) made a thorough study of the effects of inbreeding on guinea-pigs. His results are based on some 30,000 animals. He kept a control stock under the same conditions as the inbred stock, and the experiment was carried on over a period of 15 years. He showed, quite definitely, that there was a decline in vigour in every respect for the inbred animals—fertility was lowered, there was a definite decline in size and frequency of litters, and mortality was much higher than in the control stock. Both the treated and untreated stock of our series were affected by inbreeding, and the small differences in the two groups shown in Table I might be due to a higher proportion of inbred stock in the untreated group, since analysis showed that the untreated were more closely inbred. One hundred and thirty-nine of the 214 control litters were from related parents, whereas in the alcohol series there were 151 litters from related and 101 from unrelated parents (Table II). The unrelated guinea-pigs, whether subjected to treatment or not, tend on the average to give birth to rather larger litters than do related animals, but the young from related animals have a heavier mean birth-weight. The young from unrelated guinea-pigs have a slightly higher mortality in the control group, whereas the reverse is the case for the alcohol group, but the differences are not significant. The difference in the sex-ratios of young from inbred and outbred animals is considerable, and alcohol appears to accentuate the difference. Further reference to this will be made later. With the exception of a small reduction in the size of litter, there is no evidence from this table, that the greater degree of inbreeding in the control group has had an adverse effect on the results.

Distribution of Litters.

Table III gives the frequency of litters distributed by size for control stock compared with 1 generation of alcohol. Litters of 2 form 23·8 per cent. of the control series, against 13·9 of the treated series. Litters of 3, 4, and 5 occur rather more frequently in the treated than in the untreated group. Can one say, that the difference between the two frequency distributions for size of litters is greater than might be expected from chance variation? Pearson's χ^2 test for random sampling has been applied and χ^2 was found to be equal

Table II

Comparison by inbreeding.

	Controls.		Alcohols (1 generation of alcohol).		All Controls.	All Alcohols (1 generation of alcohol).
	Unrelated.	*Related.*	*Unrelated.*	*Related.*		
Number of females used	27	52	40	42	79	82
Number of litters	75	139	101	151	214	252
Total births	260	414	346	467	674	813
Average size of litter	3·47 ± 0·09	2·98 ± 0·06	3·43 ± 0·08	3·09 ± 0·06	3·15	3·23
	Diff. + 0·49 ± 0·11		Diff. 0·34 ± 0·11			
Dead births and post-natal deaths	47	71	64	89	118	153
Mortality per 100 births	18·1	17·1	18·5	19·1	17·51	18·82
	Diff. 1·0		Diff. 0·6			
Number of miscarriages	2	3	1	1	5	2
Number of malformations	0	1	1	1	1 (0·1 per cent.)	2 (0·2 per cent.)
Sex-ratio	85	107	86	117	98	102
Mean birth-weight	81·9 g.	83·8 g.	83·8 g.	88·3 g.	83·1 ± 0·75 g.	86·1 ± 0·56 g.
	Diff. 1·8		Diff. 4·5			
(Per cent. weighed of total young)	—	—	—	—	(53)	(76)
Mean birth-weight of male	—	—	—	—	84·0	88·5
Mean birth-weight of female	—	—	—	—	82·3	83·7

TABLE III

Distribution of litters

Size of litter.	CONTROLS.		ALCOHOLS (1 generation of alcohol).	
	Number of litters.	*Per cent. distribution.*	*Number of litters.*	*Per cent. distribution.*
1	13	6·1	24	9·5
2	51	23·8	35	13·9
3	72	33·6	90	35·7
4	54	25·3	73	29·0
5	18	8·4	23	9·1
6	5	2·3	7	2·8
7	1	0·5	—	—
TOTAL	214	100·0	252	100·0
	Mean size of litter = 3·15.		Mean size of litter = 3·23.	

χ^2 test for random sampling. Distribution of litters in control and alcoholic series.

Durham $\chi^2 = 8{\cdot}66$. P = 0·07 not significant.
Stockard $\chi^2 = 30{\cdot}78$. P = 0·000001 significant.

to 8·66 and p 0·07. We should expect, therefore, that differences such as those observed in the two distributions would occur 7 times in 100 trials; in other words, the chances are less than 15 to 1 that there is a real difference between the two series. We may conclude that the apparent difference between the sizes of litters in the treated and untreated groups respectively are no greater than might be due purely to chance. Chart II shows that the combined distribution of litters in the treated and untreated group can be effectively described by the normal curve.

Stockard found that small litters were relatively more frequent, and large litters less frequent, in treated animals than in untreated. He attributes this difference to ante-natal loss, such as absorption *in utero* of one or more members of an originally large litter, as a result of the action of alcohol on the developing embryos. This point has already been dealt with in the section on sterility. The value of χ^2 for his two distributions is large, 30·78, and p is less than 0·0001. In other words, there appears to be a real difference between his two samples, much greater than one would expect from mere chance fluctuations.

Mortality according to Size of Litter.

Table IV and Chart III show the distribution of mortality among guinea-pigs in different sized litters. In the untreated series, mortality is high in litters of 1, 23·1 per cent. were born dead or died within the first 3 weeks of life, but this result is only based on 13 litters. For 51 litters of 2, the mortality was only 9 per cent., and as the size of litter increases mortality also increases rapidly, but mortality for litters of 5, 6, and 7 is only based on small numbers.

For treated guinea-pigs mortality also increases with size of litter,

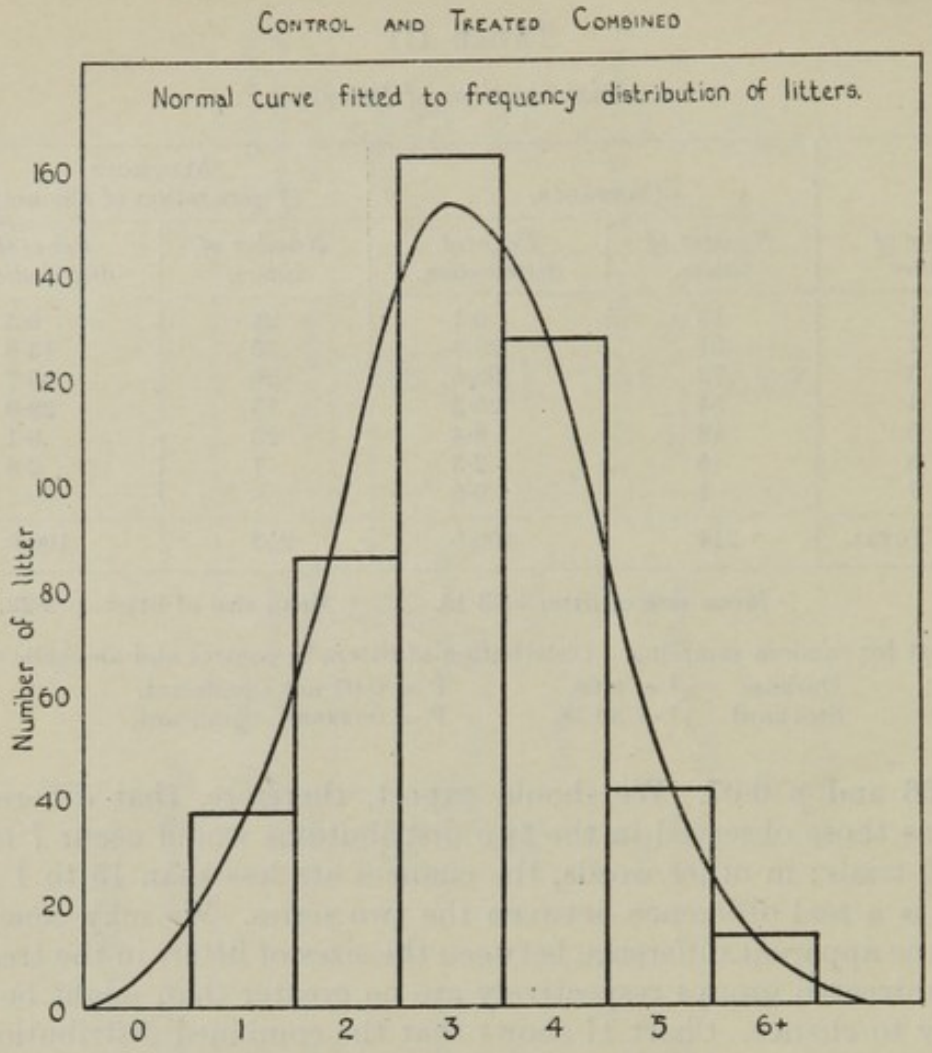

Chart II

but in 24 litters of 1 only 8 per cent. died against 23 per cent. in the untreated group. Litters of 2 had a mortality of 13 per cent., against 9 per cent. in the untreated group. The mortality in litters of 3, 4, 5, and 6 is much the same as in the untreated experience. On the whole

Table IV

Mortality by size of litter.

Size of litter.	All Controls.		1 Generation of Alcohol.	
	No. of litters.	*Deaths. Per cent. of births.*	*No. of litters.*	*Deaths. Per cent. of births.*
1	13	23·1	24	8·3
2	51	8·8	35	12·9
3	72	11·6	90	13·0
4	54	17·1	73	19·5
5	18	30·0	23	28·7
6	5	40·0	7	42·9
7	1	71·4	—	—
Total	214	17·5	252	18·8

one cannot say that the trend of mortality with size of litter is any worse in the young from alcoholic parents than in those from parents not treated with alcohol.

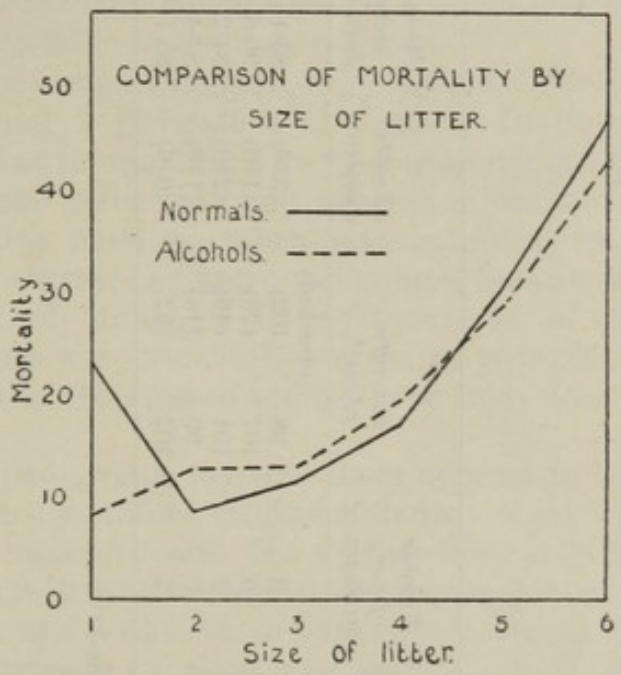

CHART III

Effect of Parental Alcoholism on Size of Litter.

The data for the alcoholic group are classified by parental alcoholism (Table V). The litters fall into 3 classes, 105 with only mother alcoholic, 82 with only father alcoholic, and 65 with both parents alcoholic. The average litter size is highest in the treated female group, but 21 per cent. of the offspring died within the first 3 weeks of life—the highest mortality of the 3 classes. In no class is the average litter less than 3. Mortality is lowest in the alcoholic father class, and the offspring have a superiority as regards weight. This is what one would expect, that where the litter is smaller, the animals are heavier and have a better chance of surviving. In this group there were more males, which probably explains the greater mean weight. In every class the mean birth-weight of the young is superior to that for untreated animals, but only significantly different in the alcoholic father class.

The Effect of Age of Female on Size of Litter and Mortality.

It is a common belief among animal breeders that the age of the parent has an important effect on certain characteristics of the progeny: that immature parents produce offspring inferior in size and constitution to those from mature parents. Sewall Wright (1929) found that age of parent (particularly the female) was an important factor in the occurrence of polydactyly and in the amount of white spotting in guinea-pigs. There was, further, a decrease in the mortality rate of offspring with advancing maturity of the doe. Wright

TABLE V

Parental alcoholism.

	Number of litters.	*Total offspring.*	*Mean size of litter.*	*Dead births and post-natal deaths.*	*Per cent. mortality of total births.*	*Number of mis-carriages.*	*Sex-ratio at birth.*		*(Number weighed.)*	*Mean birth-weight of guinea-pig.*
								(unsexed)		
Controls N♀ N♂	214	674	3·15	118	17·5	5	98	(102)	(356)	83·1 ± 0·75
N♀ A♂	82	246	3·00	36	14·6	0	107	(28)	(176)	90·8 ± 1·19
A♀ N♂	105	353	3·36	75	21·2	2	101	(46)	(270)	84·2 ± 0·76
A♀ A♂	65	214	3·29	42	19·6	0	99	(18)	(170)	84·4 ± 0·99
All alcohols	252	813	3·23	153	18·8	2	102	(92)	(616)	86·1 ± 0·56

N = untreated control animal.
A = alcoholic animal.

has studied the life history of guinea-pigs, and concludes that they are in their prime between 1 and 3 years. After 3 years there is a distinct falling-off. The best breeding period is between the ages 1–2½ years; afterwards litters tend to become smaller. First litters are smaller than later ones on the average.

In Table VI a comparison is made between fertility by age of mother for controls and 1 generation of alcohol. In human experience, fertility is not at its maximum at the beginning of reproductive life. If the age of the parent has any relation to the size of the litter in guinea-pigs, this must be taken into consideration in comparing fertility in our treated and untreated groups, because the age distributions were entirely different. Over 60 per cent. of the litters in the untreated groups were from does less than a year old, whereas 97 per cent. of litters in the treated groups were from does aged 1 year or more.

Taking the two groups together there appears to be a slight trend of fertility with age, from a minimum in does under 1 year to a maximum at 2 and under 3 years. The average litter is 3·03 for does under 12 months old, 3·19 in the group 12–24 months, 3·40 in the next group, 24–36 months, and 3·13 in those over 36 months. As far as the data for untreated animals go, there is a trend to a maximum at 2½ years, but the number of litters at this age is very small. The correlations between size of litter and age of mother are:

	r.	η.
Controls	$0{\cdot}178 \pm 0{\cdot}046$	$0{\cdot}222 \pm 0{\cdot}045$
Alcohols	$0{\cdot}087 \pm 0{\cdot}043$	$0{\cdot}102 \pm 0{\cdot}042$

There is a small but significant skew correlation between age of doe and size of litter in the control experience, but no significant correlation in the alcoholic series. If we take 12–36 months as the period of maximum fertility and under 12 months and over 36 months as the period of minimum fertility the proportion of does classified by fertility were as follows:

	Controls.	*Alcohol.*
Minimum fertility	66·3 per cent.	21·8 per cent.
Maximum fertility	33·7 „	78·2 „

On this assumption the age distribution of the treated animals was more favourable to a high fertility than that of the untreated, yet the total result as measured by average size of litter for the two groups was practically identical. If the alcoholic group had had the same age distribution of does as the control group, the 'expected' mean size of litter for all alcoholics would have been 3·43 against actual 3·23; for alcoholic mother 3·42 against 3·36, and for alcoholic father 3·41 against 3.0. It is unfortunate that the two groups were not comparable by age; it has already been pointed out that many of the animals were first used in the control group and then passed into the alcohol group. In the controls only 9 per cent. of the litters were from does over 2 years old. It is, therefore, not possible to establish definitely that alcohol had really affected fertility in 1 generation of

Table VI

Fertility and mortality by age of mother.

Age of female in months.	All Controls.			A♀ N♂.			N♀ A♂			A♀ A♂.			All Alcohols (1 generation of alcohol).		
	Total litters.	*Mean size of litter.*	*Per cent. mortality of all births.*	*Total litters.*	*Mean size of litter.*	*Per cent. mortality of all births.*	*Total litters.*	*Mean size of litter.*	*Per cent. mortality of all births.*	*Total litters.*	*Mean size of litter.*	*Per cent. mortality of all births.*	*Total litters.*	*Mean size of litter.*	*Per cent. mortality of all births.*
0–12	127	3·00	14·4	3	3·0	—	3	4·3	15·4	2	3·0	—	8	3·5	7·1
12–24	56	3·25	22·0	53	3·3	13·1	32	2·8	12·1	24	3·3	15·4	109	3·2	13·4
24–36	12	3·75	26·7	35	3·6	24·8	26	2·9	9·3	27	3·5	21·1	88	3·4	19·7
36+	7	3·30	21·7	14	3·1	47·7	21	3·2	23·9	12	2·9	28·6	47	3·1	32·2
Unknown	(12)	(3·58)	14·0	—	—	—	—	—	—	—	—	—	—	—	—
Total	214	3·15	17·5	105	3·4	21·2	82	3·0	14·6	65	3·3	19·6	252	3·2	18·8

Controls and alcohols combined.

Age of females.	*Total litters.*	*Mean litters.*	*Per cent. mortality of all births.*
0–12	135	3·03	13·9
12–24	165	3·19	16·3
24–36	100	3·40	20·6
36+	54	3·13	30·8
(Unknown)	(12)	(3·58)	14·0
Total	466	3·19	18·2

alcohol, but it will be seen that in subsequent generations there is no evidence that loss of fertility occurs as a result of alcohol (see Table XVI).

In human experience there is in general a rise in infant mortality with advancing age of the parent, although, of course, this is not solely a function of age.

Ewart's figures.

Infant mortality according to maternal age.

Age of mother.	*No. of births.*	*Deaths per 1,000 births.*
Under 19	152	171
20–24	536	132
25–29	396	166
30–34	316	170
35–39	150	220
Over 40	36	330
	1,586	

Ewart shows that infant mortality in human experience 'falls until the 24th year is reached and then slowly rises again reaching its maximum in mothers of over 40 years'. Professor Sewall Wright has kindly given us his data for guinea-pigs showing that there was a relationship between the percentage of still-births and age of doe, and some relation between deaths under 1 month and maternal age. His figures, which are given below, relate to closely inbred animals.

Age of ♀ in months.	*Number of litters.*	*Per cent. still-born.*	*Per cent. died 0–33 days.*
3–6	349	27·8	22·5
6–9	390	20·2	21·2
9–12	319	18·6	20·5
12–15	292	19·4	17·8
15–21	330	21·3	23·3
21–46	296	24·1	19·4
	1,976		

The percentage of still-births is highest for does under 6 months; there is a considerable drop in the next 3 months to a minimum at 9–12 months, after which it steadily rises. Mortality under 33 days falls gradually from 22·5 per cent. for does aged 3–6 months to 17·8 at ages 12–15 months, then tends to rise again.

In our data, mortality is high in litters born before the doe is 6 months old, lowest for does over 6 months and less than 12 months, and apparently tends to rise afterwards. Both treated and untreated groups show a rise in mortality with increasing age of doe. The combined data show a trend of mortality from 14·2 for does under 1 year to 20·6 at 24–36 months, and a further rise to 30·8 per cent. for does older than 36 months.

As a test of the significance of variations of mortality with age, an expected number of deaths has been calculated for each age-group

on the assumption that there was no differentiation with age, i.e. that the mean mortality of the whole applied at every age. The expected distribution of deaths was then compared with the actual distribution, to determine the probability whether the deviations between observed and expected might be solely due to the play of chance. In the normal

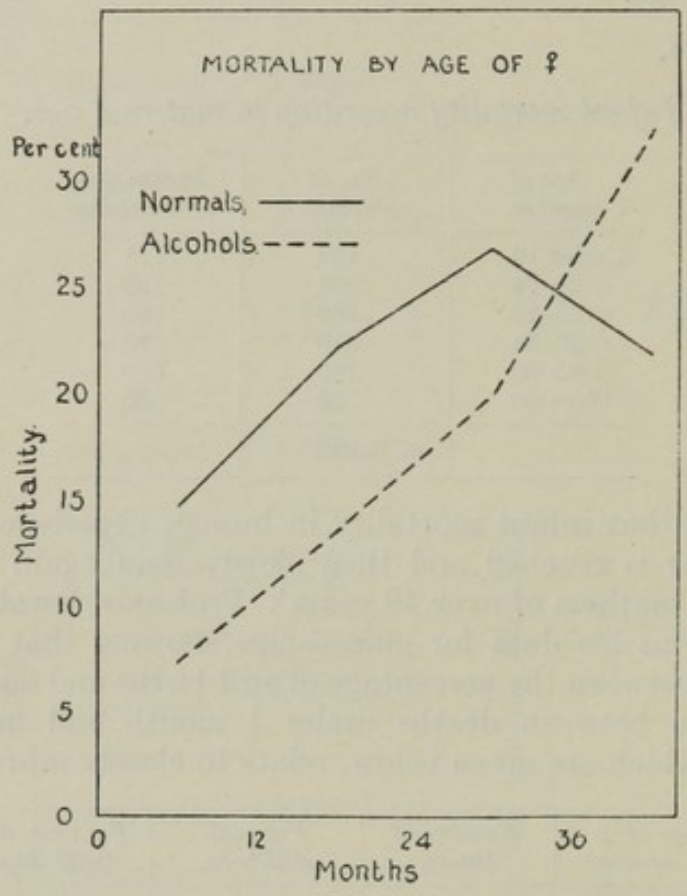

CHART IV

experience the value of $\chi^2 = 6{\cdot}58$ and $p = 0{\cdot}09$, from which one may conclude that the differences might quite well be of no significance. For the treated group $\chi^2 = 27{\cdot}32$ and $p = 0{\cdot}000001$, in other words, the differences in this series might have occurred about once in a million trials. The differences in the treated groups are greater than might be expected by mere chance fluctuations. There is, therefore, evidence that mortality really varies with age in the alcohol series. This variation may be closely associated with the duration of exposure to alcohol as well as to the increasing age of mother. Compared with the distribution of untreated litters, the alcoholic distribution is heavily weighted at those ages where we should expect mortality to be highest. The 'expected' mortality rate for the alcoholic group, had they had the same age distribution of mothers as the control groups, would have been 23·4 per cent. against actual 18·8—a substantial reduction.

Fertility and Mortality by Order of Litter.

It was thought of some interest to see whether there was any relation between the order of litter and fertility and mortality. Do

large litters alternate with small litters, or is there a tendency for guinea-pigs to breed small litters or large litters throughout? Haines (1929) concluded that the 2nd, 3rd, and 4th litters were generally superior to 1st litters and litters after the 5th, for all the characters studied except birth-weight and those born dead and those which died between birth and weaning.

Only the control data have been studied (Table VII). The correlation between the size of the 1st litter and size of the 2nd litter of 54

TABLE VII

Mean size of litter and percentage mortality by order of litter.

	CONTROLS.						
	LITTER ORDER.						
	1st.	*2nd.*	*3rd.*	*4th.*	*5th.*	*6th.*	*7th and upwards.*
Number of litters	65	57	38	15	12	5	22
						39	
Mean size	2·7	3·3	3·3	3·6	3·0	3·6	3·5
						3·36	
Number of deaths	28	28	20	8	11	4	19
						34	
Per cent. mortality	16·2	14·8	15·7	14·8	30·6	22·2	24·7
						26·0	

untreated does was $+0{\cdot}453 \pm 0{\cdot}073$. The correlation between 1st and 3rd litter was slightly higher, $+0{\cdot}507 \pm 0{\cdot}085$. In both cases, the correlations are significant and show there is a definite tendency for large litters to be followed by large litters and small by small. The trend of fertility by litter order reaches its maximum at the 4th litter and is at a minimum for 1st litters. Litter order must be closely related to the age of mother. Mortality shows no regular trend and is heaviest after the 4th litter, but the numbers are small.

Birth-weights according to Size of Litter.

In Table VIII the mean birth-weights of guinea-pigs in different-sized litters are compared. The birth-weight of both untreated guinea-pigs and alcoholics vary inversely with size of litter. The trend may be fairly satisfactorily graduated by a geometric progression for both groups. The weights for untreated guinea-pigs range from 118·8 g. for litters of 1 to 63·5 g. for litters of 5. The mean weights for alcoholics range from 128·5 g. to 60·6 g., and these guinea-pigs appear to be physically superior to untreated guinea-pigs in litters of 1, 3, 4, and 5. The differences are only significant with regard to the probable errors in litters of 4 and 5. In litters of 6 the controls have the advantage. The mean birth-weight of all alcoholic guinea-pigs was significantly greater than that of untreated guinea-pigs; but as guinea-pigs born in small litters weigh on the average more than guinea-pigs born in

Table VIII

Mean birth-weights by size of litter.

Size of litter.	Controls.			N♀ A♂.			A♀ N♂.			A♀ A♂.			All Alcohols (1 generation of alcohol).		
	Total young.	*Number weighed.*	*Mean weight. P.E.*	*Total young.*	*Number weighed.*	*Mean weight. P.E.*	*Total young.*	*Number weighed.*	*Mean weight. P.E.*	*Total young.*	*Number weighed.*	*Mean weight. P.E.*	*Total young.*	*Number weighed.*	*Mean weight. P.E.*
			g.			g.			g.			g.			g.
1	13	4	118·8±5·13	12	8	138·4±3·45	6	5	124·2±1·72	6	4	114·0±8·16	24	17	128·5±3·03
2	102	53	104·5±1·48	24	14	105·4±2·56	30	23	108·4±1·94	16	14	101·2±3·87	70	51	105·6±1·57
3	216	113	90·4±1·40	99	72	98·9±1·48	114	89	92·1±1·04	57	47	90·4±1·42	270	208	94·1±0·76
4	216	116	76·0±0·83	60	50	81·4±1·54	124	100	78·4±0·88	108	80	81·8±1·23	292	230	80·2±0·67
5	90	50	63·5±1·30	45	32	69·3±1·78	55	31	71·7±1·64	15	14	73·7±2·81	115	77	71·0±1·14
6	30	20	66·0±1·40	6	—	— —	24	22	61·8±1·55	12	11	58·2±2·77	42	33	60·6±1·40
7	7	—	— —	—	—	— —	—	—	— —	—	—	— —	—	—	— —
Total	674	356	83·1±0·75	246	176	90·8±1·19	353	270	84·2±0·76	214	170	84·4±0·99	813	616	86·1±0·56
	% 53			% 72			% 76			% 79			% 76		

Weights of controls standardized for size of litter = 86·2 g.

N = untreated.
A = alcoholic.

Differences. Controls and Alcohols.

1 −9·7±5·96 not significant
2 −1·1±2·16 not significant
3 −3·8±1·60 not significant
4 −4·2±1·06 significant
5 −7·5±1·73 significant
6 +5·4±1·98 not significant

Weight of all alcohols standardized for size of litter = 89·8 g.

large litters, this difference in total mean weight might be accounted for by unconscious selection—that a greater proportion of guinea-pigs from small litters were weighed in the alcoholic series than in the controls. The mean birth-weights were therefore corrected for differences in proportion weighed in litters of various sizes, using as a standard distribution the figures relating to 2,070 litters from Russell and Hartley's report (1930). The mean birth-weights standardized for litter size were 86·2 for untreated guinea-pigs and 89·8 for treated guinea-pigs. Even when allowance is made for differences in litter size the alcoholic guinea-pigs still maintain an advantage over untreated guinea-pigs. The higher proportion of males in the treated group probably accounts for some of the difference.

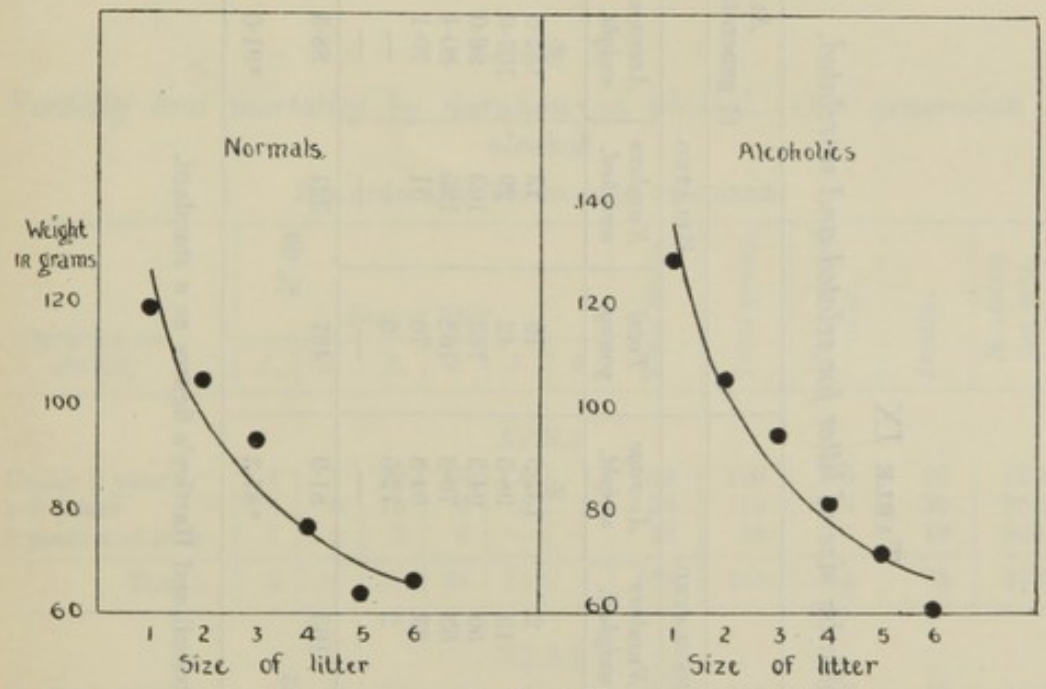

CHART V

Birth-weights of guinea-pigs. Distribution by litter size smoothed by geometric progression.

Chart V shows the trend of mean birth-weights in the two groups smoothed by a geometric progression which seems to describe the data for both controls and alcoholics fairly closely. The actual and graduated values were as follows:

Size of litter.	CONTROLS.		ALCOHOLICS.	
	Mean weight at birth.			
	Actual.	*Graduated.*	*Actual.*	*Graduated.*
1	118·8	126·1	128·5	135·3
2	104·5	97·7	104·9	102·6
3	90·3	84·2	94·0	87·2
4	76·0	75·7	80·7	77·6
5	63·5	69·7	71·0	71·0
6	66·0	65·2	60·6	66·1

When one classifies the guinea-pigs weighed by inbreeding or outbreeding (Table IX), the offspring of related parents appear to be

Table IX

Mean birth-weights by size of litter for related and unrelated.

Size of litter.	Controls.						Alcohols (1 generation of alcohol).					
	Related.			Unrelated.			Related.			Unrelated.		
	Total young.	*Numbers weighed.*	*Average weight.*	*Total young.*	*Numbers weighed.*	*Average weight.*	*Total young.*	*Numbers weighed.*	*Average weight.*	*Total young.*	*Numbers weighed.*	*Average weight.*
			g.			g.			g.			g.
1	9	2	105·0	4	2	132·5	19	13	132·6	5	4	115·0
2	82	40	106·5	20	13	98·5	42	29	107·0	28	22	103·7
3	144	63	87·2	72	50	94·3	162	103	96·0	108	105	92·2
4	120	53	75·6	96	63	76·3	168	125	80·4	124	105	80·0
5	35	17	60·9	55	33	64·8	70	51	70·1	45	26	72·9
6	24	18	65·3	6	2	72·5	6	—	—	36	33	60·6
7	—	—	—	7	—	—	—	—	—	—	—	—
Total	414	193	83·8	260	163	81·9	467	321	88·3	346	295	83·8
	% 47			% 63			% 69			% 76		
	*Weight standardized		83·4			*87·3			*91·0			*88·5

* Using Russell and Hartley's figures as a standard.

rather superior on the average, but there is no definite tendency for the related group to have the advantage when one compares litters of different sizes.

The mean birth-weights standardized for litter size remained relatively unchanged for alcoholics, but the position was reversed in the untreated group, the offspring from unrelated parents having the advantage.

Effect of Duration on Alcohol on Fertility and Mortality.

The data have been analysed to see whether any association exists between duration on alcohol of the parent and size of litter or mortality. The first part of Table X gives the distribution by parental

TABLE X

Fertility and mortality by duration on alcohol. One generation of alcohol.

RELATED AND UNRELATED COMBINED.

Duration on alcohol.	*Size of litter.* 1.	2.	3.	4.	5.	6.	*Total litters.*	*Total young.*	*Mean size of litter.*	*Deaths.*	*Mortality per cent. of all births.*
					A♀ N♂.						
Under 1 year	4	7	22	19	6	1	59	196	3·3	26	13·3
1–2 years	1	6	13	6	5	3	34	119	3·5	30	25·2
2 years and over	1	2	3	6	—	—	12	38	3·2	19	50·0
TOTAL	6	15	38	31	11	4	105	353	3·4	75	21·2
					N♀ A♂.						
Under 1 year	8	6	18	6	4	—	42	118	2·8	17	14·4
1–2 years	1	4	8	5	5	1	24	84	3·5	13	15·7
2 years and over	3	2	7	4	—	—	16	44	2·8	6	13·6
TOTAL	12	12	33	15	9	1	82	246	3·0	36	14·6
					A♀ A♂.						
Under 1 year	3	3	11	11	2	1	31	102	3·3	13	12·7
1–2 years	2	3	5	11	1	1	23	78	3·4	20	25·6
2 years and over	1	2	3	5	—	—	11	34	3·1	9	26·5
TOTAL	6	8	19	27	3	2	65	214	3·3	42	19·6
					ALL ALCOHOLS.						
Under 6 months	8	6	29	18	7	1	69	220	3·2	30	13·6
6–12 ,,	7	10	22	18	5	1	63	196	3·1	26	13·3
12–18 ,,	2	9	18	15	6	3	53	182	3·4	28	15·4
18–24 ,,	2	4	8	7	5	2	28	99	3·5	35	35·4
24–30 ,,	3	3	5	8	—	—	19	56	2·9	17	30·4
30+ ,,	2	3	8	7	—	—	20	60	3·0	17	28·3
TOTAL	24	35	90	73	23	7	252	813	3·2	153	18·8

N = untreated.
A = alcoholic.

alcoholism. For all alcoholics combined, the average litter is never less than 3 up to 2 years on alcohol, over 2 years the average litter is 2·98.

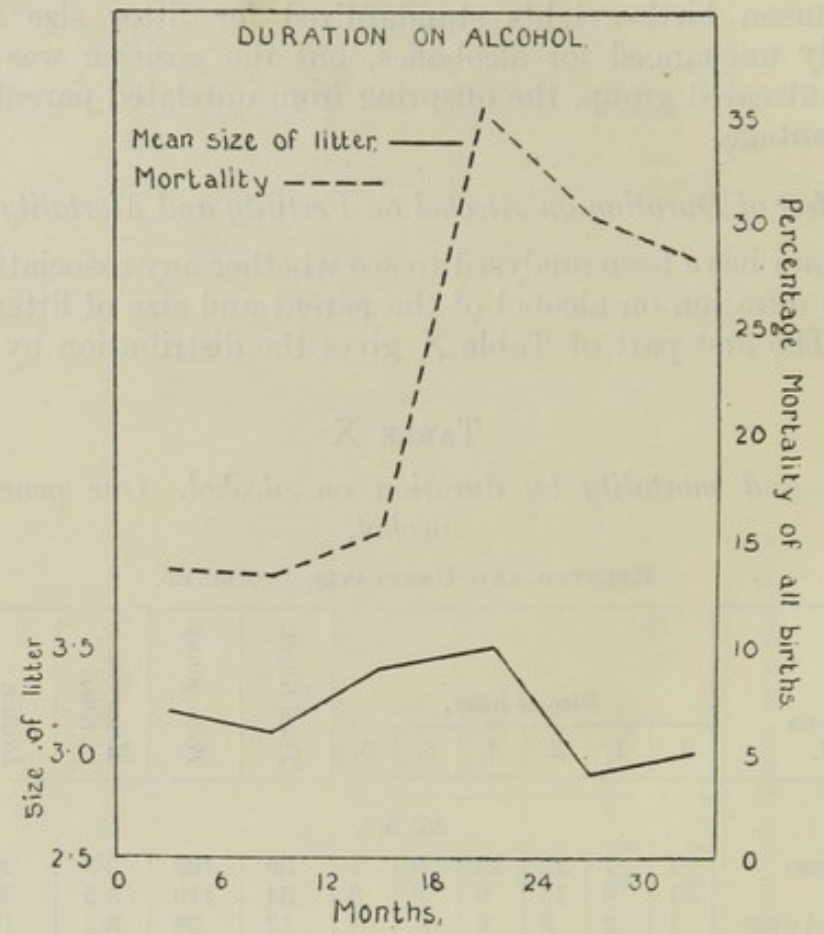

CHART VI

Duration on alcohol seems to be definitely associated with mortality, the longer the parent has been on alcohol the higher the mortality. Under 6 months' treatment the percentage mortality of the young is 13·6, 12–18 months it rises to 15·4, 18–24 there is a heavy increase to 35·4, and after 24 months the average mortality is 30·0 (Chart VI). How much of this rise in mortality is due to advancing age of the parent is not possible for us to determine.

Effect of Alternative Mating with Treated and Untreated Animals.

This is an attempt to correct for possible selection. If the same control females are mated alternatively with treated and untreated males, is there any difference in the records of the two groups? In our data 21 untreated males were mated 111 times with untreated does and 95 times with treated does (Table XI). If alcohol has an injurious action on the germ-cells one would expect some evidence of inferiority in the offspring from the treated guinea-pigs. Using the same males in both groups acts as a control against selection.

The results of mating with untreated does show a slightly lower fertility and a lower mortality than those from matings with treated

TABLE XI

Matings of 21 *control* ♂*'s with control* ♀*'s.*

Age of ♀ *in months.*	*No. of litters.*	*Total young.*	*Deaths.*	*Mis-carriages.*	*Average size of litter.*	*Per cent. mortality.*
0–12	60	183	32	3	3·1	17·5
12–24	33	109	14	—	3·3	12·8
24+	18	62	15	—	3·4	24·2
TOTAL	111	354	61	3	3·2	17·2

Matings of same 21 *control* ♂*'s with alcoholic* ♀*'s.*

Age of ♀ *in months.*	*No. of litters.*	*Total young.*	*Deaths.*	*Mis-carriages.*	*Average size of litter.*	*Per cent. mortality.*	*Expected deaths at normal rates.*
0–12	2	8	1	—	4·0	12·5	1·4
12–24	48	155	15	1	3·2	9·7	19·8
24+	45	159	45	2	3·5	28·3	38·5
TOTAL	95	322	61	3	3·4	18·9	59·7

does, but the differences are not significant with regard to their probable error in either case. The does in the treated group have a higher mean age than the untreated, and, if one corrects for age of does by calculating the expected deaths among alcoholic offspring had they died at the same rate for age of mother as did the young of untreated mothers, the expected deaths would have numbered 59·7 against the actual number of 61.

Sixteen untreated females were mated 55 times with untreated males and 54 times with alcoholic males (Table XII). Here, again,

TABLE XII

Matings of 16 *control* ♀*'s with control* ♂*'s.*

Age of ♀ *in months.*	*No. of litters.*	*Total young.*	*Deaths.*	*Mis-carriages.*	*Average size of litter.*	*Per cent. mortality.*
0–12	25	76	14	—	3·0	18·4
12–24	16	51	7	—	3·2	13·7
24+	14	48	15	—	3·4	31·2
TOTAL	55	175	36	—	3·2	20·6

Matings of same 16 *control* ♀*'s with alcoholic* ♂*'s.*

Age of ♀ *in months.*	*No. of litters.*	*Total young.*	*Deaths.*	*Mis-carriages.*	*Average size of litter.*	*Per cent. mortality.*	*Expected deaths at normal rates.*
0–12	9	27	—	—	3·0	—	5·0
12–24	18	56	7	1	3·1	12·5	7·7
24+	27	91	11	—	3·4	12·1	28·4
TOTAL	54	174	18	1	3·2	10·3	41·1

there is no difference in fertility, but mortality is twice as high in the young from untreated fathers. The mean age of the doe in alcoholic matings is again higher. Had the alcoholic offspring died at the same rate for age of mother as the untreated group, the expected deaths would number 41 against 18 actually recorded.

In both tables the results are quite consistent with those found in Table V for all matings (see comparison by parental alcoholism).

	Mean size of litter.	*Mortality.*	*Difference.*	*Difference compared with all normals.*
Controls, Table V	3·15±0·07	17·5±0·99		
Selected matings, Table XI	3·2 ±0·08	17·2±1·35		
,, ,, Table XII	3·2 ±0·11	20·6±2·06		
All matings, A♀ N♂, Table V	3·36±0·08	21·2±1·47	6·6±2·11	+3·7±1·77
Selected, A♀ N♂, Table XI	3·4 ±0·08	18·9±1·47		+1·4±1·77
All matings, N♀ A♂, Table V	3·00±0·09	14·6±1·52	8·6±2·14	−2·9±1·81
Selected, N♀ A♂, Table XII	3·2 ±0·09	10·3±1·55	-	−7·2±1·84

In both groups, all matings and selected matings, the differences in mortality of offspring from alcoholic mothers and of offspring from alcoholic fathers are significant with regard to their probable errors. The mortality of offspring in the selected group of alcoholic fathers is also significantly lower than that of the controls. One might expect deleterious effects to be more pronounced through a drunken mother, but one would not expect that having an alcoholic father should appear to have such an advantage on the survival of the offspring.

In discussing Table I, it was suggested there was some evidence that the does used first in the control and later in the alcoholic series were selected. The data given here show that the effect of selection, if any selection existed at all, was not important.

The Descendants of Alcoholized Guinea-pigs

This section deals with all the descendants of alcoholized guinea-pigs, F_1, F_2, F_3, and F_4, and the results of alcoholizing members of these series, producing 2 generations of alcohol, 3 generations of alcohol, and 4 generations of alcohol, respectively, and their descendants.

It was not possible to analyse all of these groups as fully as in the case of 1 generation of alcohol, as the number in the separate classes was very small. All the F_3 generations, of whatever origin, have been grouped together, and the same is true for the F_4 generations.

The F_1 of one generation of alcohol (offspring of alcoholic parents) is represented by the formulae $F_1A♀N♂$, $F_1N♀A♂$, and $F_1A♀A♂$ where N = untreated and A = alcoholic parent. Matings have been made in the straight line.

$F_1A♀N♂ \times F_1A♀N♂$, $F_1N♀A♂ \times F_1N♀A♂$, $F_1A♀A♂ \times F_1A♀A♂$, both brother × sister, and unrelated F_1 × unrelated F_1. This gives

rise to the F_2 generation in the straight line. Other matings were made between the different F_1 groups, thus

$F_1A♀N♂ \times F_1N♀A♂$, $F_1A♀N♂ \times F_1A♀A♂$, and $F_1N♀A♂ \times F_1A♀A♂$.

These matings were made both ways, bucks and does of each sort being used in order that the effects of alcohol on either parent or both could be tested. These matings are referred to as F_1 one generation of alcohol various, and the offspring are F_2 one generation of alcohol various. The F_3 and F_4 generations were obtained in similar ways.

It must be noted here that the terms F_1, F_2, F_3, and F_4 are applied strictly to the alcoholic line and not to actual stages in the heredity of the animal. An F_1 animal from alcoholic × control may be as regards its descent an F_2 or F_3 from the original mating of the controls, which were its forbears. Inbred animals were alcoholized as well as outbred.

If, as Stockard stated, alcohol damages the germ-cells, it seems probable that the longer the exposure to alcohol the greater the damage will be. If the effects are inherited then the offspring from the later stages of alcoholism should be worse off than those from the early stages. In order to test this, F_1 young were chosen for breeding purposes at different stages of parental alcoholism. In some cases, the parents had been a few weeks only on alcohol; in others, over two years, and so on.

This was the only kind of selection used; otherwise the animals used for breeding were taken at random as required, without regard to qualities of size and vigour, &c., or litter size. Matings of all kinds were made in order to get as much variety as possible. If alcohol affects germ-cells of succeeding generations, the effects would be more the strikingly shown by using as large a variety as possible than by taking many litters from a few, when it might be argued that selection has been practised. Many of the F_1 animals were used for 2 generations of alcohol, in straight as well as mixed lines. Two generations of alcohol on straight lines are expressed by the formulae

$$\begin{cases} A♀\,N♂ \\ A♀\,\mathbf{N}♂ \end{cases} \quad \begin{cases} N♀\,A♂ \\ \mathbf{N}♀\,A♂ \end{cases} \quad \begin{cases} A♀\,A♂ \\ A♀\,A♂ \end{cases}$$

where N = untreated.

N = untreated F_1 alcoholic descendant.

A = alcohol.

The mixed 2 generations of alcohol were obtained by alcoholizing the male $F_1A♀N♂$ or the female $F_1N♀A♂$ or either male or female $F_1A♀A♂$. The possible combinations for these matings are as follows: Alcoholized ♀ $F_1A♀N♂$ might be mated with untreated ♂ $F_1N♀A♂$ or untreated ♂ $F_1A♀A♂$. Alcoholized ♀ $F_1N♀A♂$ might be mated with untreated ♂ $F_1A♀N♂$ or untreated ♂ $F_1A♀A♂$, and so on. These matings are referred to as 2 generations of alcohol various. Three generations and 4 generations of alcohol were obtained by similar methods and as the numbers in the various classes were few, they have been grouped together under the headings 3 generations and

4 generations of alcohol, respectively. Their descendants are treated in like fashion.

Stockard (1918) expressly states (p. 183) that he chose large, strong specimens, that were particularly good breeders, for alcoholization among his F_1 animals (and presumably for the other generations of alcohol). 'Such a choice has been made on account of the severity of the treatment to which the descendants are to be subjected through a number of generations. Only the best animals are likely to produce descendants sufficiently strong to be treated with alcohol and to continue to reproduce for one generation after another.'

Every breeder is aware that the fact of animals being large and vigorous and good breeders is no sure guide as to what their offspring or descendants may do, when the genetic behaviour of the stock from which they are derived is unknown. Moreover, in outbreeding, new combinations are made at each mating. Stockard has not bred his generations in strictly straight lines from any of the descendants of the original alcoholized stock. His F_1 animals were mated with control, untreated animals instead of with other F_1 animals. His so-called F_2's are out of $F_1 \times$ control, and he mated these also to control, and so on, for all succeeding generations. When he alcoholized an F_1 animal to get 2 generations of alcohol, he mated this also with a control instead of using F_1 untreated. Thus his F_1, F_2, F_3, and F_4 do not really belong to these classes. The young of $F_1 \times$ control is not the equivalent of $F_1 \times F_1$, and the same is true for the other generations.

Our experiments, therefore, cannot be compared with those of Stockard when dealing with the descendants of the original alcoholized stocks. A further difficulty also arises from the method in which Stockard and Papanicolau (1918) have tabulated their results. In Table III, p. 169, they have pooled all their generations together except F_1. Stockard (1923) in Table II, p. 314, gives further results from alcoholics of more than one generation, and again the results are pooled, with the exception of F_1 and F_4. The alcoholic group is a complicated one. In the 1918 (p. 167) report it is stated—'the nearer to the direct alcohol treatment an animal is produced, the more inferior in quality it will be as a result of the high amount of modified germ-plasm contained in the germ-cell complex from which it arises. Therefore, the records of F_1 individuals are worse than the records of the sum of all alcoholic generations. . . . The later generations being further and further removed from the treatment, and having less and less modified germ-plasm on account of the constant introduction of normal stock, are progressively improved until finally the F_4 generation has its modified germ-plasm diluted to such a degree that its record is on a par with the control.'

In order to test this statement we have made as complete an analysis as possible, by dealing with the groups separately, although, as our breeding has proceeded on different lines, the results are not comparable with those of Stockard.

We can, however, make comparisons between our groups. For

instance, the results from 2 generations of alcohol can be compared with those from F_1 of 1 generation of alcohol, those from 3 generations of alcohol with F_2 of 1 generation of alcohol, those from 4 generations of alcohol with F_3 from 1 generation of alcohol, since the groups follow the same lines. Some allowance must be made for outbreeding when it has occurred, since the effect of inbreeding becomes noticeable after the first generation. We have, however, bred a certain number of F_1 and F_2 animals with control untreated, unrelated animals, and these will be dealt with separately.

Two Generations of Alcohol.

In Table XIII it will be seen that in the straight lines the combined results from F_1 one generation do not differ markedly from those of 2 generations of alcohol. The mean size of the litters is for F_1, 3·1, and for 2 generations of alcohol, 3·3. The percentage mortality for 2 generations of alcohol is considerably higher, 21·1 against 13·3, but it must be noted that the average age of the alcoholized does in this group was above that of the does used in the F_1 experiments. In the case of 2 generations of alcohol various, the mortality is 14 per cent. The litter size and mortality are in no instance significantly different from those for control, untreated animals, but there are some instances in which the birth-weights do differ significantly from the controls, for example in offspring from N♀A♂, all alcohols, F_1 A♀A♂ and $\frac{\text{A♀ A♂}}{\text{A♀ A♂}}$ the mean birth-weights are significantly greater.

Three Generations of Alcohol.

Three generations of alcohol in the straight line are compared in Table XIV with F_1 two generations of alcohol and F_2 one generation of alcohol. There is no difference in the mean size of litter between the three groups. The mortality rate is higher for 3 generations (20·9 per cent.) than for the other two groups (15·9 per cent. and 16·5 per cent., respectively). But here the age of the doe must be taken into consideration because in the F_1 two generations of alcohol and F_2 one generation of alcohol the does used were young, whereas in the 3 generations of alcohol nearly 50 per cent. were 2 years old or more.

The average birth-weight for 3 generations of alcohol is 88 g. which is significantly higher (8·7 $\pm$ 1·13 g.) than for the other two groups (79·2 g. and 79·3 g., respectively). This would suggest that the effect of alcohol has been to counterbalance the effect of inbreeding as regards birth-weight. When the results from 1 generation, 2 generations, and 3 generations of alcohol are compared, it is impossible to show any striking differences between them. The mortality rate, when the buck only is alcoholized for 1 generation (Table XIII), is 14·6 per cent., for 2 generations it is 17·3 per cent., and for 3 generations 25 per cent., which looks like a progressive increase as the result of

TABLE XIII

Comparison between controls, 1 generation of alcohol. F_1 one generation and 2 generations of alcohol.

	Number of litters.	*Total offspring.*	*Mean size of litter.*	*Dead births and post-natal deaths.*	*Per cent. mortality of total births.*	*Number of mis-carriages.*	*Sex-ratio at birth.*	*Number weighed.*	*Mean birth-weight of guinea-pig.*	*Probable error of difference in weights from controls.*
									g.	g.
Controls N♀ N♂	214	674	3·15±0·07	118	17·5±0·99	5	98	356	83·1±0·75	
N♀ A♂	82	246	3·00±0·09	36	14·6±1·52	—	107	176	90·8±1·19	+7·7±1·41
A♀ N♂	105	353	3·36±0·08	75	21·2±1·47	2	101	270	84·2±0·76	+1·1±1·07
A♀ A♂	65	214	3·29±0·10	42	19·6±1·83	—	99	170	84·4±0·99	+1·3±1·24
All alcohols	252	813	3·23±0·05	153	18·8±0·92	2	102	616	86·1±0·56	+3·0±0·94
F_1 N♀ A♂	117	363	3·1 ±0·07	47	12·8±1·19	—	107	307	79·8±0·80	−3·3±1·09
F_1 A♀ N♂	93	307	3·3 ±0·08	41	13·4±1·31	3	92	277	79·7±0·94	−3·4±1·20
F_1 A♀ A♂	77	217	2·8 ±0·10	30	13·8±1·58	4	105	194	88·9±0·65	+5·8±0·99
TOTAL	287	887	3·1 ±0·05	118	13·3±0·77	7	101	778	82·0±0·54	−1·1±0·92
F_1 various	73	245	3·4 ±0·09	37	15·1±1·54	1	144	210	82·5±0·76	−0·6±1·07
N♀ A♂ **N**♀ A♂	66	214	3·2 ±0·09	37	17·3±1·74	3	125	186	82·3±1·08	−0·8±1·31
A♀ N♂ A♀ **N**♂	21	76	3·6 ±0·15	19	25·0±1·06	1	73	64	80·5±1·46	−2·6±1·64
A♀ A♂ A♀ A♂	27	90	3·3 ±0·14	24	26·7±1·00	—	135	70	91·1±1·48	+8·0±1·66
TOTAL	114	380	3·3 ±0·07	80	21·1±1·40	4	115	320	83·9±0·74	+0·8±1·05
2 gen. alc. var.	116	397	3·4 ±0·08	56	14·1±1·18	2	104	351	83·1±0·76	

Table XIV

Comparison of 3 generations of alcohol with F_2 one generation of alcohol, and F_1 two generations of alcohol.

Three generations alcohol.	*Number of litters.*	*Total offspring.*	*Mean size of litter.*	*Dead births and post-natal deaths.*	*Per cent. mortality of total births.*	*Number of Mis-carriages.*	*Sex-ratio at birth.*	*Weights at birth.*	
								Number weighed.	*Mean weight.*
									g.
A♀ N♂ A♀ **N**♂ A♀ **N**♂	14	48	3·4	10	20·8	2	67	44	83·1
N♀ A♂ **N**♀ A♂ **N**♀ A♂	35	100	2·9	25	25·0	5	107	79	90·0
A♀ A♂ A♀ A♂ A♀ A♂	25	82	3·3	13	15·9	1	129	72	89·1
Totals	74	230	3·1	48	20·9	8	103	195	88·0±0·92
Three generations various	39	130	3·3	27	20·8	1	89	101	96·3±1·22
F_2 one generation of alcohol.									
F_2 N♀ A♂	59	162	2·7	35	21·6	2	111	143	76·9
F_2 A♀ N♂	36	113	3·1	18	15·9	1	130	94	85·1
F_2 A♀ A♂	31	101	3·3	9	8·9	1	106	96	77·2
Totals	126	376	3·0	62	16·5	4	115	333	79·3±0·65
F_2 one generation of alcohol various	28	82	2·93	3	3·6	—	105	74	83·56
F_1 two generations of alcohol.	96	295	3·1	47	15·9	5	113	274	79·2±0·65

continued alcoholization. When the size of the sample is taken into account the differences in the mortality rate are significant for the 1st and 3rd generation. When the doe only is alcoholized for 1 generation (Table XIII) the rate is 21·2 per cent., for 2 generations 25 per cent., and for 3 generations 20·8 per cent., showing no increase of mortality for increase of alcoholization. When both buck and doe are alcoholized for 1 generation the mortality rate is 19·6 per cent., for 2 generations 26·7 per cent., and for 3 generations 15·9 per cent.

There is nothing to show, therefore, that alcoholizing the male gives worse results than alcoholizing the female; for when both male and female are treated the results should be worse, or at any rate, as bad as, when the buck only is treated, whereas in the 3 generations of alcohol, the mortality rate for both sexes treated is as low as the rate for does only in F_2 one generation of alcohol.

Four Generations of Alcohol.

There were only 20 litters in this group (Table XV). The mean litter size was 3·8, mortality was low, 9·3 per cent., sex-ratio only 63, and mean litter weight 76·9 g. The proportion of females was exceptionally high, and as would be expected the birth-weight was low. The evidence as far as it goes shows no harmful effects from continued alcoholization for 4 generations.

The groups which may be compared with 4 generations of alcohol are F_3 one generation of alcohol, F_2 two generations of alcohol, F_1 three generations of alcohol. Fertility appears to be much the same in these groups but mortality is higher and ranges from 15·6 in the F_2 two generation group to 22·1 in F_1 three generations of alcohol. There is considerable variation in the sex-ratios; in F_3 one generation of alcohol, it is as high as 144. The birth-weight in this group is the lowest in the series.

With such a high proportion of males the mean birth-weight is exceptionally low, and it must be concluded that the offspring of F_3 one generation of alcohol showed signs of physical inferiority compared with the other groups.

F_4 various group includes all F_4 animals from 1 and 2 generations of alcohol. The numbers in each group were too small for separate classification. The mean size of litter was 3·2. It is perhaps of interest to record that among the litters there was one of 9; three of the young were born alive but subsequently died. Nine is a record for guinea-pigs, Sewall Wright gives 8 as his maximum litter. Mortality was high, 30·3 per cent., sex-ratio 112, and the mean birth-weight 78·3 g. Stockard claims that his F_4 generation showed a recovery from the effects of alcohol on the ancestry. Unfortunately his data are not comparable with ours as he mated each successive generation with controls. Our figures show some deterioration with regard to mortality. If the litter of 9, all of whom died, is omitted the mortality is still rather high, 26 per cent.

TABLE XV

	Number of litters.	*Total offspring.*	*Mean size of litter.*	*Deaths.*	*Mortality percentage.*	*Mis-carriages.*	*Sex-ratio.*	*Number weighed.*	*Average birth-weight.*
									g.
Four generations of alcohol	20	75	3·8	7	9·3	—	63	72	76·9±1·32
F_3 one generation of alcohol	39	121	3·1	24	19·8	2	144	113	75·6±1·16
F_2 two generations of alcohol	67	199	3·0	31	15·6	2	85	184	78·5±1·35
F_1 three generations of alcohol	125	399	3·2	88	22·1	7	114	330	82·2±0·69
F_4 various	45	142	3·2	43	30·3	5	112	114	78·3±0·99

Results of Outbreeding

$F_1 \times$ *Control.*

Some F_1 animals were mated with controls. This was done both ways. F_1♀ × control ♂ and control ♀ × F_1♂. The F_1 animals used were of various origin from 1, 2, or 3 generations of alcohol. They have been grouped together as the numbers are too small for separate handling. These matings are all outbred, since none of the F_1 animals were related to the controls. One hundred and fifty-nine young were produced, giving an average litter size of 3·26, mortality per cent. 4·5, and average birth-weight $84{\cdot}6 \pm 0{\cdot}94$ g. These animals did not appear to be in any way inferior to the results from control mated to control.

Fertility and Mortality in Different Generations of Alcohol analysed by Age of Female and by Duration on Alcohol

Table XVI

Fertility and mortality by age of ♀'s.

Age of ♀'s in months.	*Size of litter.*							*Miscarriage.*	*Total litters.*	*Total young.*	*Mean size of litter.*	*Deaths.*	*Per cent. mortality.*
	1.	*2.*	*3.*	*4.*	*5.*	*6.*	*7.*						
1 Generation of Alcohol.													
0–12	—	—	6	—	2	—	—	—	8	28	3·5	2	7·1
12–24	13	14	36	36	9	1	—	1	109	344	3·2	46	13·4
24–36	6	13	32	23	9	5	—	1	88	295	3·4	58	19·7
36+	5	8	16	14	3	1	—	—	47	146	3·1	47	32·2
Total	24	35	90	73	23	7	—	2	252	813	3·2	153	18·8
F_1 One Generation of Alcohol.													
0–12	15	36	77	45	11	3	1	4	188	578	3·1	74	12·8
12–24	8	21	27	20	8	2	1	2	87	270	3·1	31	11·5
24–36	2	1	3	4	2	—	—	1	12	39	3·3	13	33·3
36+	—	—	—	—	—	—	—	—	—	—	—	—	—
Total	25	58	107	69	21	5	2	7	287	887	3·1	118	13·3
Two Generations of Alcohol.													
0–12	—	—	1	1	1	—	—	—	3	12	4·0	2	16·7
12–24	2	7	18	18	6	1	—	—	52	178	3·4	34	19·1
24–36	2	3	13	16	5	1	—	4	40	142	3·6	31	21·8
36+	5	3	7	4	—	—	—	—	19	48	2·5	13	27·1
Total	9	13	39	39	12	2	—	4	114	380	3·3	80	21·1
Three Generations of Alcohol.													
0–12	2	1	—	—	—	—	—	—	3	4	1·3	—	—
12–24	3	6	11	13	3	1	—	6	37	121	3·3	15	12·4
24–36	3	6	8	11	3	—	—	2	31	98	3·2	31	31·6
36+	—	2	1	—	—	—	—	—	3	7	2·3	2	28·6
Total	8	15	20	24	6	1	—	8	74	230	3·1	48	20·9

Table XVI is an analysis of the data relating to different generations of alcohol by age of ♀'s. There is again some indication that maximum fertility occurs at about 2½ years. It will be seen that fertility is maintained up to this age in the subsequent generations of alcohol. Mortality definitely increases with advancing age of ♀, but there is no evidence of a greater increase in the later generations of alcohol.

Table XVII shows the trend of fertility and mortality by duration on alcohol. Fertility tends to fall after the parent has been 18 months

TABLE XVII

Fertility and mortality by duration on alcohol.

Duration on alcohol in months.	*Size of litter.*						*Miscarriage.*	*Total litters.*	*Total young.*	*Mean size of litter.*	*Deaths.*	*Mortality per cent. of all births.*
	1.	*2.*	*3.*	*4.*	*5.*	*6.*						
1 GENERATION OF ALCOHOL.												
0–6	8	6	29	18	7	1	—	69	220	3·2	30	13·6
6–12	7	10	22	18	5	1	—	63	196	3·1	26	13·3
12–18	2	9	18	15	6	3	—	53	182	3·4	28	15·4
18–24	2	4	8	7	5	2	—	28	99	3·5	35	35·4
24–30	3	3	5	8	—	—	—	19	56	2·9	17	30·4
30+	2	3	8	7	—	—	—	20	60	3·0	17	28·3
TOTAL	24	35	90	73	23	7	—	252	813	3·2	153	18·8
2 GENERATIONS OF ALCOHOL.												
0–6	—	3	9	14	2	—	—	28	99	3·5	18	18·2
6–12	1	2	7	4	3	1	1	18	63	3·5	11	17·5
12–18	1	1	5	9	1	—	1	17	59	3·5	16	27·1
18–24	2	3	9	5	—	—	2	19	55	2·9	5	9·1
24–30	4	1	4	1	2	—	—	12	32	2·7	12	37·5
30+	1	3	5	6	4	1	—	20	72	3·6	18	25·0
TOTAL	9	13	39	39	12	2	4	114	380	3·3	80	21·1
3 GENERATIONS OF ALCOHOL.												
1–6	3	6	4	7	2	1	1	23	71	3·1	7	9·9
6–12	3	3	7	8	1	—	5	22	67	3·0	12	17·9
12–18	1	1	5	6	2	—	1	18	52	2·9	20	38·5
18–24	1	3	3	3	1	—	1	8	33	4·1	7	21·2
24–30	—	1	—	—	—	—	—	1	2	2·0	—	—
30+	—	1	1	—	—	—	—	2	5	2·5	2	40·0
TOTAL	8	15	20	24	6	1	8	74	230	3·1	48	20·9

on alcohol and mortality becomes high, but the effect is no worse in 3 generations of alcohol than in 1 generation.

SEX-RATIO

Very little is known about the causes which affect sex-ratio in normal life. MacDowell and Lord (1925) are of opinion that there is no correlation between the amount of pre-natal mortality and the

sex-ratio at birth in mice, and that the primary ratio at conception is almost identical with the ratio at birth.

Parkes (1926) criticizes their findings, since from earlier experiments (1923) he found that pre-natal mortality falls more heavily on the male sex, but he admits that more information is required before a coherent story of the factors governing sex-ratio can be told. Litter size would appear to be uncorrelated with any definite variation in sex-ratio. Sewall Wright (1922) inbred guinea-pigs for 13 years, and showed that there were no significant fluctuations from year to year in sex-ratio, in spite of a diminished fertility due to inbreeding. The sex-ratio for the inbred animals and for the control outbred animals showed no appreciable difference.

MacDowell and Lord (1925, 1927) deal with the effect of alcohol on sex-ratio in mice. Their experiments were controlled as far as possible to meet suspected influences on the sex-ratio. In all cases any modifications which might be due to alcohol were small, and about the low limit of significance. They concluded that as long as the actual causes of normal variation in sex-ratio are undetermined it is impossible to know when they are experimentally controlled. Even when the enquiry is planned to meet suspected influences, the causal relations may be undisclosed.

Hanson and Heys (1925) used rats. Their data cover 10 successive generations of control and alcoholic animals. All the animals used, both for control and experiment, were bred from a single pair, and comparisons were made generation by generation from both control and treated animals. They used 3,123 animals in all, and all the matings were sister and brother. Alcohol was given by the fume method. They concluded that, taken generation by generation, the evidence was overwhelming against any modification of sex-ratio by alcohol.

Inspection of the sex-ratio for 10 successive generations did not show a tendency to a higher ratio for the later generations as might be expected, if alcohol were adversely affecting the female-producing sperm. The sex-ratios of the treated were rather higher in the earlier generations, though not significantly higher than those of the controls. Any shift of the sex-ratio by means of alcohol must have taken place during the first 5 generations at most. But if it occurred, it was lost during the last 5 generations. The authors point out that if the modification were an actual one, it would be difficult to see how the subsequent loss could have taken place, as generations 6–10 were treated daily, and should have either retained the gain or even increased it, but this did not occur.

Bluhm (1921) used mice and gave alcohol as a subcutaneous injection to the males only. Her mice were intensively inbred, and the control and treated animals were derived from the same stock. She got a high sex-ratio for her controls, and a low one for the treated animals. The descendants of the treated animals were inbred for 7 generations, and also showed a low sex-ratio. She considered that alcoholization of the male had a lasting and inherited influence on sex-ratio. Her results are, therefore, not in accord with the findings

of MacDowell and Lord, and of Hanson and Heys. The explanation of this discord may be that she had not really established a homozygous race, and that her controls were not genetically similar to the treated animals.

Our results (in Table V) are not in accord with those of Stockard (1918, p. 188).

	Durham & Woods.	*Stockard.*
Control untreated	98	113
Father alcoholic	107	102
Mother ,,	101	97
Both alcoholic	99	121

Stockard maintains that alcohol acts differently on the male- and female-producing sperm, that, when both parents are alcoholic, the female cells suffer most, and therefore the pre-natal mortality was mainly female. Our figures do not support this. In Table II it was shown that inbreeding appeared to affect the sex-ratio, and as our animals were more closely inbred than Stockard's one would have expected a higher sex-ratio in our stock than in his. Moreover, Hanson and Heys (1925) have tested Stockard's data and calculated the probable error of each sex-ratio percentage for the 6 groups of treated guinea-pigs and compared the results with the controls. They showed that alcoholic fumes cannot be said to have modified the sex-ratio in guinea-pigs.

There is very little known about the effect of the age of the parent on the sex of the offspring. Sewall Wright (1926) states that sex-ratios show no reliable changes as a result of increasing age of the parents, although a slight excess of males (above the average ratio) in the first litters is in harmony with human statistics as far as it goes. In our experiments many guinea-pigs have been alcoholized for over 2 years, and they can therefore no longer be considered as young animals. The sex-ratio, derived from such material, may be influenced not so much by alcohol as by age.

King (1924) found that young female rats (3–7 months old) tend to produce litters with an approximately equal proportion of sexes. The relative number of young ♂'s increases with the age of mother up to 12–14 months old. An abrupt and striking change then appears in the sex-ratio, ♀'s tending to predominate in all the subsequent litters. The maximum sex-ratio is attained at a time when the mothers have passed the zenith of reproductive activity and fertility is lessening, as shown by fewer and smaller litters. According to King's suggestion it is possible that the factor involved in the change in sex-ratio, at this time, causes differential mortality in the sexes among the foetuses. If so, it acts at an early period of gestation, and the foetuses are absorbed in situ, since there is no marked increase in still-births at this time. Males are largely in excess of females among still-born regardless of the age of the mother.

In our data the sex-ratio for 1 generation of alcohol showed no significant variation from that of the control, 102 against 98. In Table II it was shown that inbreeding appeared to affect the

sex-ratio; there was a rise in the proportion of males when the parents were related. Both control and alcoholic animals showed an excess though the rise was more accentuated in the treated group.

	Sex-ratio.
Unrelated controls	85±7·71[1]
,, alcoholics	86±6·49
Related controls	107±7·72
,, alcoholics	117±7·89

As the difference between the related controls and alcoholics is not statistically significant, it seems unlikely that alcohol played any part in altering the sex-ratio.

The sex-ratio for all F_1 animals for 1 generation of alcohol was almost equality, but for F_2 animals it rose to 115, and it was as high as 144 for the F_3's.

	Sex-ratio.
1 generation of alcohol	102± 5·11
F_1 ,, ,,	101± 4·75
F_2 ,, ,,	115± 8·31
F_3 ,, ,,	144±18·25

It would appear from these figures that alcohol results in raising the proportion of males, but the numbers are small in the F_3 group, and the differences between each group are all within their probable errors.

When we compare the sex-ratios for 2 generations of alcohol the tendency is for females to be in excess in the F_2 and F_3 groups, but again the numbers in these groups are small and the differences are in no instance significant.

2 generations of alcohol	115± 8·53
F_1 ,, ,,	113± 9·01
F_2 ,, ,,	85± 9·32
F_3 ,, ,,	89±13·04

The sex-ratio for 3 generations of alcohol starts at equality and rises to 114 in F_1 animals.

3 generations of alcohol	103±9·58
F_1 ,, ,,	114±8·08

A comparison has been made of the sex-ratios in different generations by parental alcoholics.

One generation of alcohol.

	Sex-ratio.	*Differences.*
N♀A♂	107± 9·80	
A♀N♂	101± 7·70	
A♀A♂	99± 9·57	9±10·6
F_1 N♀A♂	107± 7·97	
F_1 A♀N♂	92± 7·24	
F_1 A♀A♂	106±10·12	38±19·1
F_2N♀A♂	111±12·24	
F_2A♀N♂	130±17·61	
F_2A♀A♂	106±14·38	

[1] The probable errors throughout this section have been calculated from the formula $(1+Z)\sqrt{\frac{Z}{N}}$ where Z = the ratio. (See Yule, *Theory of Statistics*, xiii, Ex. 11).

There was no change among the young of alcoholized fathers. When the mother was alcoholized the sex-ratio fell from equality to 92 in the F_1 animals, and in the next generation males were in excess, 130, but there were only 113 young in the F_2 A♀ N♂ group, and the probable error from such a sample is large. The differences are not statistically significant. When both parents are on alcohol there is no evidence of any important change in the sex-ratio.

To test the effect of time exposure to alcohol a further analysis has been made in the alcoholics. Here the alcoholics are arranged according to length of time of treatment, the related and unrelated groups being classed together.

Treatment for 1–12 months of all alcoholics (1 generation of alcohol), sex-ratio 114.
Treatment for 13–24 months of all alcoholics (1 generation of alcohol), sex-ratio 92.
Treatment for 25 months and over of all alcoholics (1 generation of alcohol), sex-ratio 90.

As stated previously the question of age of the parent must be taken into account in dealing with these ratios, since animals treated for 2 years and upwards are no longer young. These figures therefore ought to be compared with results taken for controls of corresponding ages. Unfortunately we are unable to do this, as insufficient controls of the right ages were used.

If length of time of exposure to alcohol materially affects the sex-ratio and this effect is transmitted to the offspring and descendants, analysis of the sex-ratios of F_1 and F_2 on this basis should show it.

In Table XVIII, figures for F_1 and for F_2 are given.

F_1 ancestor on alcohol	1–12 months	Sex-ratio	94 ± 6·52
,, ,,	13–24 ,,	,,	114 ± 8·49
,, ,,	25–36 ,,	,,	112 ± 13·6
,, ,,	37 months and over	,,	100 ± 10·6
F_2 ancestor on alcohol	1–12 months	Sex-ratio	113 ± 16·5
,, ,,	13–24 ,,	,,	81 ± 11·4
,, ,,	25–36 ,,	,,	160 ± 25·1
,, ,,	37 months and over	,,	89 ± 10·4

Comparison of these figures with those from the alcoholics themselves given above shows that there is no evidence of inheritance of any result which might be attributed to the effect of length of time of exposure to alcohol.

The Effect of Time of Alcoholization of Ancestors on Subsequent Generations

The F_1's and F_2's from 1 generation of alcohol have been studied further to see the effect of time of alcoholization of the ancestors. The F_2 animals are the offspring of the F_1 generation and therefore grandchildren of the original alcoholized stock. Four periods have been taken in each case, i.e. parents on alcohol 1–12 months inclusive, 13–24 months, 25–36 months, and 37 months and over, and similarly for the next generation, the grandchildren.

TABLE XVIII

The effect of time of alcoholization of ancestors on subsequent generations.

F_1 ONE GENERATION OF ALCOHOL.

Parents on alcohol in months.	*Number of offspring.*	*Average litter size.*	*Sex-ratio.*	*Mortality per cent.*	*Number weighed.*	*Average birth-weight.*
1–12	414	3·19	94	13·0±1·1	365	84·7 g.
13–24	430	3·12	114	12·8±1·1	407	82·5 „
25–36	127	3·4	112	19·7±2·4	119	81·8 „
37+	161	3·5	100	13·0±1·8	97	81·3 „

F_2 ONE GENERATION OF ALCOHOL.

Grandparent on alcohol in months.						
						g.
1–12	123	3·09	113	11·2±1·9	109	80·5±1·34
13–24	103	2·86	81	14·6±2·4	82	81·2±1·24
25–36	84	2·9	160	22·6±3·1	66	75·2±1·37
37+	148	2·96	89	11·6±1·8	131	80·3±1·04

In the F_1 one generation of alcohol there is no striking difference between the 4 groups. Mortality is highest in the 25–36 group, but not significantly higher than in the other groups. It would not appear that the offspring of parents over 3 years on alcohol have produced worse young than those whose parents were on alcohol 1 year or less.

In the next generation mortality is again highest for the 25–36 group, being 22·6 per cent. This figure is significantly higher than the rates for the 1–12 group (11·4±3·6) and the 37 months and over group (11·0±2·6). In the F_1 generation the differences were all within their probable errors. If the effect of long alcoholization causes an increase of mortality among the offspring, which is inherited by later generations, then the highest rate of mortality should be shown by the descendants of the 36+ group. But this is not the case.

It is unfortunate that we have not sufficient animals in the F_3 generation to be analysed in the same way. We are unable to say whether this apparent increase in mortality among descendants of the 25–36 group is shown in generations later than F_2. The results from 2 or 3 generations of alcohol cannot be arranged in comparable groups owing to the varying times of alcoholization of the animals concerned.

COMPARISON BETWEEN THE RATES OF GROWTH IN CONTROL AND ALCOHOLIC STOCK

Stockard chose the weights at birth, at 1 month, and at 3 months for comparison in estimating the rates of growth. But it must be remembered that a guinea-pig does not attain its full weight until

about a year old; and the weight at 3 months is not necessarily a guide to the weight it will acquire later. Thus a guinea-pig which weighs 500 g. at 3 months may weigh less than a kilo when a year old, while another guinea-pig, which weighs less than 500 g. at 3 months, may weigh over 1 kilo when it has reached its full weight.

The weights at birth, 1 month and 3 months, are compared for offspring of control and the 1st generation of alcoholized stock. The weight of young animals increases, of course, with age, but the rate of increase slackens as the animal reaches maturity. The equation which describes the curve of growth is therefore curvilinear, not linear, although within certain limits the linear equation gives a close approximation. In the data analysed the guinea-pigs were weighed up to maturity, i.e. 3 months of age, and are outside the range in which a linear assumption would be valid. The rates of growth have accordingly been calculated from the growth curve, that is, by geometric progression. It will be seen in Table XIX that with three exceptions the weights of the alcoholic stock on the average are superior to those of the control at every period whether one considers small or large litters. In the 1st month of life the alcoholic stock showed on the whole the advantage as regards daily rate of growth. This advantage appears to be lost later. From 1 month to maturity the control stock had a somewhat higher rate of growth but the numbers weighed in the various litters at 3 months were in most cases very small.

In Tables XX–XXII a similar study of weights and rates of growth has been made for various generations of alcoholized stock. There is some evidence of deterioration in weight in the descendants of alcoholized stock. Take for example the data relating to the offspring from 1 generation of alcohol and compare the results for litters of 3 and 4 (the groups in which the largest number of weights were made). The figures in brackets are the percentage rates of growth per day.

Size of litter. 3.

	Birth.	*1 month.*	*3 months.*
1 gen. alc.	94·1±0·8 (3·6)	265±3·3 (1·0)	489± 5·2
F_1 ,,	87·2±0·5 (3·3)	236±2·5 (1·1)	460± 4·7
F_2 ,,	82·7±0·8 (3·4)	227±4·0 (1·1)	449± 7·1
F_3 ,,	80·9±1·6 (3·3)	214±3·6 (1·4)	496±14·8

Size of litter. 4.

1 gen. alc.	80·2±0·7 (3·7)	230±2·8 (1·3)	467± 4·2
F_1 ,,	76·1±0·7 (3·4)	210±3·3 (1·2)	423± 4·8
F_2 ,,	73·6±0·8 (3·4)	200±3·0 (1·3)	432± 7·4
F_3 ,,	71·3±1·6 (3·6)	206±4·6 (1·2)	413±10·5

In almost every case there is a reduction in mean weight with each successive generation. The same is true when one compares the weights of the ancestors of 2 generations of alcohol. The weights at

Table XIX

Comparison between rate of growth in controls and 1 generation of alcohol.

Size of litter.	Birth.				1 Month.				3 Months.			
	Controls.		*Alcohols (1 generation).*		*Controls.*		*Alcohols (1 generation).*		*Controls.*		*Alcohols (1 generation).*	
	Number weighed.	*Mean weight.*	*Number weighed.*	*Mean weight.*	*Number weighed.*	*Mean weight.*	*Number weighed.*	*Mean weight.*	*Number weighed.*	*Mean weight.*	*Number weighed.*	*Mean weight.*
		g.		g.		g.		g.		g.		g.
1	4	118·8±5·13	17	128·5±3·03	2	309±17·2	14	329±11·9	1	555	6	579±18·5
2	53	104·5±1·48	51	105·6±1·57	18	266± 7·8	36	298± 8·2	12	520±17·8	24	502±13·6
3	113	90·8±1·40	208	94·1±0·76	68	250± 4·1	147	265± 3·3	54	503± 6·4	99	489± 5·2
4	116	76·0±0·83	230	80·2±0·67	69	203± 5·1	142	230± 2·8	69	403± 7·4	114	467± 4·2
5	50	63·5±1·30	77	71·0±1·14	22	191± 6·0	60	204± 4·7	15	429± 6·7	41	454± 8·8
6	20	66·0±1·40	33	60·6±1·40	11	196±11·3	15	200± 5·4	9	412±11·4	15	423±10·7

	Rate of growth per day from birth to 1 month.		*Rate of growth per day from 1 month to 3 months.*	
	Controls. per cent.	*Alcohols. per cent.*	*Controls. per cent.*	*Alcohols. per cent.*
1	3·31	3·17	0·98	1·05
2	3·15	3·51	1·12	0·87
3	3·43	3·61	1·27	1·03
4	3·33	3·68	1·15	1·29
5	3·71	3·68	1·36	1·34
6	3·70	4·04	1·25	1·26

Table XX

Comparison between F_1 one generation of alcohol, 2 generations of alcohol.

Size of litter.	F_1 *one gen. of alcohol. Birth-weight.*		*2 gen. of alcohol. Birth-weight.*		F_1 *one gen. of alcohol. 1 month.*		*2 gen. of alcohol. 1 month.*		F_1 *one gen. of alcohol. 3 months.*		*2 gen. of alcohol. 3 months.*	
	Number weighed.	*Average weight.*	*Number weighed.*	*Average weight.*	*Number weighed.*	*Average weight.*	*Number weighed.*	*Average weight.*	*Number weighed.*	*Average weight.*	*Number weighed.*	*Average weight.*
		g.		g.		g.		g.		g.		g.
1	16	116·7±2·48	4	125·0±2·16	4	259±34·82	1	270	2	430±14·31	—	—
2	98	101·1±1·65	25	107·0±2·05	44	277± 5·26	16	283±7·33	28	469±11·16	14	506±12·19
3	283	87·2±0·50	105	93·6±1·08	162	236± 2·49	56	241±4·09	122	460± 4·68	44	442± 8·76
4	244	76·1±0·69	131	79·7±0·92	121	210± 3·26	56	236±4·09	89	423± 4·81	46	444± 6·31
5	96	66·9±0·84	44	63·2±1·47	44	197± 6·60	33	163±5·33	32	425±10·33	29	410±10·76
6	29	58·6±1·34	11	55·0±1·37	9	164± 3·46	10	162±6·93	8	410±11·37	10	377± 8·01
7	12	63·9±1·14	—	—	—	—	—	—	—	—	—	—

Rates of growth.

per day.

F_1 *one gen. of alcohol. Birth–1 month.*	*2 gen. of alcohol. Birth–1 month.*	F_1 *one gen. of alcohol. 1–3 months.*	*2 gen. of alcohol. 1–3 months.*
%	%	%	%
2·74	2·36	0·85	—
3·26	3·56	0·88	0·97
3·27	3·12	1·12	1·02
3·40	3·62	1·17	1·06
3·56	2·96	1·29	1·55
3·47	3·43	1·54	1·42

TABLE XXI

Comparison between rates of growth of F_2 one generation of alcohol, F_1 2 generations of alcohol, 3 generations of alcohol.

Size of litter.	Birth-weights. F_2 one gen. of alcohol. Number weighed.	Mean weight.	F_1 two gen. of alcohol. Number weighed.	Mean weight.	3 gen. of alcohol. Number weighed.	Mean weight.	Rates of Growth. Birth–1 month. F_2 one gen. alcohol.	F_1 two gen. alcohol.	3 gen. alcohol.
		g.		g.		g.	%	%	%
1	14	108·2 ± 2·27	26	113·3 ± 2·28	6	107·5 ± 6·92	3·68	3·17	2·74
2	74	94·7 ± 1·35	107	100·6 ± 1·12	36	107·9 ± 1·48	3·32	3·01	3·21
3	160	82·7 ± 0·75	212	86·4 ± 0·71	85	99·0 ± 1·11	3·41	3·16	3·22
4	178	73·6 ± 0·83	216	71·2 ± 0·68	132	85·2 ± 0·93	3·37	3·56	3·26
5	38	65·1 ± 1·46	138	66·5 ± 0·88	31	75·9 ± 1·92	3·40	3·92	3·39
6	9	67·8 ± 2·51	32	54·1 ± 4·44	6	55·9 ± 1·26	3·96	3·92	4·96
		Weights at 1 Month.						1–3 Months.	
1	4	319 ± 17·79	3	288 ± 25·90	2	243 ± 7·75	0·97	0·80	0·66
2	53	253 ± 5·04	33	246 ± 6·61	25	281 ± 6·82	1·00	0·99	0·91
3	72	227 ± 4·04	49	219 ± 4·69	82	259 ± 4·81	1·14	1·04	0·95
4	74	200 ± 3·04	37	203 ± 4·48	83	225 ± 2·90	1·29	1·18	1·12
5	17	177 ± 7·42	19	212 ± 8·82	35	209 ± 4·59	1·40	1·22	1·07
6	5	218 ± 6·92	12	171 ± 6·31	3	239 ± 6·53	1·29	1·38	0·96
		Weights at 3 Months.							
1	4	571 ± 23·88	3	465 ± 2·07	1	360			
2	29	458 ± 8·11	25	443 ± 1·08	19	484 ± 8·99			
3	66	449 ± 7·07	43	407 ± 7·60	64	457 ± 7·66			
4	56	432 ± 7·43	34	401 ± 6·03	67	439 ± 5·53			
5	11	407 ± 20·14	12	439 ± 14·19	30	405 ± 4·68			
6	5	469 ± 11·58	4	390 ± 4·92	5	425 ± 26·05			

Table XXII

Comparison between rates of growth of F_1 three generations of alcohol, F_2 two generations of alcohol, F_3 one generation of alcohol, 4 generations of alcohol.

Size of litter.	Birth-weights.								Rates of Growth. Birth–1 Month.			
	Number weighed.	F_1 three gen. alcohol.	Number weighed.	F_2 two gen. alcohol.	Number weighed.	F_3 one gen. alcohol.	Number weighed.	Four gen. alcohol.	F_1 three gen.	F_2 two gen.	F_3 one gen.	4 gen.
		g.		g.		g.		g.	%	%	%	%
1	8	110·6± 5·57	12	114·6± 1·93	4	115 ± 3·95	—	—	3·56	3·47	2·34	—
2	36	98·5± 1·66	75	98·5± 1·19	14	86·1± 3·30	4	98·7± 5·22	3·21	2·73	2·99	2·52
3	127	87·0± 0·91	159	82·6± 0·96	38	80·9± 1·60	12	83·0± 2·07	2·98	3·35	3·29	3·58
4	93	78·0± 1·10	154	68·9± 1·28	36	71·3± 1·57	48	74·2± 1·40	3·32	3·17	3·61	3·37
5	50	69·2± 1·36	42	62·4± 1·11	8	61·2± 1·95	4	93·7± 3·40	3·09	3·67	3·27	2·32
6	10	59·0± 1·57	6	67·5± 2·95	6	60·8± 1·93	4	53·8± 3·14	3·81	—	—	4·38
7	6	55·8± 2·96	—	—	7	55·0± 2·89	—	—	4·13	—	4·31	—
		1 Month.							1–3 Months.			
1	3	317 ± 4·79	1	330	1	230	—	—	0·87	0·94	1·34	—
2	12	255 ± 9·03	19	222 ± 5·26	6	208 ±13·91	4	209·20±18·40	0·86	1·03	1·21	1·89
3	59	210 ± 4·02	54	223 ± 3·87	20	214 ± 3·63	12	237·05± 8·16	1·19	1·07	1·41	1·20
4	27	208 ± 5·37	47	176 ± 5·14	16	206 ± 4·57	46	200·40± 3·89	0·98	1·39	1·17	1·78
5	5	172 ± 3·52	2	183 ± 5·98	2	160	3	186·70± 3·40	1·23	1·66	1·45	3·18
6	8	181 ± 6·33	—	—	—	—	3	195 ± 6·93	1·55	—	—	2·01
7	4	188 ± 7·96	—	—	2	195	—	—	1·36	—	1·34	—
		3 Months.										
1	3	533 ±21·59	1	560	1	510	—	—				
2	12	426 ±13·97	14	410 ±13·89	4	429 ±23·58	4	410 ±31·84				
3	57	426 ± 7·82	49	428 ± 6·72	12	496 ±14·84	12	450 ±19·06				
4	21	373 ±10·81	41	403 ± 7·28	9	413 ±10·47	44	389·7± 7·70				
5	5	357 ±12·37	2	490 ±23·85	2	380	2	392·5± 1·26				
6	8	452 ± 8·90	—	—	—	—	3	385 ±10·43				
7	4	421 ±14·84	—	—	2	433	—	—				

3 months of the F_1's and F_2's in litters of 3 and litters of 4 are inferior to those for the parental stock.

Size of litter.	3.		
	Birth.	*1 month.*	*3 months.*
2 gen. alc.	93·6±1·1 (3·1)	241±4·1 (1·1)	442±8·8
F_1 ,,	86·4±0·7 (3·2)	219±4·7 (1·0)	407±7·6
F_2 ,,	82·6±1·0 (3·4)	223±3·9 (1·1)	428±6·7
Size of litter.	4.		
2 gen. alc.	79·7±0·9 (3·6)	236±4·1 (1·2)	444±6·3
F_1 ,,	71·2±0·7 (3·6)	203±4·5 (1·2)	401±6·0
F_2 ,,	68·9±1·3 (3·2)	223±3·9 (1·4)	403±7·3

Stockard (1918) compared the birth-weights and rates of growth in the young from controls and alcoholic stock without distinguishing ancestry, the total stock of all generations were combined. He concluded that the alcoholic animals as a group were at a disadvantage in birth-weight and rate of growth. The normal offspring weigh more and grow more rapidly shortly after birth than do the young alcoholic specimens.

In our data the offspring from the parental alcoholic stock were actually superior to those from the control stock but descendants from the alcoholic stock show deterioration in weight. It is not possible to say whether this deterioration was caused by alcohol or inbreeding. There was no parallel control stock with which to make a comparison.

SUMMARY

Table XXIII sums up the results for all the groups of alcoholized animals and their descendants. There is no evidence of progressive deterioration in either fertility or mortality. In only one instance is the average litter less than 3, and this particular group, F_3 two generations of alcohol, was very much inbred. Mortality varies in the different groups but there is no definite increase with alcoholization. The mortality for 2 generations of alcohol was 21 per cent. compared with 18·8 per cent. for 1 generation of alcohol. The F_1's and F_2's of the former group had a lower rate of mortality, 15·9 and 15·6 per cent. respectively, and it still remained low for the F_3's, 20·5 per cent.

The sex-ratios have already been discussed on p. 49, and no effect could be shown as a result of exposure to alcohol. As regards weight, the young from the descendants of 1 generation of alcohol showed signs of physical inferiority. There was a progressive decline in weight in the successive generations from 86 to 76. The difference between these two weights is statistically significant. The next alcoholized group, 2 generations of alcohol, also showed a decline in weight from 84 to 79. But when we come to an even more alcoholized group, 3 generations of alcohol, there seems to be a recovery in weight, and the average weight was as high as 88. There was again a decline in the F_1's of this group. The mean weight for 4 generations of alcohol

Table XXIII

	Number of litters.	*Total offspring.*	*Mean size of litter.*	*Dead births and post-natal deaths.*	*Per cent. Mortality of total births.*	*Number of mis-carriages.*	*Sex-ratio at birth.*	*Number weighed.*	*Mean birth-weight of guinea-pig*
									g.
Controls . . .	214	674	3·2±0·07	118	17·5±0·99	5	98	356	83·1±0·75
All Alcoholics.									
1 gen. alcohol . .	252	813	3·2±0·05	153	18·8±0·92	2	102	616	86·1±0·56
F_1 one gen. alcohol .	287	887	3·1±0·05	118	13·3±0·77	7	101	778	82·0±0·54
F_2 one gen. alcohol .	126	376	3·0±0·07	62	16·5±1·29	4	115	333	79·3±0·65
F_3 one gen. alcohol .	39	121	3·1±0·14	24	19·8±2·44	2	144	113	75·6±1·16
2 gen. alcohol . .	114	380	3·3±0·07	80	21·1±1·40	4	115	320	83·9±0·74
F_1 two gen. alcohol .	96	295	3·1±0·09	47	15·9±1·44	5	113	274	79·2±0·65
F_2 two gen. alcohol .	67	199	3·0±0·08	31	15·6±1·73	2	85	184	78·5±1·35
F_3 two gen. alcohol .	35	88	2·5±0·13	18	20·5±2·90	6	89	73	79·3±1·65
3 gen. alcohol . .	74	230	3·1±0·09	48	20·9±1·38	8	103	195	88·0±0·92
F_1 three gen. alcohol	125	399	3·2±0·07	88	22·1±1·40	7	114	330	82·2±0·69
4 gen. alcohol . .	20	75	3·8±0·13	7	9·3±2·26	—	63	72	76·9±1·32

was low. As evidence against the harmful effect of alcohol the mean weight for F_1 three generations of alcohol is identical with that of F_1 one generation of alcohol, and superior to that of F_1, F_2, and F_3 two generations of alcohol, while the weight of offspring from 3 generations of alcohol is considerably better than that of the controls.

CONCLUSIONS

We can now consider the answers to the questions discussed (see p. 11).

1. There is no evidence to show that exposure to alcohol injures the germ-cells, so that abnormal young are produced. Guinea-pigs of both sexes have been treated with alcohol for varying periods from 1 month to 5 years, and also 4 successive generations of guinea-pigs have been treated. One abnormality occurred among the 674 control animals and 10 abnormalities among 6,309 alcoholic stock. Four of the abnormal young appeared among the offspring of directly treated animals; in 1 case the mother only was treated, in 1 case the father only, and in 2 cases both parents were treated; the remaining cases were among F_1, F_2, or F_4 families.

2. All the abnormalities appeared in families which were related and bred from the same original stock. It is suggested that these abnormalities may be the result of inheritance and not due to the effect of alcohol on the parents.

3. Fertility would appear to be unaffected by alcohol. There is no evidence to show that the males are more affected than the females, and any loss of fertility occurring can be equally well attributed to the genetic qualities of the stock itself, when the effect of inbreeding is also taken into consideration.

4. Some evidence was found of deterioration in weight in the descendants of the alcoholized stock. This deterioration might also be due to inbreeding but there was no parallel control stock with which to make a comparison.

5. The mortality rate for still-births and deaths in the first 3 weeks after birth is greater for the offspring of directly treated than for those of untreated animals. But the highest mortality among the offspring of alcoholized stock occurs when the animals have been treated for a long period (over 2 years). The animals themselves are then no longer young, and the increased mortality may be due to the age of the parents. A proper comparison, however, cannot be made, as insufficient control animals of like age were used for breeding.

6. Sex-ratio would appear to be unaffected by alcoholic treatment.

There is therefore no evidence to show that alcohol has had a deleterious effect on the genetic behaviour of guinea-pigs and our results would appear to agree with those of the majority of observers in the same field.

No conclusions should be drawn from these experiments by either advocates of alcohol for human use or by those in favour of total

abstinence. The behaviour of an intoxicated person upsetting social life is a question with which our experiments are not concerned.

In conclusion, we wish to express our very grateful thanks to Miss M. Rogers for the care and trouble she has taken in preparing the charts and tables and for the many calculations she has made; to Miss J. E. Marchal and Miss W. I. Strangeways and Miss M. Lane for help in taking charge of the experiments and weighing the animals at various times, and to Mr. F. Baxter who had charge of the feeding and cleaning of the animals.

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By FREDERICK W. ANDREWES and
ETHEL M. CHRISTIE

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PREFACE

THE haemolytic streptococci, as Sir Frederick Andrewes explains in the first sentence of this report, 'stand in the very forefront of the bacteria harmful to mankind'. He might have said as truly that they are among the most numerous and deadly of all the living enemies of man. They inflict damage or death in many different ways. They cause diseases as various as scarlet fever, erysipelas, pneumonia, heart disease, puerperal fever after child-birth, and sepsis after accident or surgical operation. As secondary invaders, moreover, after infections of other kinds, these organisms kill multitudes by giving a fatal end to temporary weakness. Yet the streptococci were first identified and described only fifty years ago. During those relatively few years it has been one of the chief tasks of the bacteriologist, as his methods of study have progressively developed, to group and classify the streptococci for the guidance and help of the clinician who is confronted by their various, and apparently distinct, manifestations in disease.

Sir Frederick Andrewes gave the last working years of his life to promote advance in this direction. He hoped that by using the most refined methods of analysis he might improve the classification of the streptococci and make more secure the identification of given streptococci with particular varieties of disease. The results of more than seven years of laborious effort are presented here. He died on the 24th of February, 1932, when the writing of his report was practically completed. Almost the whole of it had been written in first draft by himself, and he had revised and re-written everything down to the end of the section upon acid agglutination. The remainder needed only a few trifling corrections, though doubtless it would have gained if Sir Frederick Andrewes had been able to re-write the whole. The preparation of the report for press, however, has been undertaken by his colleague, Mrs. Christie, and his son, Dr. Christopher Andrewes, who have had the advantage not only of the notes he had left but also of close intercourse with him. They have themselves added by the aid of his notes the latter half of the section dealing with puerperal fever, erysipelas, and surgical sepsis. They have also interpolated a paragraph on 'minor variations in serological behaviour', describing work which had just been completed at the time of his death. In the concluding summary they have been careful to draw only those conclusions which it is known that he himself drew; in many instances, indeed, they have been able to use his own words.

It is a matter of much interest that the work here described has not attained the principal objective held in view at its beginning. Accurate study by the most refined methods has shown that these haemolytic streptococci have such inherent powers of adaptation to their chemical environment as to make it impossible, for the present at least, to find any firm foundation for a permanent systematic classification. The very methods used for discrimination between forms of different origin may themselves bring change to the

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characters of the organisms under observation. The worker may find himself at first identifying an apparently distinctive type of streptococcus as being associated with a certain form of disease, and later discover by more critical work that it was his own methods that produced the type characters on which he first depended. The work here reported, nevertheless, has produced a body of trustworthy and valuable results. It has cleared up many past confusions that have arisen from want of correlation between the results of numerous earlier workers using different methods. It has revealed many pitfalls in work of this kind, and although these serological studies, made by methods more accurate and quantitative than most workers have hitherto used, have failed, in Sir Frederick's words, 'to bring order into the chaos', their chief interest and value may be found actually to lie in having shown that success is probably unattainable in this direction.

The menacing and protean dangers of the haemolytic streptococcus to human life appear to arise from the very instability of its physico-chemical behaviour which is revealed so clearly in this report. The organism as an invading parasite appears to have special facility in adapting the refinements of its living chemistry to the particular host environment in which it finds itself. Studies of different 'types' in terms of formal definition become fruitless or meaningless if each type is a passing chemical phase quickly assumed and readily abandoned. Progress will seem to lie in gaining deeper knowledge of the exact chemical events which are involved in this interplay between the parasitic organism and the environment of the invaded host. Sir Frederick Andrewes's work, taken as a whole, would seem to show that serological methods, however refined, are not likely to do more than state the problems of which other methods must bring the solution.

The Council would express their grateful acknowledgements to Mrs. Christie and to Dr. Andrewes for their work in preparing this report for publication. They have made it possible for it to appear in almost exactly the form desired by its chief author. The Council have lost heavily by Sir Frederick Andrewes's death. He had served for many years as a member of the Council, and had brought aid in many other ways to their work from the beginning. Memory of these services enhances the gratitude the Council feel towards those who have assisted in giving worthy presentment to this last work of his.

Medical Research Council,
38, Old Queen Street,
Westminster, S.W.1.

21*st July*, 1932.

THE HAEMOLYTIC STREPTOCOCCI: THEIR GROUPING BY AGGLUTINATION

BY

FREDERICK W. ANDREWES

AND

ETHEL M. CHRISTIE

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1. INTRODUCTION

The haemolytic streptococci stand in the very forefront of the bacteria harmful to mankind. Not only are they the direct exciting causes of a number of acute inflammations in various regions of the body, receiving different clinical names in accordance with their localization, but they also possess faculties which render them of especial danger to the patient. The first is their ability to invade the lymph stream and thus to spread widely from the primary focus of infection; the second is their tendency to invade the blood stream, and to cause septicaemia and metastatic infections in different organs. They are 'serophytes' in Wright's sense, able to grow and multiply in the unaltered body fluids. Nor is this all. They are apt to lurk on the mucous surfaces of the apparently healthy body, especially in the nasopharynx; when the resistance of the body is lowered by some other infection, these streptococci commonly step in as secondary infections, and only too often, as in measles or influenza, administer to the unhappy patient the *coup de grâce*.

The haemolytic streptococci have so far adapted themselves to a parasitic existence that we know of them only as tenants or invaders of the animal body, and it is now beginning to be perceived that they maintain themselves in a human population much after the fashion of the meningococcus or the pneumococcus—namely by 'carriers'. Not every person is a carrier, and if he is, the condition may be a temporary one, but in certain circumstances carrying may be wide-spread in a community.

The question whether there is more than one sort of haemolytic streptococcus has been much discussed, and the problem of specificity is at its most difficult amongst the streptococci. We must, nevertheless, endeavour to make clear our own position, in this respect.

(i) Nomenclature

The first to recognize a streptococcus, which we now know to belong to this type, was Ogston in 1881, but he did not give it a definite name. In 1883, however, Fehleisen demonstrated the specific relation of a

streptococcus to erysipelas, and he bestowed the name '*Streptococcus erysipelatos*' on this organism. A year later Rosenbach (1884) applied the name '*Streptococcus pyogenes*' to the chain coccus which he found in acute suppurative conditions. This he did in all good faith, for the two cocci were at that time believed to be different species. In the course of years they were found to be identical, and the wider implications of Rosenbach's name gradually led to its being preferred to Fehleisen's. Although, by the laws of strict taxonomic pedantry the name *Streptococcus erysipelatos* must be accorded priority, it is hard to upset the usage of forty years, and Rosenbach's name is now in general use.

In 1903 Schottmüller established the haemolytic properties of this coccus as seen on the blood-agar plate, and the importance and convenience of this character are now so well recognized that many modern writers employ the term '*Streptococcus haemolyticus*' in a specific sense. It need not be said that there is no scientific justification for this. The most righteous taxonomist may consent to condone the dropping of Fehleisen's name, but we cannot see Rosenbach's similarly treated after nearly half a century of useful life. Further, the new name is a bad one, for there are streptococci other than *Str. pyogenes*, which haemolyse blood corpuscles under certain conditions, and there is every degree of haemolysis. It is perfectly legitimate to speak of 'haemolytic streptococci' in our daily talk, but to use the name '*Streptococcus haemolyticus*' in scientific terminology is surely wrong.

The true *Str. pyogenes* of Rosenbach presents fairly definite characters apart from that of causing β-haemolysis in the blood-agar plate. Its 'sugar reactions', as defined by Gordon (1905), and confirmed by Andrewes and Horder (1906), and by many other subsequent workers, are very constant. It ferments the disaccharides, lactose and saccharose, and also the glucoside, salicin. Any one of these fermentative powers may be found absent—usually temporarily, and extra powers may be sometimes present—as for instance that of fermenting mannite, and even starch. Litmus milk is always rendered acid and sometimes clotted; the latter is really a question of degree, and wider experience has shown that the proposal to use it as a character for separating certain forms under the name of '*Streptococcus anginosus*', made by Andrewes and Horder in their original paper (1906), has no sufficient warranty. Even the reduction of neutral red under anaërobic conditions, a power long denied to *Str. pyogenes*, may be found very occasionally present if a sufficient number of strains be tested. But not withstanding all this it must be repeated that the biological characters of this coccus are sufficiently constant to enable one to recognize it.

It is well recognized that certain outbreaks of septic sore-throat in man, have been traced to milk from infected cows. In some of these outbreaks the infecting agent has been found to be a strongly haemolytic streptococcus differing from *Str. pyogenes* in the possession of a well-marked capsule, and growing in large, moist colonies, spreading on the surface of an agar medium. It was described in 1912 by D. J. Davis,

under the name of '*Streptococcus epidemicus*'. The sugar reactions are those of *Str. pyogenes*, and in other respects it seems to resemble that organism. It is a little rash to separate off a species merely on the presence of a capsule, until we know more precisely the constancy of the structure, and the conditions in which it develops. We have ourselves had no opportunity of comparing the two organisms, though one of us was shown the capsules by the Indian ink method, in a laboratory in the University of Wisconsin where this streptococcus has been carefully studied.

With this doubtful exception *Str. pyogenes* seems a good species in the natural history sense, but as it is the typical 'haemolytic streptococcus' we must examine a little more closely into its haemolytic powers.

(ii) Haemolysis

The ordinary way in which the fact of haemolysis is shown is in the blood-agar plate. This is not a wholly satisfactory way for there are many degrees of haemolysis. Since the publication of Brown's monograph in 1919, the slighter degrees forming only a narrow zone round the colonies (α-haemolysis) are distinguished from the wide and conspicuous bands of clearing seen around the colonies of *Str. pyogenes* (β-haemolysis). Moreover, the kind of blood used is not without influence on the intensity of the phenomenon: horse's blood is probably best, and rabbit's blood worst of those commonly used.

The haemolytic powers of *Str. pyogenes* depend upon the production of a definite toxin passing out into the medium, and capable of disintegrating the limiting membrane of the erythrocytes. It is the production of this haemotoxin which is now regarded as the true test of a haemolytic streptococcus in the stricter sense in which most people (including ourselves) use it. There is another streptococcus (*Str. faecalis*)—the 'enterococcus' of continental writers—which (under anaërobic, though rarely under aërobic conditions) may produce a haemolysis on blood-agar plates, quite similar to that caused by *Str. pyogenes*.

A. *Technique of Testing for Haemolysis.*

The demonstration of a filterable haemolysin must be carried out in a liquid medium. We have employed the test on at least 150 occasions, including repetitions and control tests on non-haemolytic streptococci.

The medium we have used has been ordinary peptone broth, containing 10 per cent. of horse serum. Some writers recommend 20 per cent. of serum, but we have found 10 per cent. ample. Ascitic fluid may quite well be substituted for serum, and indeed we have found the test to come off with plain broth almost as well as if serum were present. The addition is, however, advisable, though there is probably no magic in it beyond the encouragement of vigorous growth. We have usually incubated at 37° C. for 24 hours after inoculation, but in the case of a weakly growing strain, 48 hours may be required for a good reaction. The pH of our medium was 7·13.

When good growth has occurred the fluid is centrifuged till apparently clear. Some strains take much longer than others, and in no case are all the cocci removed, for subculture reveals many colonies on agar.

The erythrocytes we have used are those of the horse well washed in saline with care against contamination. One drop of a dense suspension is added to each 10 c.c. tube of broth—enough to render the fluid opaque and bright red on gentle shaking. The tubes are now put in the incubator at 37° C. and examined after 20, 30, and 60 minutes, and thenceforward hourly. The interest of the test is much enhanced by the use of a pocket spectroscope. An oblong glass container is useful for slight reactions, so that a deeper layer of fluid may be surveyed, but this, of course, only when contamination is no longer of consequence.

B. *Changes in Medium.*

(a) *Haemolysis.*

Three changes may be observed—haemolysis, reduction and the formation of methaemoglobin—and these are largely independent of one another.

An actively haemolytic strain gets to work in about 20 minutes, and haemolysis is usually complete in an hour, when the fluid should be completely cleared. A more sluggish strain may take 2, or even 3 hours, to accomplish the haemolysis, seldom longer. It depends largely upon vigour of growth under the conditions of the experiment, which cannot always be exactly controlled. It is not uncommon for a strain to give excellent haemolysis on a second trial when at the first attempt the reaction had been imperfect and unconvincing.

Of the strains which, on other grounds we had put down as examples of *Str. pyogenes*, over 100 in number, only in two did we finally fail to satisfy ourselves of the presence of a soluble haemolysin. Two other strains were of interest; they were of bovine origin, and had caused metastatic suppurative lesions in guinea-pigs inoculated with milk sediments. They resembled *Str. pyogenes* in most respects, but differed in fermenting raffinose. We had regarded them as haemolytic from the appearances on blood agar, but they both totally failed to respond to the test for soluble haemolysin.

(b) *Reduction.*

Observation of the colour of the tubes, and, more certainly, the use of the spectroscope reveals that, in the mode of carrying out the test which we have described, a good deal of reduced haemoglobin is commonly found. If the matter be further investigated it is revealed that this reduction is brought about only in presence of living cocci. If the culture is passed through an adequate filter, and shown by subsequent culture to have been rendered sterile before adding the blood corpuscles, haemolysis occurs as usual, but no trace of reduction can be detected, even after 24 hours. Inasmuch as centrifuging removes a varying proportion of cocci the amount and rate of reduction in a

series of tubes vary widely. It occasionally happens that reduction is marked before any haemolysis can be detected. It is manifest that it is a part of the respiratory function of the active coccus, and is not due to any product secreted into the culture medium.

(c) *Methaemoglobin formation.*

The haemolytic streptococci are not so active as many non-haemolytic ones in their ability to form methaemoglobin. Different forms of mouth streptococci vary in their capacity in this respect. From the buccal secretions of one of us four streptococcal colonies were isolated, and tested against washed corpuscles. No haemolysis occurred, and no reduction, but at the end of an hour in the incubator the haemoglobin was almost completely converted into methaemoglobin. Three colonies similarly isolated from the other of us showed no methaemoglobin formation at any time. With haemolytic streptococci we have never detected methaemoglobin formation in so short a time as an hour though it may be quite well marked in 1½ hours, and is more often seen than not after 4 or 5 hours' incubation. If after this, the tubes be left on the bench overnight the conversion of oxyhaemoglobin into methaemoglobin is usually complete, though different strains vary in this respect. We have observed the formation of methaemoglobin even after a culture has been rendered sterile by passage through a Seitz filter.

It may be pointed out here that the soluble haemolysin of *Str. pyogenes*, though it will pass a porcelain filter is near the border line. The readiness with which it passes depends on the permeability of the filter. We furnished Dr. Gordon with four similar cultures of the same strain, and he was good enough to pass them through four different filters: these were L_1, L_2, Berkefeld, and Seitz. After addition of corpuscles to the filtrates, they were incubated in the ordinary way. Haemolysis occurred in the L_1 and L_2 filtrates as readily as if no filtration had taken place—i.e. in about 30 minutes; in the Berkefeld filtrate the process was deferred, and in the Seitz filtrate it took still longer to be manifest.

As regards the formation of methaemoglobin it is plain that the factor of acidity must be taken into account. We made a few observations on this in the absence of streptococci. One drop of washed corpuscles was put into each of 11 test tubes, and then 1 c.c. of distilled water to bring about haemolysis: the tubes were then filled to 5 c.c. from our stock bottles of acetate-acetic acid solutions—ranging by jumps of 0·3 from pH 4·7 to pH 6·8. For the less acid tubes around neutrality, phosphate mixtures were used. The tubes were now incubated at 37° C. and studied with the spectroscope at suitable intervals. After 1 hour, the pH 4·7 tube showed the methaemoglobin band, the pH 5·6 tube following suit at 1½ hours. After 3 hours incubation traces of methaemoglobin could be found up to pH 6·5, but none at 6·8. The tubes remained at 37° C. for about 7 hours after which they were left overnight at room temperature: next morning there was a good methaemoglobin band in the tubes at pH 7·2 and 7·4. The band

was also manifest in an uninoculated control tube of serum broth. It is therefore likely that some of the methaemoglobin formed in the tubes containing streptococci may be due to acid formation by the cocci.

C. *Effect of Anaërobiosis*

The influence of anaërobiosis on the haemoglobin changes was studied in two opposite ways. (1) Twelve strains of *Str. pyogenes* were grown in serum broth in a McIntosh and Fildes jar in which was a methylene blue control. After 24 hours' incubation at 37° C. the tubes were removed from the jar, red corpuscles added as usual, and the rest of the experiment allowed to proceed aërobically at 37° C. (2) In the second procedure the growth of the organisms was aërobic; corpuscles were then added and the tubes forthwith placed in the anaërobic jar at 37° C. to undergo the further changes. We were unable to detect any notable differences between the haemoglobin changes in the tubes thus treated and those treated in the ordinary manner, except that reduction was more marked in the tubes treated anaërobically in the later stages. It was interesting to note that methaemoglobin formation though an oxidative process, took place freely under anaërobic conditions.

It may be added that, by the technique we have used, soluble haemolysin appears in the cultures in between 12 and 14 hours after inoculation. This is later than was found by De Kruif and Ireland (1920). The reason is that they used as their inoculum a culture in the logarithmic phase of growth: we did not.

2. SEROLOGICAL OBSERVATIONS: PRELIMINARY STUDIES

We have gone in some detail into the matter of haemolysis, not because there is anything novel in our observations, but to show that we have been vigilant in excluding dubious organisms from our series. We believe that all our strains may strictly be classed as belonging to one species, viz. *Str. pyogenes*.

(i) Problems awaiting Solution

We pass now to the main study to which we have addressed ourselves. Is it possible, and if so, is it clinically worth while to establish the existence of constant serological varieties in the species? Such serological races are well known to exist in the diphtheria bacillus, but inasmuch as they all produce a common toxin, no importance attaches to the fact in ordinary clinical practice. In the case of the pneumococcus such races also exist, but here they are important, for a knowledge of the precise type present in any given case helps greatly in prognosis and especially in serum treatment.

When we speak of serological diagnosis we mean practically diagnosis by agglutination, for the use of such methods as complement

fixation, and precipitin formation, and still more the more elaborate method described by Mrs. Lancefield (1925), though they may be made to work, are too slow and cumbrous for clinical purposes.

There is no doubt whatever, that there exist, amongst the haemolytic streptococci, serological races comparable to those met with amongst many other bacteria. Our problem was to ascertain, by using the more refined methods of antigenic analysis, whether we could construct a grouping likely to be of practical value in clinical work, and especially whether we could establish serological varieties correlated with apparently distinct diseases.

Previous attempts at such serological classification are well summarized by McLeod in his recent article on the haemolytic streptococci in the Medical Research Council's *System of Bacteriology*, vol. ii, p. 61. It must be confessed that no clear picture arises in the mind from the study of what has been done, and the absence of even an attempt at correlation of the results of different workers is confusing.

(ii) Material Studied

Our own material has been drawn from the widest possible sources within the limits of the species—*Str. pyogenes*. We have had under our hands 200 strains in all, derived from the following sources:

Scarlet Fever	64
Puerperal sepsis	48
,, ,, (suspected contacts)	15
Erysipelas	11
Surgical infections, and various	62

Many of the surgical group, especially of the ear cases, and excised tonsils, may well have owed their origin to scarlet fever, though there was no history of this. A very large proportion of our strains were derived from the wards and out-patient departments of St. Bartholomew's Hospital. We owe a large number of strains to Dr. F. Griffith, of the Ministry of Health Laboratories, and others to Professor W. W. C. Topley: both these gentlemen were also good enough to supply us with samples of immune sera which they had prepared. To other friends, too numerous to set down here, we are indebted for cultures of streptococci included in the above series.

It is not to be supposed that we examined each of these strains with equal elaborateness. So far as possible each was tested by agglutination with the sera in our possession, and as regards sugar reactions and haemolytic power. It was only practicable for us to make a limited number of sera from amongst these strains, but at different times we made 32 for ourselves. Dr. Griffith generously gave us about 30, in such quantities as he could supply from his limited stores and Professor Topley gave us 5. We were not thus limited in the use of different strains for absorbing purposes, and we thus employed at least 80 strains and mutations from strains.

(iii) Difficulties in the Technique of Agglutination Tests with Streptococci

The technique of agglutination amongst the haemolytic streptococci presents many difficulties, as may be judged from the literature, for almost every writer has devised a special technique of his own, which is one reason why the results of different authors are not comparable. No sooner had we begun our work than we were arrested by the difficulties in question and it is hardly too much to say that in our attempts to overcome them we have tried almost all the devices recommended in the writings of others. The greater part of our work, during the first two years and more, lay in the study of technique; not till we had sufficiently mastered this, could we feel any sort of confidence in our results.

A. *Application of Dreyer's Technique to Streptococci.*

The method of agglutination which we desired to use was that introduced by Dreyer, of which one of us had many years' experience, and the delicacy of which seemed especially well adapted to antigenic analysis. This method is, however, one which demands a saline suspension of sufficient stability to stand up in a water-bath at 56° C. for 2 hours or more, and a primary difficulty lies in the preparation of such a suspension. This particular difficulty we eventually in large measure overcame, as we shall shortly describe.

Dr. Griffith, in his valuable work on the types of streptococci in scarlet fever, needing a rapid, if rough and ready, method for determining his types, short-circuited the difficulty by abandoning Dreyer's method altogether, and putting up his emulsions on a slide with drops of sera only slightly diluted and from which group agglutinins had been absorbed. He finds this naked-eye method effective, and it must be conceded that it has enabled him to carry out valuable work, which could hardly have been done on the same scale by ordinary means. We found it, however, unsuited to the aims of our own work, for it is lacking in refinement and is unadapted to exact quantitative methods.

After a brief trial of the naked-eye method, we again devoted ourselves to the Dreyer technique, which is fully described in the Medical Research Council's Special Report Series, No. 51. This technique was primarily designed for the measurement of the H-agglutinins of *B. typhosus* and its allies, but has been also adapted to the O-agglutinins of the dysentery bacilli. We saw no reason why it should not be applied to the haemolytic streptococci if only good stable emulsions could be made. Much of the detail of Dreyer's method is a matter of convenience designed to render it as fool-proof as possible, but the central feature lies in the adoption of an arbitrary 'standard' degree of agglutination, of which permanent sample tubes are sent out from the 'Standards Laboratories' at Oxford. The dysentery standard representing 'somatic' or O-agglutination seemed to us most comparable

with that yielded by the streptococci, and we have adhered to this throughout.

Dreyer has put forward a reduction table by which degrees of agglutination greater or less than standard can be calculated as standard. This table was not, it is true, calculated for streptococci, but the discrepancy involved, if any, cannot be large, and is in any case uniform throughout the whole of our work. The other details of the method—measurement by drops, a water bath at 56° C. &c., &c., were just as advised by Dreyer. The time allowed in the water-bath was usually 2 hours, but with some strains this had to be extended to 4 hours. We have attached comparatively little importance to simple agglutination *per se* in our attempts at grouping, but an accurate technique was essential for reading the results of the absorptions on which we have laid chief stress.

We must now approach the chief difficulties which we have encountered, and describe how we have endeavoured to meet them.

B. *Suspension Stability of Streptococci.*

One may make an emulsion in two ways (*a*) by using broth cultures, and (*b*) by rubbing down the growth on solid plate cultures in some suitable fluid. A good uniform broth culture apparently leaves little to be desired, but such cultures are the exception rather than the rule. A large proportion of newly isolated strains yield a fluffy deposit of long chains, with a clear supernatant fluid and with such cultures nothing can be done. Worse than this, those apparently favourable cultures, of uniform turbidity may yield positively misleading results. When we come to discuss the facts of dissociation it will be seen that it is precisely the smooth forms with group properties, which yield uniform turbidity in broth, while the rough, and specific forms tend to lack saline stability. Professor Topley pointed out to us several years ago that he could only achieve specific results with these cocci by using agar suspensions. He showed us a long series of tests in parallel in which he had used broth cultures and suspensions of the same strains, and the figures fully bore out his contention. We have, therefore, followed Topley's advice, and have used suspensions made from solid media, first dried for an hour or two in the 37° C. incubator, and we must admit that, whereas we had previously been obtaining erratic and worthless results with our broth cultures, we now felt ourselves on much firmer ground.

The nature of *suspension stability* is now generally admitted to be reasonably explained by the hypothesis of Northrop and De Kruif (1921–3). It is a matter of electrical charge. Bacteria suspended in saline solution or broth bear a uniform negative charge, measurable by cataphoretic methods. These charges exercise a mutually repelling influence, which prevents the bacteria from running together as they otherwise would from the attracting force (probably surface tension) which subsists between minute solid particles suspended in a fluid. Suspension-stability means a condition in which the electric charge

keeps the upper hand; spontaneous agglutination means that it fails to do so. One may picture the bacteria as bearing a certain number of free electrons on their surfaces, constituting the negative charge; it is the robbery of these electrons by the kations of the electrolyte which diminishes or abolishes the charge and so permits the attractive forces to gain the upper hand. We may take a stable suspension to be one in which the negative charges are active and well developed, and the spontaneously agglutinating suspension to be one in which the bacteria are deficient in free electrons.

C. *Acid Agglutination of the Haemolytic Streptococci.*

Before going any further it will be well to discuss the acid agglutination of streptococci, which, so far as we are aware has not previously been studied. As with serum agglutination a uniform and reasonably stable suspension is requisite for the study.

(a) *Technique of testing acid agglutination.*

There are several mixtures which can be prepared in advance to give a range of hydrogen-ion concentrations of any desired degree of closeness, and so well buffered that the addition of a streptococcus suspension in water or saline scarcely affects the pH. We have made extensive trial and comparison of three such sets of solutions, the composition of which is described in Clark's book, *The Determination of Hydrogen Ions* (1928).

(1) Sørensen's mixtures of sodium acetate and acetic acid. Mixtures of this nature were proposed by Michaelis when he first put forward acid agglutination as a differential test amongst bacteria. On the whole we prefer such mixtures to any others; our series ranges by jumps of pH 0·3 from pH 3·2 to 6·8. When the mixtures are made up a crystal of thymol should be added to the pH 5·0 bottle and all on the alkaline side of it, or moulds are liable to grow, with disastrous results on the hydrogen-ion concentration. The mixtures were preserved in bottles lined with paraffin.

(2) The solutions devised by Clark and Lubs, which have a basis of acid potassium phthalate (or acid potassium phosphate, at a certain level) to which suitable amounts of HCl or NaOH are added as the case may be.

(3) The salts sold by 'British Drug Houses', prepared according to the formula of Prideaux and Ward, under the name of 'Universal Buffer Solution'. The results yielded by the last-named mixtures have seemed to us less satisfactory than those given by the other two, as there is a liability to agglutination round about the neutral point, absent, or nearly so, with the other two sets of solutions.

The accuracy and constancy of the mixtures has been checked throughout with the simple form of portable potentiometer devised by Brown and Broom (1929), which makes use of the quinhydrone electrode. We are greatly indebted to these gentlemen for having an instrument made for us under their own supervision. We find that

with a little practice this form of potentiometer will yield results accurate to pH 0·02, which is all that is claimed for it.

The emulsions we have used have been prepared in the following manner. From the stock egg-culture of the strain to be tested, a culture is made in peptone broth containing a few drops of sterile rabbit's blood. The blood-broth culture, after a night in the warm incubator is microscopically examined, and then stored at room temperature. It remains viable for many weeks, and from it dried agar plates are prepared as needed. The agar growth is emulsified in sterile saline, and diluted down to a strength of about 400 million per c.c. For the test itself equal volumes of emulsion and standard pH solutions in series are placed in ordinary Dreyer agglutination tubes, and kept in a water-bath at 55° C. for 1½ to 2 hours. The results can be read after standing 10 minutes or so on the bench, and can be expressed in the same way as one records a serum agglutination. The excellent buffering of the solutions we have used was shown in experimental tests. The pH of a mixture of equal parts of a standard solution of pH 3·2 and of a saline emulsion of streptococci having a pH of 6·13, was found to be 3·36. On many occasions the actual hydrogen concentrations of the mixtures at the close of the test were determined by potentiometer, and found to vary but little from what had been intended. In one test the figures were as follows:

Intended pH.	*pH actually found.*
3·5	3·56
4·4	4·22
5·6	5·47
6·5	6·38

If it is remembered that the amount of fluid available for each reading was only about 1 c.c. it will be admitted that the figures are close enough for practical purposes.

The technique above outlined was the result of a large number of experiments in which alternative methods were tried. Emulsions of the cocci in plain distilled water were compared with those in saline. Although it occasionally happened that a strain would stand up in water better than in saline we found that on the whole acid agglutination came off better and extended further towards the alkaline side when sodium chloride was present. In tests with salt solutions varying from 0·2 to 0·85 per cent., no noteworthy differences were found in acid agglutination.

A comparison was made between emulsions grown on plain agar, and on the legumin agar devised by Gordon, Hine and Flack (1916). Legumin agar, into which extract of pea-flour enters as an ingredient, contains starch—a substance which is fermented by some haemolytic streptococci, but not by most. Speaking generally, we found that a more intense acid agglutination was given by the suspensions grown on legumin agar, but this was not always the case. Such emulsions, however, were more liable to spontaneous agglutination, and we have pre-

ferred, as a routine, to use plain agar emulsions, especially since these avoided any possible fallacy due to acid formation from starch in the medium.

(b) *Results of acid agglutination tests.*

The results of pure acid agglutination of the haemolytic streptococci, as far as possible freed from disturbing circumstances, are displayed in Fig. 1, and here we may say that throughout this report we have preferred to state our results by the graphic method, rather than by rows of figures, because, when once the principle of the graph is grasped, it seems to us at once more concise and more informative. Moreover, it is excellently adapted for the comparison of results. We shall have to employ graphs constructed on more than one principle, but each will be fully explained in due course.

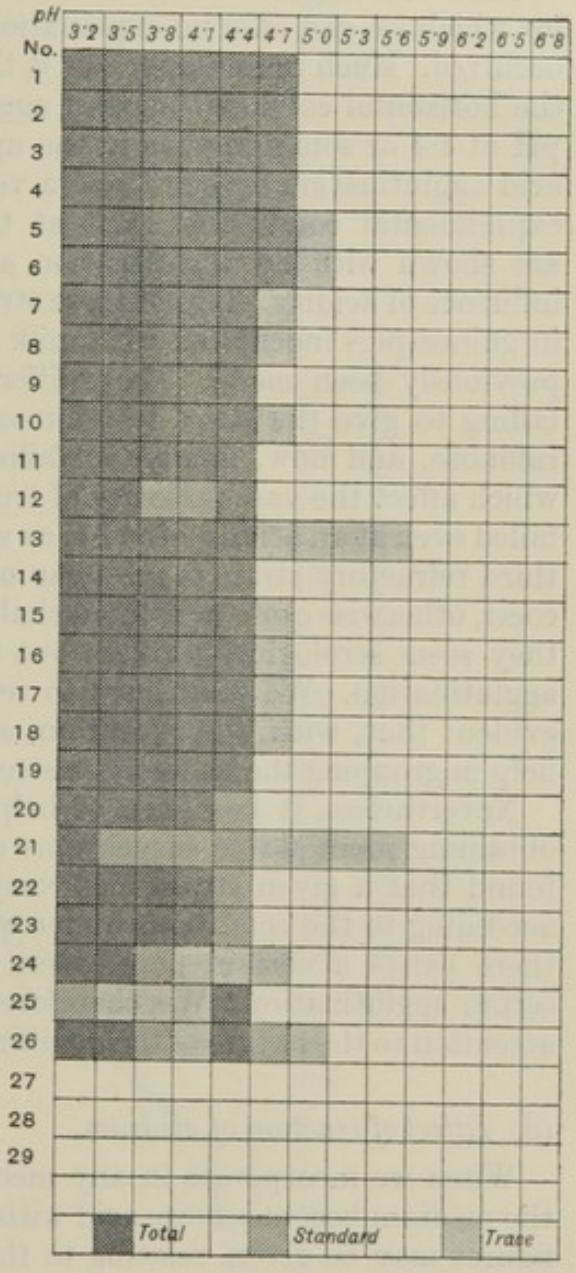

FIG. 1. GRAPH OF ACID AGGLUTINATION
29 strains

Fig. 1 represents the acid agglutination of 29 unselected strains of haemolytic streptococci, and they were tested towards the close of our investigations on acid agglutination when experience had taught us how best to avoid fallacies. They are, in fact, the best and most trustworthy results that we obtained. They were obtained with saline emulsions off young agar plates, and the controls with neutral saline were in every case perfect, showing no trace of agglutination after the sojourn in the water-bath at 55° C. The series of pH solutions was prepared according to Sørensen—with sodium acetate and acetic acid.

The principles on which the preceding graph is constructed are these. The numbered horizontal rows of squares are the 29 individual strains of streptococci tested, and the vertical rows represent the series of hydrogen-ion concentrations indicated at their head. The method of shading indicates the intensity of agglutination. The cross-hatching represents 'total agglutination' in Dreyer's sense, 'standard' is indicated by oblique parallel lines, and mere 'traces' by dots; absence of shading means absence of any sign of agglutination.

(c) *Discussion of results.*

The series of cocci being unselected shows some anomalous results for which we can offer no explanation: they are depicted just as they occurred. Much more important is the regularity of the great bulk of the horizontal columns: marked agglutination ceases sharply after a pH of 4·4 or sometimes, as in the upper 6 rows, at 4·7. The limit of acid agglutination is seen to be a very uniform one under the given experimental conditions. But at the foot of the graph 3 strains are shown with no agglutination at all—strains refractory to this influence of acidity. Two of these strains are the bovine ones obtained in guinea-pigs inoculated with milk sediments, to which allusion has previously been made. They differ from the true *Str. pyogenes* in failing to give the strict test for soluble haemolysin, in fermenting raffinose, and now, finally, in failing to agglutinate with acidities which affect the vast majority of true haemolytic streptococci. They failed even at an acidity of pH 1, as we have shown in other tests. The third refractory strain is a human one belonging to a small group of cocci, otherwise quite indistinguishable from *Str. pyogenes*, except that they seem serologically allied, but tending to be refractory to acid agglutination. We shall have to refer to this group later, but it is evident that, with this exception, acid agglutination affords no real help in grouping the haemolytic streptococci.

Nevertheless, it has been of help to us in evolving a method of obtaining more stable suspensions of these organisms. For we soon found that a given strain may vary in its reaction to the acid test according to the conditions under which it has been grown, and that there exists a certain parallelism between acid agglutination and serum agglutination. We, therefore, began to devote a good deal of attention to the nature of the medium from this point of view.

(d) *Effect of reaction of medium.*

When we incorporate in any medium a fermentable carbohydrate the medium becomes more acid with the growth of the coccus. Actual figures may be given, relating to the scarlatinal Type IV of Griffith. A plain broth tube of pH 7·1 before inoculation underwent little change, for when growth was completed the pH was still 6·8. A 1 per cent. glucose-broth tube, on the other hand, of pH 6·8 before inoculation showed after completed growth an acidity of pH 5·2, and acidities of pH 5·0 are sometimes met with, or even 4·8. Subjoined is a graph (Fig. 2) showing the development of acidity in glucose broth produced by 3 strains of haemolytic streptococci, but it must be noted that the steepness of the curve depends to some extent on the condition of the inoculum. If the tube be inoculated from an ancient culture there is a considerable lag period before the cocci get to work in earnest, a lag absent when the inoculation is made from a vigorous young culture in the logarithmic phase.

In this graph the ordinates represent time after inoculation in hours and the abscissa acidities in terms of pH.

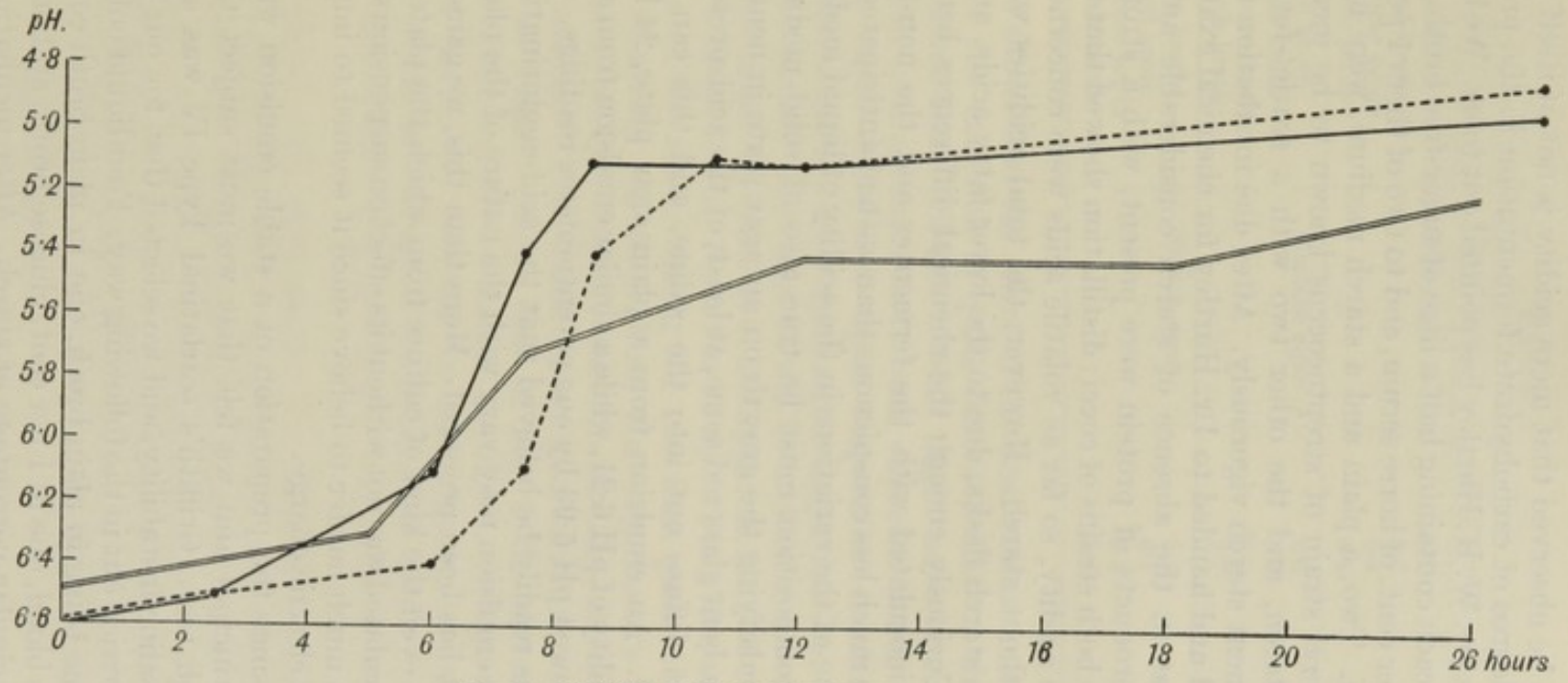

FIG. 2. ACIDITY CURVES IN GLUCOSE BROTH (1%)

It is here to be observed that mere acidity is not of itself a perfect guide to the degree of carbohydrate fermentation in the presence of raw protein, as Dr. W. H. Hurtley has pointed out to us. We had made up four flasks each containing half a litre of sugar-free broth. To each was added 1 per cent. of horse serum, and to two of them 1 per cent. of soluble starch. Two, a plain and a starch medium, were inoculated with a puerperal strain of streptococcus known to be incapable of fermenting starch, and the other two with a scarlet-fever strain known to ferment starch vigorously. After due incubation the flasks were sterilized and handed to Dr. Hurtley for chemical examination. He found that in the absence of starch considerable amounts of degradation products of protein were present, with a strong Millon reaction, with both strains of cocci: distillation showed that there was actually more acidity, so far as volatile acids were concerned, in the two flasks without starch. However, the total acidities were much greater in the starch flasks, due to the lower fatty acids, and also to lactic acid. Curiously enough the chemical differences between the starch flasks inoculated with the fermenter and the non-fermenter of starch were much less conspicuous than we had anticipated.

What is true of the variations in the acidity of liquid media according to their composition must be true also of solid media. In the process of emulsifying the growth on an agar plate in neutral saline by means of a bent glass rod some, at least, of the acid present in the medium must diffuse out into the saline, and this can easily be demonstrated. An emulsion from a plain agar plate, 24 hours old, showed an acidity of pH 6·21, while a similar emulsion from a legumin-agar plate showed pH 5·93 by our potentiometer readings.

It may thus readily be believed that the acid-agglutination results yielded by an emulsion may vary with the nature of the plate culture from which it has been prepared. More than this, we gained the impression that even the kind of culture from which the plate had been originally inoculated was not without its effect on suspension stability—a proposition much harder to believe since it seemed to imply a kind of heredity of electric charge.

However, since the preparation of a stable emulsion was, at this stage, our principal aim we felt that we must subject the matter to a thorough test. Griffith's scarlatinal Type IV was a strain of pronounced saline instability, and we selected that for our first tests, which were carried out in the following way. Four liquid cultures were first made, one being in plain broth, one in plain broth containing a little rabbit's blood, one in 1 per cent. glucose broth, and one in broth containing a similar percentage of starch. After incubation of these four cultures overnight, four plain agar plates were prepared, one from each of the four kinds of broth, and similarly four 1 per cent. glucose-agar, and four legumin-agar plates. From each of the twelve plates, suspensions were prepared, with the same lot of sterile saline; we thus had twelve suspensions each differing from the rest in its cultural history during the last two generations. The results as regards acidity of emulsion are shown in the following table:

TABLE I

Penultimate Medium.	*Final Medium.*	*pH of Emulsion.*	*Average pH.*
Plain broth	Plain agar	6·5	6·42
Blood broth	,,	6·4	
Glucose broth	,,	6·4	
Starch broth	,,	6·4	
Plain broth	Glucose agar	5·6	5·75
Blood broth	,,	5·7	
Glucose broth	,,	5·9	
Starch broth	,,	5·7	
Plain broth	Starch agar	5·8	6·0
Blood broth	,,	6·1	
Glucose broth	,,	6·1	
Starch broth	,,	6·0	

The table shows that the effect of the final culture medium upon the acidity of the emulsion is quite definite, that of the penultimate medium much less. All this is what might have been expected, but what might not, perhaps, have been expected is the effect of these relatively small differences upon suspension stability, upon acid agglutination, and still more upon serum agglutination. How astonishing the last-named effect may be will be understood from the fact that in the experiment shown, Fig. 4, the apparent titre of the homologous serum was found to vary from 1 in 75 up to 1 in 1730, according to the treatment of the strain during the two final generations. We shall have to illustrate the facts from our experiments with Griffith's scarlatinal Type I, as Type IV was too unstable in saline to allow of reliable readings. Figs. 2 and 3 are constructed on exactly the same principle as Fig. 1, except that in Fig. 3 the increases in acidity proceed by jumps of pH 0·5 instead of pH 0·3. In Fig. 4 there is added a series of horizontal columns on the right, indicating the different titres one would have assigned to the serum in reading by the ordinary Dreyer method, according to the previous cultural treatment of the emulsion.

Fig. 3 shows acid agglutination by our ordinary technique. No differences of any significance are seen to be produced by the penultimate culture, but the differences due to the composition of the final culture plate are marked. Legumin agar gives an emulsion with a much greater intensity of agglutination, yet one extending no further on the neutral side than is customary. The column at pH 7·0, which may be taken as a control, shows no trace of any tendency to spontaneous agglutination.

Much more important are the serum-agglutination results shown in Fig. 4, obtained with the same emulsions as those used in Fig. 3. The serum used was, of course, our stock Type I serum, homologous with the strain under test. Only 3 grades of agglutination are shown, and the graph does not, in all respects, correspond with the calculated titres on the right-hand side, which are based on the more numerous degrees of agglutination in the original readings. It is seen that whereas in that part of Fig. 4 which shows serum dilutions, the

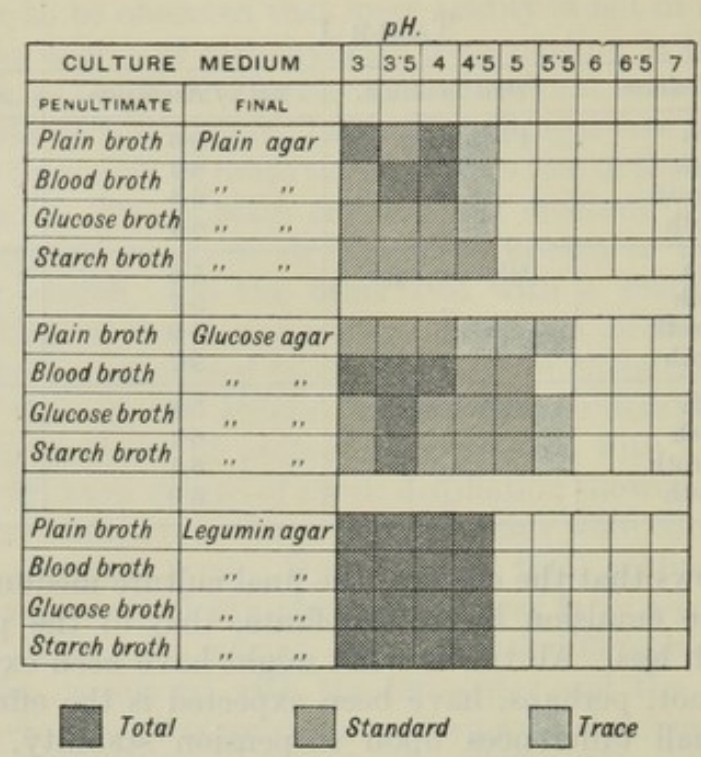

FIG. 3. Type I. Scarlet Fever Streptococcus.
INTENSITY OF ACID AGGLUTINATION
according to previous treatment

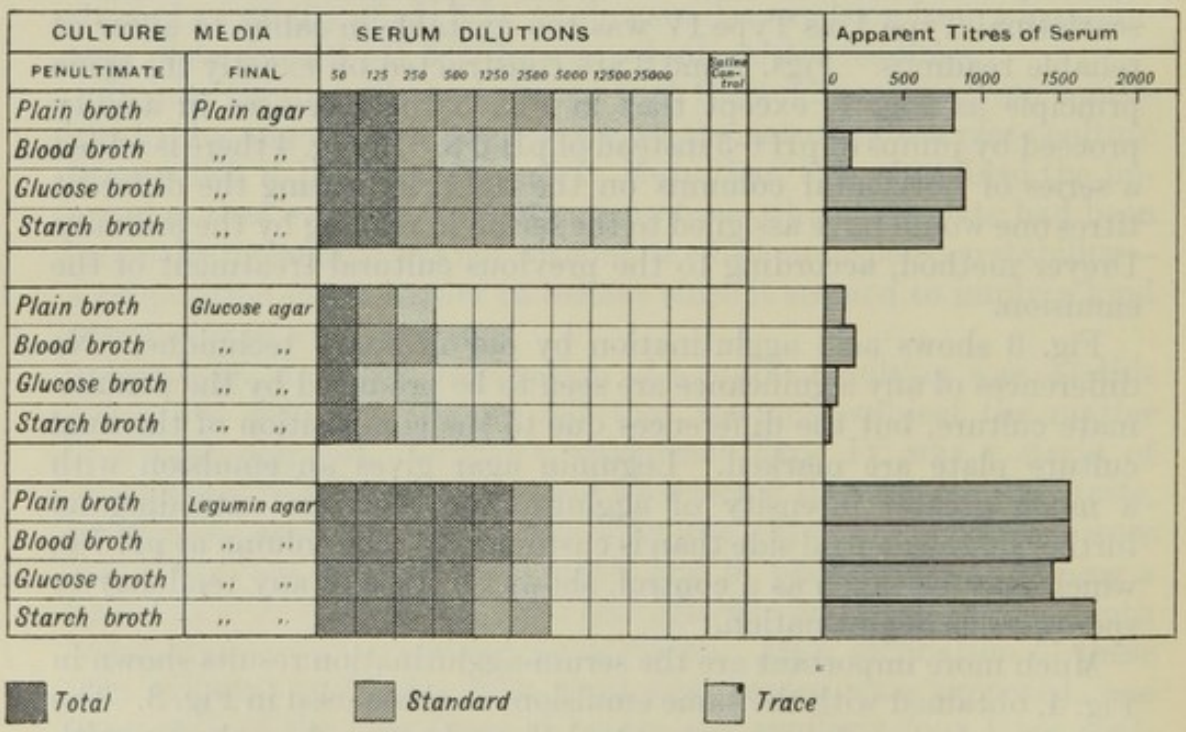

FIG. 4. Type I. Scarlet Fever Streptococcus
INTENSITY OF SERUM AGGLUTINATION
according to previous treatment

influence of the penultimate medium is distinct, its effect upon the apparent titres of the serum as shown on the right-hand side of the figure is much more so. As regards the final medium it is seen that glucose agar seems the least, and legumin agar the most favourable medium to use; with the latter the agglutination is more intense and gives a sharper end-point, while the saline control is as blameless as in the other emulsions.

Results such as these were so surprising to us and seemed so likely to lead us nearer the goal of a stable emulsion, that we pursued the subject further. In all we have employed the above penultimate and final methods of culture on 12 different strains of haemolytic streptococci, testing the effects on acid agglutination and, in the 6 strains of which we possessed sera, on serum agglutination also. The results were often erratic, but on the whole they led us to certain conclusions which have stood the test of experience. They are as follows: (1) suspension stability is definitely greater in emulsions prepared from glucose-agar plates than from plain agar. This leads to more perfect controls, and one feels greater confidence in the results of serum agglutinations, but on the other hand the sera show greatly lower titres. In acid agglutinations a similar but less marked result is seen—the agglutination reaching less far on the alkaline side. (2) Emulsions from starch-agar plates exhibit a weaker suspension stability than those from glucose-agar. The emulsions are liable to come down in saline alone and serum titres are perhaps unduly high. On the supposition that suspension stability depends upon electric charge it would seem that growth on glucose agar (and also in glucose broth) yields an emulsion in which the micro-organisms are richly supplied with free surface electrons, while after growth on legumin agar the supply of such electrons is more scanty.

The praises of glucose, as an ingredient in media for growing haemolytic streptococci, have been sung by many workers, and we ourselves would join in the chorus. There can be little doubt as to the reason for its virtues, which is to be sought in the active acid production attendant on its fermentation, which is even greater than in the fermentation of starch. Moreover, all these streptococci ferment glucose, while only a few break down starch.

There is a way of putting this matter of acidity to the test. The curves of acid production, as seen in Fig. 2, show the rapidity of the acid change during the logarithmic phase of growth, and we can 'tap' a glucose-broth culture at short intervals during the rise, making agar plates from the samples removed, and comparing the acid agglutination of the emulsions made from them. Fig. 5 exhibits in duplicate the result of one such experiment, in which the acidity of the standard solutions proceeds by jumps of pH 0·2. The emulsions were tested both against Clark and Lubs series of solutions and against the acetic acid and sodium acetate series, and the results are seen to be much the same in both. A control column at pH 7 shows the absence of any tendency to spontaneous agglutination at any level. A plain blood-broth culture without any glucose acts as a

control in the uppermost horizontal row. The strain of coccus in this dual experiment is that which we shall term later 'Rough Cutbush'. The same emulsions were also tested against the 'Universal Buffer' solutions of British Drug Houses, but the results, though in general similar, were confused by traces of auto-agglutination about the neutral point. Of the two experiments shown in Fig. 5 those yielded by Sørensen's acetic fluids are the cleanest and most convincing. They indicate that with increasing acidity of the penultimate glucose culture the suspension stability, as judged by acid agglutination,

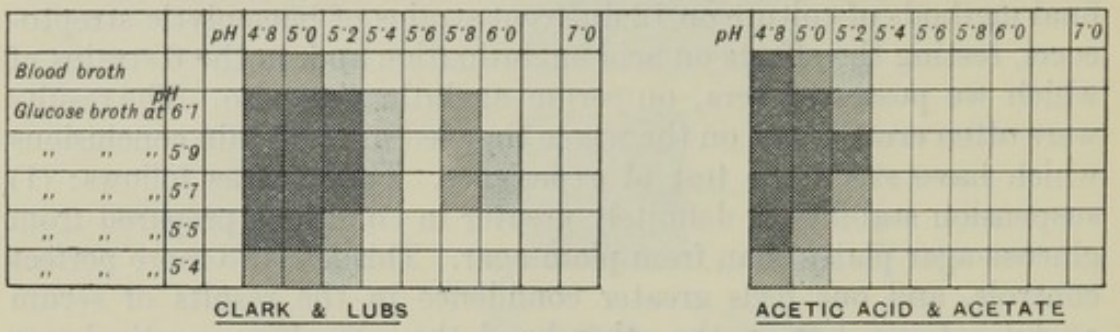

FIG. 5. VARIATIONS IN ACID AGGLUTINATION
in accordance with acidity of penultimate culture

diminishes at first, up to the point of pH 5·7, and then increases again. It looks as if the optimum moment for inoculating the final plate was when the penultimate glucose culture had reached the acidity of pH 5·7, when this culture was some 8 or 9 hours old (see Fig. 2).

We formed the opinion that the most advantageous stage at which to employ glucose was at the penultimate broth culture and not in the final agar plate. The serum titres found with emulsions grown on glucose agar are commonly so low as to be largely useless. One may have too much of a good thing, and it has happened to us when we have used glucose broth in the penultimate stage and glucose agar in the final stage, to get an emulsion which will stand up in saline with a suspension stability so extreme that it will not agglutinate even with its own homologous serum. The best means we have discovered of dealing with an obstinately salt-sensitive strain is to grow it in glucose broth and finally on legumin agar, a procedure which has more than once succeeded in our hands when all else has failed. Now if the virtue of glucose depends upon the acid reaction it induces, the question arises whether the same effects could not equally be brought about by the artificial addition of acid to the broth cultures.

Six plain-broth tubes were inoculated with a strain (Rough Cutbush), and on the following day their pH was found to be 6·1. Weak acid was now added (N/20 HCl) so bringing the pH in 5 of the tubes to 5·8, 5·5, 5·4, 5·3, and 5·0, while the sixth tube was left at 6·1 as a control. After standing an hour at room temperature, they were inoculated on to legumin-agar plates: all the plates grew equally well, and the resulting emulsions were compared as to acid and serum agglutination. The differences were slight, and, in the case of serum agglutination,

negligible, though in the acid-agglutination series there was a very slight decrease in suspension stability in the case of the suspensions from the tubes which had been brought to pH 5·3 and 5·4. Further experiments on these lines were, therefore, abandoned as unprofitable.

It was ascertained in another experiment that the deposit from a two-days old, glucose-broth tube, with a natural pH of 5·2, when washed in saline and resuspended was highly unstable, whereas a legumin-agar plate made from it yielded an emulsion which was perfectly stable and agglutinated normally with the homologous serum.

(iv) Technique finally evolved for our Serological Studies

A. *Preparation of Stable Emulsions.*

Basing our practice on preliminary observations such as those which we have now described, we presently evolved a technique for the preparation of streptococcal suspensions which has given us a high percentage of success. If we take as criteria of success the ability to remain stable in saline emulsion for 1½ to 2 hours in the water-bath at 55–56° C., combined with readiness of specific agglutination with the appropriate serum, we must confess that no technique we have employed has proved a certain panacea. But out of 140 strains of haemolytic streptococci of which we have full enough records, 67 (48 per cent.) have yielded, at one time or another, though often only after a good deal of patience, perfect suspensions, and a further 58 (42 per cent.) have yielded us suspensions which, if not absolutely perfect, could still be used for agglutination, while only 15 (or a little over 10 per cent.) proved absolutely intractable. Those who have worked with this troublesome group of organisms will concede that a 90 per cent. success, or approximate success, represents a not unsatisfactory achievement.

Our routine technique may be summarized thus:

(1) Stock cultures were made in blood broth from single colonies of the type required, selected under a low power of the microscope. These remained viable for many weeks.

(2) Penultimate cultures were made from these, as required, into peptone broth containing 1 per cent. of glucose.

(3) After not more than 24 hours' incubation at 37° C. the glucose-broth cultures were used to spread legumin-agar plates, using several large drops and being careful to leave one small area sparsely inoculated for subsequent microscopic study, to determine whether the plate is pure and uniformly of the type desired. The importance of the latter consideration will be apparent when we come to discuss dissociation.

(4) Plates were emulsified after 24 hours' incubation at 37° C. using a bent glass rod and a minimum of fluid (normal saline containing 0·1–0·2 per cent. of neutral formol), to be added a few drops at a time.

One should get 2 or 3 c.c. of an emulsion from a 4½ in. plate, and this must be freely diluted for agglutination purposes. A convenient density is 300 million per c.c. (substantially Tube 1 on the Brown[1] scale), and this will be further diluted on adding the other ingredients of the agglutination tubes. The acidity of emulsions thus prepared should lie between pH 6 and 7.

A long series of observations was made by one of us to see whether the value of an emulsion could be predicted by microscopic examination under an ordinary high power: both broth cultures and legumin-agar suspensions were examined. It was thought that length of chain might be a factor in suspension stability, but this seemed quite definitely not the case. Emulsions containing chains of 8 to 16 elements frequently stood up perfectly, while others, containing no longer chains than 2 to 4 elements, often proved of marked instability. The sign of an unstable emulsion proved to be the running together of the single elements, or short chains, into little clumps of 4 or 5. It was found that this microscopic appearance was invariably the herald of instability. We often tried the centrifuge as a remedy for a bad emulsion, but it was very seldom that it did much good, except when a few flakes were present in an otherwise good emulsion.

B. *The Control Tube.*

One other lesson has been enforced by our studies on the acid agglutination of streptococci; it is that of the vital importance of a perfect control tube. Some people have thought that the best control for a series of tubes in which a specific serum is being tested is a tube containing an equivalent amount of normal serum. We do not agree with this practice, because any sort of serum is apt to exercise a shielding effect upon the action of the electrolyte upon particles suspended in a fluid. It is quite a common thing for a series of tubes put up to show an ordinary serum agglutination to appear negative in those containing the strongest dilutions of serum and then to show progressively increasing agglutination as we get to the higher dilutions. A control tube containing only electrolyte shows this up for what it usually is—spontaneous agglutination; one would feel more doubt if protein had been introduced into the control tube also. By a 'perfect' control we mean one which shows no trace of granularity even under a hand lens with good dark-background illumination. It is surprising to find what a difference appears in the apparent titre of a serum, when there are even the smallest traces of granularity in the saline control. In Fig. 4 we have shown such differences even with perfect controls. At the risk of repetition we will now give the figures for another precisely similar experiment with a different strain of coccus (see Table II).

In 11 of the 12 tubes the controls are perfect: it can hardly be due to chance that in the last tube but two, where the control is not quite perfect, the titre of the serum has to be calculated at something like double that of the adjacent figures.

[1] Cf. Brown and Kirwan (1915).

TABLE II

Penultimate Culture.	*Final Culture.*	*Saline Control.*	*Apparent titre of Serum.*
Plain broth	Plain agar	0	1 in 500
Blood broth	,,	0	,, 330
Glucose broth	,,	0	traces
Starch broth	,,	0	1 in 80
Plain broth	Glucose agar	0	,, 80
Blood broth	,,	0	,, 30
Glucose broth	,,	0	traces
Starch broth	,,	0	1 in 570
Plain broth	Legumin agar	0	,, 1600
Blood broth	,,	Slight traces	,, 2800
Glucose broth	,,	0	,, 1300
Starch broth	,,	0	,, 1350

When, a few pages back, we spoke of our results in the preparation of stable emulsions we spoke of 48 per cent. of them as 'perfect' and a further 42 per cent. as being not absolutely perfect—but yet 'usable'. It can now be seen what we meant. Upon the one result we should venture to base a secure conclusion, from the other we should gather guidance only but often guidance of great help.

We ought, perhaps, to apologize for the large amount of space we have devoted to suspension stability, but it is a space not incommensurable with the importance of the matter. Although we have by no means solved all our difficulties we venture to think that we have pointed out some troubles which others may thus be enabled to avoid.

C. *The Preparation of Agglutinating Sera.*

We found no serious difficulties here as soon as we ceased to hearken to the voices of those who maintained that it was necessary to use living cocci for immunization. It is not necessary, it is not humane, and it is wasteful of life. Our experience with living cocci was that more than half our rabbits died.

We found that high titre sera could be produced easily and quickly by the intravenous, or even by the subcutaneous route, with killed cocci. The procedure was as follows. The streptococci were grown on legumin-agar plates because this medium yielded vigorous growth. Emulsions were made in normal saline containing 0·1 or 0·2 per cent. of formol. By using a number of plates and only a small volume of fluid a very dense emulsion could be made. The number of cocci was estimated by abstracting a few drops and diluting down to something within the limits of Brown's scale. The original emulsion was then diluted down to 3,000 million per c.c. and this was preserved in the ice chamber till sterile, which required about a week. The product constituted our standard emulsion and could be used not only for immunizing, but was often sufficiently stable to be diluted further for purposes of agglutination, remaining unchanged for many weeks.

Rabbits were immunized by intravenous injection of the undiluted (3,000 million) emulsion on 3 days a week—the dose rising from 0·1 c.c. at the beginning of the course to 2 c.c. at the end. A series of 12

injections was found enough, after which the animals were allowed to rest for 10 days and then bled from the ear vein. Titres of from 1 in 2,000 to 1 in 5,000 were usually obtained, and, if desired, a second course of immunization could be carried out later. The sera were preserved in the ice chamber in the dark after the addition of 0·5 per cent. phenol, and were available for use for 2 or 3 years, though with slow loss of titre.

It has been asserted as a special difficulty attending agglutination work amongst the haemolytic streptococci that 'the response of the animal to immunization is generally "group" in character, while the type-specific agglutinins present in the serum may be negligible in amount' (see section on haemolytic streptococci in the *Annual Report of the Chief Medical Officer of the Ministry of Health for 1930*, p. 190). With such a generalization we feel unable to agree. It may be true in those cases in which an animal is immunized with a crude strain of streptococcus without regard to the properties of the individual colony from which the immunizing emulsion was bred. But, as we shall show later, the phenomenon of dissociation is well marked in these cocci, and one finds, on a pure plate, colonies, often different in aspect, and widely different in serological properties, some having group and some specific features. By making a sufficient stock of formolized emulsion from an individual colony and carefully testing its properties, we have found that a rabbit, immunized throughout with the same emulsion, yields us a serum which reflects with fair accuracy the properties of the coccus used. Out of 18 sera, all but one of our own manufacture, 11 have been reasonably specific while 7 have presented preponderant group characters. And this has, for the most part, been intentional: it is perhaps too much to say that we have been able to make specific or group sera at will, but we have gone a good way towards it.

D. *The Technique of Agglutinin Absorption in Work with the Haemolytic Streptococci.*

We brought to this work a strong conviction of the importance of strictly quantitative methods. We used throughout Dreyer's technique, the application of which to the study of haemolytic streptococci we have already discussed (p. 13). For a long while, it is true, our absorbing doses, though accurately measured, were not uniform from case to case, but even by this rough method it quickly became apparent that some strains of haemolytic streptococci were highly specific and others the reverse. To this matter we shall recur later, but we must first set forth the methods of absorption finally adopted.

The absorbing dose was first determined by absorption of a serum with its homologous coccus in graduated doses. The manifest advantages of a system of absorptions by graduated doses of the antigen over a single dose led us ultimately, as will be explained later, to adopt this plan in all cases. The principle is to absorb similar volumes of serum with increasing doses of cocci and to plot out the results on squared paper—as a curve of absorption: for purposes of comparison

it is convenient to calculate the figures as percentages of the initial titre of the serum (see Table IV and Fig. 6). This method of working will be illustrated later by graphs. Here we will give the percentage figures in a graduated absorption of Griffith's Type I scarlatinal serum

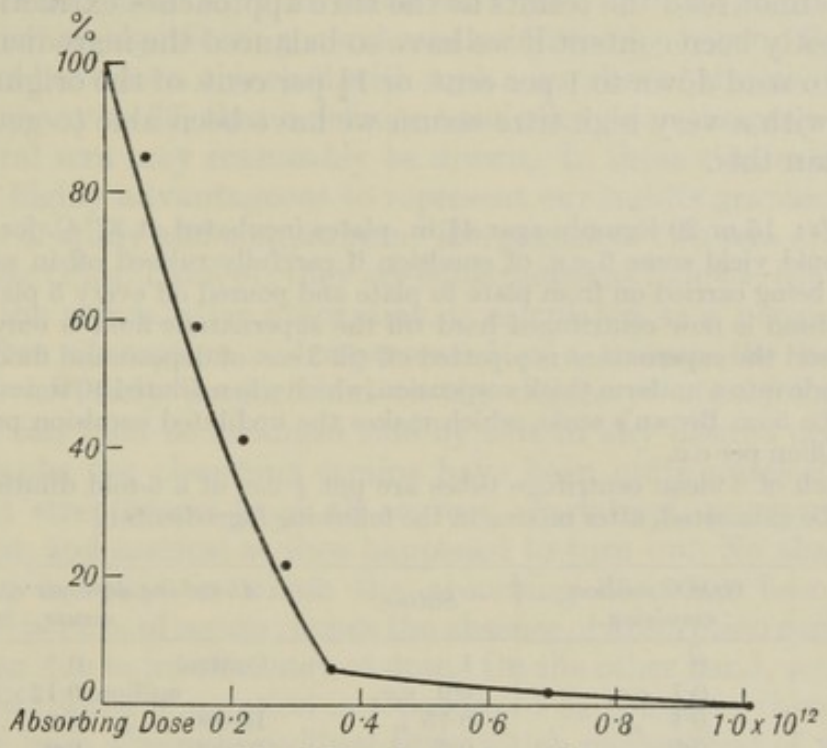

FIG. 6. Homologous absorption
Griffith's Type I serum absorbed with Type I

by its own coccus. Doses are stated in 10^{12}s per c.c. of *pure serum*.

Fig. 6 shows a favourable example of a graduated absorption; the evenness of the curve speaks well for the technique, but one cannot always get such good results. It is seen that in this case a dose of

TABLE IV

Absorbing doses.	*Calculated titre.*	*Residual percentage of specific agglutinin.*
Control	1 in 2,130	100 %
Dose $0{\cdot}07 \times 10^{12}$	,, 1,816	86
,, 0·14 ,,	,, 1,250	59
,, 0·21 ,,	,, 865	41
,, 0·28 ,,	,, 450	21
,, 0·35 ,,	,, 159	4·7
,, 0·70 ,,	,, 33	1·5
,, 1·05 ,,	,, <28	<1·0 %

350,000 million cocci per c.c. of serum has been insufficient to secure complete absorption; 700,000 million has been almost enough, but it takes $1{\cdot}05 \times 10^{12}$ to exhaust the last traces (within the limitations of the technique employed). In other cases of autologous absorption doses of 300,000 million to 400,000 million have proved sufficient. Very similar doses are required for homologous but not actually autologous absorptions.

The question arises how such colossal doses can be best attained in practice with scantily growing organisms such as streptococci. One secures as much growth as one can, but one's main resource lies in dilution of the serum. The latter, however, cannot be carried very far, or one cannot read the results as the titre approaches extinction. We have mostly been content if we have so balanced the ingredients as to be able to read down to 1 per cent. or 1½ per cent. of the original titre, though with a very high titre serum we have been able to get a little lower than this.

Example: 15 or 20 legumin-agar 4½ in. plates incubated at 37° C. for 24 or 48 hours should yield some 6 c.c. of emulsion if carefully rubbed off in saline, the washings being carried on from plate to plate and poured off every 5 plates or so. This emulsion is now centrifuged hard till the supernatant fluid is only slightly clouded, and the supernatant is pipetted off till 3 c.c. of deposit and fluid remain. This is made into a uniform thick suspension, which when diluted 40 times ought to match tube 5 on Brown's scale, which makes the undiluted emulsion practically 60,000 million per c.c.

Into each of 6 clean centrifuge tubes are put ¼ c.c. of a 5-fold dilution of the serum to be exhausted, after mixing in the following ingredients:

	60,000 *million emulsion.*	*Saline.*	*Absorbing dose per c.c. pure serum.*
Tube 1	0	1	Control 0
2	0·1 c.c.	0·9 c.c.	million $0{\cdot}12\times10^{12}$
3	0·1 ,,	0·75 ,,	15,000 ,, 0·3 ,,
4	0·5 ,,	0·5 ,,	30,000 ,, 0·6 ,,
5	0·75 ,,	0·25 ,,	45,000 ,, 0·9 ,,
6	1 ,,	0	60,000 ,, 1·2 ,,

The ingredients are mixed in the following order: saline, emulsion, and lastly, the serum. The total bulk of fluid in each tube is 1·25 c.c. The serum dilution is 1 in 25, enabling one to read exhaustions down to about 1½ per cent. of the value of a 2,000 titre serum.

The centrifuge tubes are allowed to stand on the bench overnight, and are then whirled till the supernatant fluid is clear, when it is pipetted off and titrated. There is always enough of the fluids to test against the strain with which the serum was prepared, which is the chief thing that matters.

It is, of course, a drawback that the amount of fluid is so small, since this increases the risk of errors of measurement, but with practice we have found ourselves able to secure sufficiently uniform results, and when the readings of a graduated absorption are plotted as a curve, gross errors are at once betrayed. Another drawback of the small volume of fluid is that, when once it has been titrated against the strain with which the serum was prepared, only an insufficient amount remains for complete titration against any other strain, a thing which in the analysis of a complex entity one would often be glad to carry out.

3. RESULTS OF ABSORPTION EXPERIMENTS

(i) Absorptions with Single Adequate Doses

The preceding account of the method of graduated absorptions was needful to make clear what constitutes an adequate absorbing dose.

We have now to pass back to the results we have obtained by absorption with a single large dose such as 0·5 to $1{\cdot}0 \times 10^{12}$ cocci per c.c. of pure serum.

The total number of sera we have tested by the single absorption method is 27,[1] but many of these have been tested against so few strains that no significant general conclusion can be drawn. But 15 of these sera have been absorbed with such a number of strains (from 12 up to as many as 66) that a definite conclusion as to the properties of the several sera may reasonably be drawn. In these studies we have found it highly advantageous to represent our results graphically for purposes of study and comparison. The graphs of two sera are reproduced here (Figs. 7 and 8); they are constructed as follows: the specific titre of the serum, after absorption, is calculated as a percentage of that before absorption and represented as a column the height of which is inversely proportional to the degree of absorption. The columns can then be arranged side by side in any desired order. In these graphs the absorbing strains have been quite unselected and represent streptococci from all sources, scarlatinal, puerperal, erysipelatous, and surgical as they happened to turn up. No absorption has been included in which the absorbing dose has been below $0{\cdot}5 \times 10^{12}$ per c.c. of serum; hence the absence of absorption can hardly have been due to insufficiency of dose. On the other hand, we cannot affirm that when absorption is represented as complete there might not have been a minute residual titre, which our method of working did not allow us to detect.

A. *Specific and Group Sera.*

The two figures here chosen for reproduction are somewhat extreme examples of specificity and unspecificity in a serum, and the methods of working and representation show what wide differences may exist amongst sera in this respect.

As regards Griffith's Type II (Fig. 7) it is seen that of the 39 miscellaneous strains used for absorption, only 1 effects anything approaching complete absorption, and even here there is a small residue of specific agglutinin. Curiously enough this effective strain was an erysipelatous one (Eagles No. 3 in the National Collection of Type Cultures). On the other hand, 33 out of the 39 entirely failed to reduce the specific titre of this serum in any degree and 3 more did so to such a slight extent as to be almost within the limits of experimental error.

The example of Griffith's Type III serum (Fig. 8) offers a strong contrast. The absorbing strains were substantially the same in the two cases (though not arranged in the same order in the two figures), but here it is seen that no less than 35 out of the 39 miscellaneous strains effect a marked reduction in the titre of the serum, and 10 of them appear to effect complete exhaustion. One must infer that those

[1] The majority of these sera have been of our own manufacture, but we owe a number of them to the generosity of Dr. F. Griffith of the Ministry of Health.

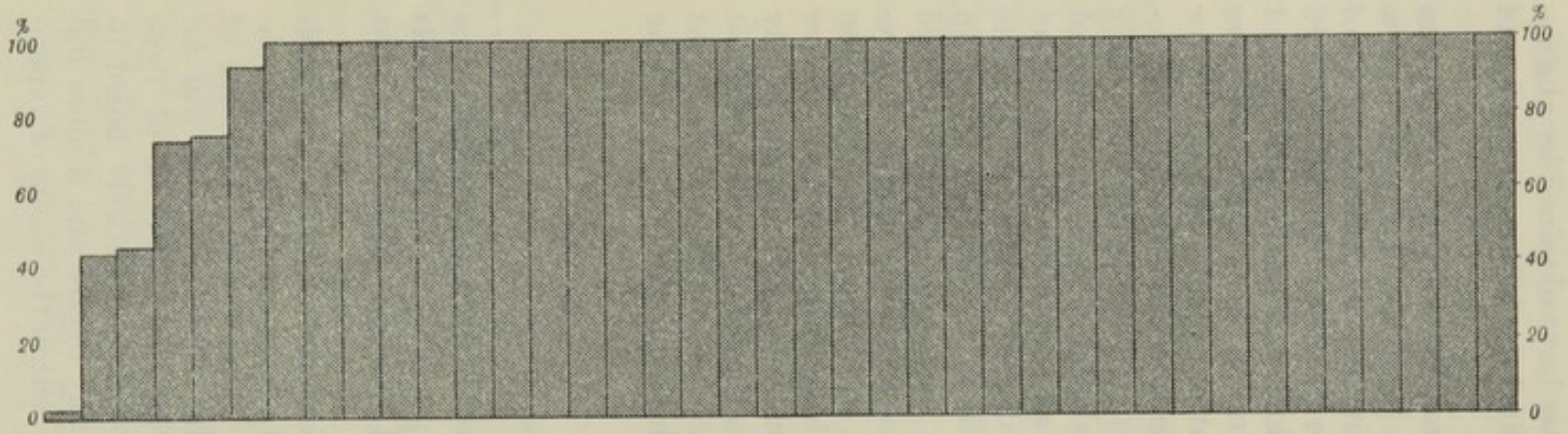

FIG. 7. SERUM: TYPE II (Scarlatinal) Griffith
Example of a highly specific strain

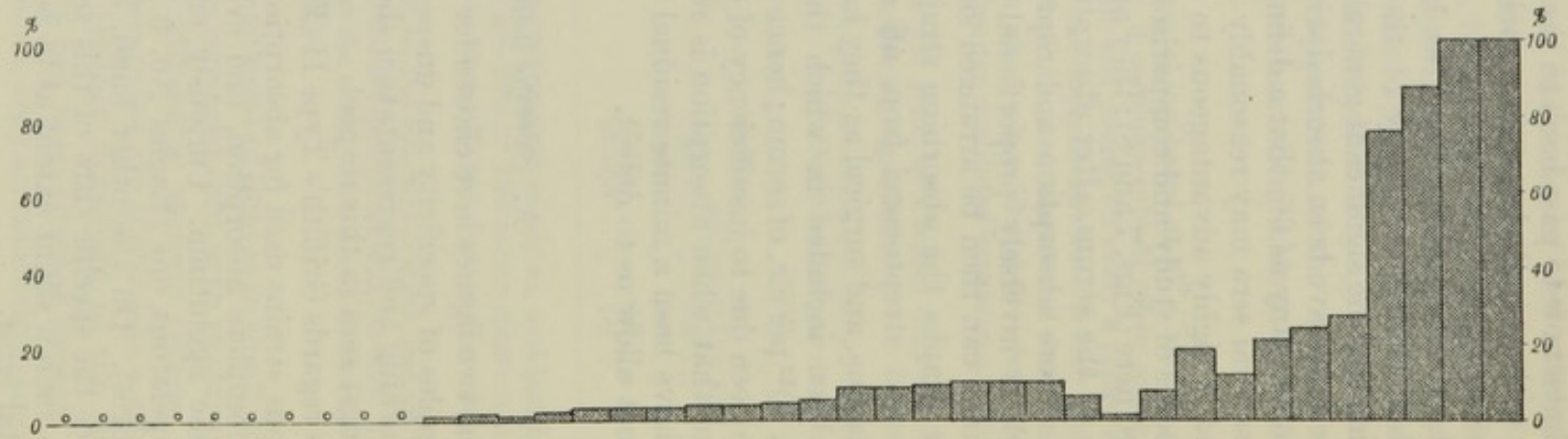

FIG. 8. SERUM: TYPE III (Scarlatinal) Griffith
Strain Lewis, highly unspecific

few strains which hardly lower the titre of this serum contain hardly any of the group antigen of which this Type III strain so largely consists.

There are other sera intermediate between those depicted in Figs. 7 and 8, neither so rigorously specific as the one, nor so unspecific as the other. For example, a serum which we made from Griffith's Type I scarlatinal strain, when tested by absorption with large doses ($0{\cdot}5 \times 10^{12}$ or more) of 62 miscellaneous strains showed no reduction of specific titre in about half the experiments, but in 20 instances this titre was lowered to from 25 to 75 per cent. of its original value; in 6 cases absorption was so nearly complete as to suggest identity of the absorbing strain with Type I. It looks as if there were more group antibody in Type I than in Type II serum, but the explanation of the facts is difficult, and any discussion may conveniently be deferred till we come to consider the results of graduated and mirror absorptions.

Griffith's Type IV scarlatinal type has yielded, in our hands, a serum very nearly as sharply specific as Type II. Hence, of these four main scarlatinal types—in this country at least, the best-known strains of haemolytic streptococci—our observations fully confirm those of Griffith as regards the type-specificity of Nos. I, II, and IV.

Type III presents a more difficult problem: we have failed to isolate from several examples, kindly given us by Dr. Griffith, any strain, or to prepare any serum, presenting anything approaching the type-specificity of the other three, though Dr. Griffith has given us a strain and serum which is certainly somewhat more specific than those we have prepared. Facts similar to those just recorded of the four main scarlatinal types have been met with amongst the much larger series of strains studied by us outside these limits.

B. *Absorbing Powers.*

There is another way of studying the antigenic constitution of a strain, namely the range of absorbing power which it exhibits amongst a number of other sera. Instead of grouping our facts according to the individual sera, we can construct graphs on the same principle as those already exhibited, but grouped according to the absorbing strain.

Amongst the sera which we have had available for such trials there have been some that were highly specific and others 'group' in character. One might anticipate that a group serum would be exhausted of its agglutinin by (1) other group strains and (2) by any specific strains which possessed as antigenic component a sufficient amount of group antigen (which most of them do possess—even so specific a strain as Type II). But we should expect a specific serum to be unaffected in titre by any purely group strain and by any specific strain other than those if its own kidney. And this is practically what we find to happen.

Thus, still confining ourselves to Griffith's Types, we find the serum of Type III to be easily exhausted not only by all examples of Type III, but also by Types I, II, and IV, while no Type III strain exercises

the slightest effect on the titres of Types I, II, and IV sera. We are confronted with perfect examples of unilateral absorption. Our other examples of purely group sera behave in precisely the same way. But a fact which continues to puzzle us is that a number of outside strains, unrelated to Type I, do, as a fact, reduce the specific titre of a Type I serum by 50 per cent. or more. This is a matter which we must discuss later.

It must be noted, at this point, that the conclusions to be drawn from the behaviour of an absorbing strain and of an absorbed serum are of a somewhat different kind. A serum is a static thing, not liable to vary qualitatively, but only to deteriorate quantitatively as time goes on. But a living coccus is subject to qualitative variation owing to internal mutation in successive subcultures. We can have no guarantee that a present subculture is identical in its properties with a stock culture from which it has descended, as we shall now see in discussing the subject of 'dissociation'.

C. *Dissociation.*

As long ago as 1922, Miss Cowan published observations on the occurrence of two forms of colony amongst streptococci. It was known that these organisms showed sometimes turbidity in broth and sometimes a deposit with a clear fluid above. Miss Cowan (1922), working with a number of strains, chiefly of the haemolytic form, succeeded by a process of selective subculture in isolating what she termed 'smooth' and 'rough' colonies—the smooth ones yielding a uniformly turbid growth in broth and the rough ones a deposit at the bottom of the tube. It took her 4 months before the differences became so stable that they could be relied on to appear in every culture. Both forms were equally haemolytic, but Miss Cowan found the smooth forms of much higher virulence for the mouse than the rough ones, though the latter sometimes gave rise to abscesses at a late stage.

Towards the close of 1925 we had in our possession a highly virulent strain of streptococcus from the finger of a nurse named Miss Shilling. Dr. R. R. Armstrong, to whom this strain had been passed on, informed us that he had found on plate cultures of this coccus well-marked examples of smooth and rough colonies intermingled, but that he found the rough colonies far more virulent for mice than the smooth. These observations we confirmed. There was no need for any selective subculture, the differences in the colonies were apparent by transmitted light with a hand-lens, or even with the naked eye. The correspondence with Miss Cowan's description was complete except for the reversal of virulence—we found the rough strain some 200 times more virulent than the smooth, and for this reason we could not at first reconcile our findings with those of Miss Cowan.

We at once proceeded to extend these observations to other strains, and from most we succeeded in obtaining dissociation into colonies of two kinds, with some intermediate varieties. We will refer to them as rough' and 'smooth' colonies. When we put the matter to the test

with suitable agglutinating sera we at once obtained evidence of serological unconformity between the roughs and the smooths of a single strain, and we selected 4 strains for more intensive study. Before, however, we give the detailed results of this study it will be well to refer to the corroborative evidence of dissociation obtained by other workers. In 1927, F. Griffith, describing the characters of the streptococci from the scarlatinal throat as seen on blood-agar plates, refers to colonies of three kinds, (1) the commonest is opaque and flat with a rough surface and a tough texture, so that it may be picked up as a whole from the surface of the agar, (2) the second, at first resembling No. 1, never becomes tough, but remains soft in consistency, with a translucent margin, (3) the third is described as a 'watery' colony resembling a shiny transparent dome, not, apparently, breeding true on subculture. Strains of Types I and II habitually yield colonies of the first variety; those of Type III of the second. Subcultures of the coherent and of the watery type did not, in Griffith's hands, show difference in virulence or in serological properties.

E. W. Todd (1927) also finds colonies of two chief kinds amongst these streptococci. He quotes, and supports, an observation of Fleming's that when grown on plates of blood agar, poured at 80° C., one finds 'matt' and 'glossy' colonies. The matt colonies have central eminences and clearly defined margins; grown on broth they form a granular deposit; they are virulent to mice. The glossy colonies are rounded and give uniform turbidity in broth; they are less virulent to mice than the matt ones. Todd was unable to identify his two forms of colony with those of Miss Cowan, but solely because of the reversal of virulence.

G. H. Eagles (1928) describes and figures colonies of several different kinds in the study of 2 strains of haemolytic streptococci—(*a*) a toxic erysipelas strain from New York and (*b*) the 'Aronson' strain of 1903 maintained in the Hygienisches Institut at Berlin. Broadly, Eagles finds two chief types of colony, rough and smooth, but by selective subculture, alternately in serum broth and on serum agar, he found himself able to intensify the characters of these. In the evolution of the rough series he obtained, in order, the following stages: (1) papillary colonies, (2) wrinkled, (3) opaque, and (4) rough. The smooth strains could similarly be made smoother by selective subculture and possessed only short chains, whereas the rough ones had very long and tortuous chains both in liquid and on solid media.

Eagles found the rough forms far more readily saline-agglutinable than the smooth; he gives the following figures:

'Rough' colonies	agglutinated	by	0·1	per cent. NaCl.
'Opaque'	,,	,,	0·2	,,
'Wrinkled'	,,	,,	0·4	,,
'Smooth'	,,	,,	0·4–0·5	,,

As regards haemolysis and toxin production he found no serious differences between rough and smooth forms, but as regards virulence to mice his results are instructive. In the case of the New York

erysipelas strain he found the smooth form somewhat more virulent than the rough, but in the case of the much more highly lethal Aronson strain the rough form was enormously more virulent than the smooth. It was concluded that no necessary correlation existed between virulence and colonial form.

D. *The Differing Serological Properties of the diverse Colonial Forms.*

From the evidence of so many witnesses it must be admitted that these differing forms of colony have a real existence amongst the haemolytic streptococci. None of the authors quoted, however, has been much concerned with the serological differences between them. And yet such differences are often striking and go a long way to explain the difficulties which beset the serological classification of these organisms.

We have mentioned that, when we first found these evidences of dissociation, we selected 4 strains for intensive study. These were as follows: (1) the *Dochez* strain, originally isolated in New York in 1923 from a scarlet-fever case and since widely distributed in laboratories on account of its high toxigenic value. (2) the *Shilling* strain from a grave case of wound infection in a nurse, highly virulent for mice and capable of forming a toxin which though more potent than that of most pyogenic strains, was, nevertheless, inferior to that of the best scarlet-fever strains. (3) and (4)—these were strains cultivated from the blood; one from a patient named *Napier* was from a case of septicaemia in otitis media: in the other, *Cutbush*, the primary source of the septicaemia was not known. These strains did not all present an equally strong contrast between the 'rough' and 'smooth' colonies. In the Shilling strain the differences were very striking, while in the Dochez strain they were much less distinctive though quite appreciable. We later came to recognize that there are degrees of 'roughness', and we do not doubt that the quality is one which may be emphasized by selective subculture, as was found by Cowan and by Eagles.

We must, however, place on record that whereas these strains readily yielded rough and smooth forms when first isolated they largely ceased to do so with lapse of years. We originally isolated our rough and smooth variants in 1926, but when in 1930 and 1931 we endeavoured to repeat the observations, we had great difficulty in doing so, only succeeding after several attempts. They had now settled down on prolonged subculture into a moderately rough condition of which the emulsions stood up fairly well in saline.

Having selected a rough and a smooth colony of the 4 strains under study a good supply of dense emulsion was prepared from each and formolized (0·1 to 0·2 per cent.); it was then ascertained that these four pairs of emulsions still retained the differential properties of the colonies from which they had been prepared (at this stage, by their respective reactions with the sera we already possessed). Agglutinating sera were then prepared from each and were found when first prepared to have homologous titres as follows:

Rough Dochez 1 in 2,750	Smooth Dochez 1 in 1,250
,, Shilling no reading	,, Shilling 1 in 2,600
,, Napier 1 in 7,550	,, Napier 1 in 1,650
,, Cutbush 1 in 3,200	,, Cutbush 1 in 1,600

When, however, these sera were tested upon all 8 emulsions very remarkable results were obtained, as shown in Table V. The figures

TABLE V

Sera.

Strains.	*Rough Dochez.*	*Rough Cutbush.*	*Rough Shilling.*	*Rough Napier.*	*Smooth Dochez.*	*Smooth Cutbush.*	*Smooth Shilling.*	*Smooth Napier.*
R. Dochez	1,800	0	0	0	0	0	0	0
R. Cutbush	500	2,500	2,800	1,725	800	2,500	2,800	1,280
R. Shilling	50	0	1,500	0	50	0	0	0
R. Napier	0	0	0	1,250	0	110	565	75
Sm. Dochez	0	0	0	0	570	800	1,400	870
Sm. Cutbush	0	0	0	0	256	376	675	570
Sm. Shilling	0	0	0	0	500	1,250	1,450	1,250
Sm. Napier	0	0	0	0	600	1,800	2,800	1,660

there given were obtained in December 1927, using the same sera, but freshly prepared emulsions. The results are in substantial agreement with those earlier observed, but there is one anomaly to note. At this later date the Rough Shilling strain was behaving badly in two ways: it was so strongly saline-agglutinable that its emulsions could not be used unless the cocci were washed in acetone, and it would not come down with its own serum unless the latter had been extracted with kaolin—a procedure which we have more than once found effective in removing some unknown inhibiting agency in a serum. The results obtained after these doping processes may be in a measure incommensurable with those yielded by the other 7 emulsions, though they are probably not far from the truth.

The following unexpected facts were revealed:

(*a*) In all 4 strains the rough and smooth forms exhibited serological unconformity: in no case was the smooth race agglutinated by the rough serum, while in the case of 2 strains, Dochez and Shilling, the rough race was not agglutinated at all by the smooth serum, and in a third case—Napier—only to a small fraction of the full smooth titre of the serum. In the fourth strain—Cutbush—the rough form was agglutinated equally by its rough and smooth sera.

(*b*) There appeared to be much in common between the smooth forms of all 4 strains—all being fairly well agglutinated by any one of the smooth sera. This was not so with the rough forms, which (with the exception of the Rough Cutbush form) showed a considerable degree of specificity.

At first it was believed that the rough forms were the specific ones and the smooth ones group forms with much in common, and at the

meeting of the Pathological Society of Great Britain and Ireland in January 1928, one of us (Andrewes, 1928) made a preliminary communication suggesting this point of view. Further experience, however, showed that the matter was not so simple, and when we came to study these sera more elaborately by the absorption method the Rough Cutbush form was found to be amongst those which presented 'group' properties in a pronounced form (being substantially identical with Griffith's Type III), while in lack of specificity the Rough Shilling strain fell not far short of it. The smooth race of Cutbush actually exhibited a higher degree of specificity than the rough.

It may be added that in June 1931 the preceding observations were repeated with the same sera—now much debased in titre—but with fresh emulsions which may well have now presented somewhat different properties to those which they had possessed 3½ years previously. The results were on the whole surprisingly similar to those obtained before, though naturally some discrepancies were observed.

As regards saline stability we have found the smooth forms to give uniform turbidity in liquid media and to emulsify well in saline, while the rough forms showed as a rule, to which there were not infrequent exceptions, the reverse properties. These differences are at least partly due to differences in length of chain—the smooth colonies consisting of short-chained and the rough ones of long-chained forms. Under a low power of the microscope (2/3), as may readily be seen if a transparent unscratched Petri dish be inverted on the stage, the contorted chains of the rough colonies may be seen to explain their coarsely granular aspect and irregular edges. We must agree with Eagles that there is often no sharp distinction into rough and smooth colonies. Intermediate forms are seen, and frequently the colonies on a plate cannot be referred with confidence to either category.

E. *Summary of Facts as to Dissociation.*

We may be pardoned for recapitulating the chief facts concerning these colony-mutations, on account of their important bearing on attempts at the serological classification of the haemolytic streptococci. It is shown that there are revealed in plate cultures of many strains different forms of colony, which, in their extreme forms, may be termed 'rough' and 'smooth', though intermediate forms exist. Typical roughs and smooths in any given strain are sharply contrasted in their serological properties. They may be contrasted, too, and usually are, in saline stability. There is no consistent contrast in virulence to mice since sometimes the rough and sometimes the smooth forms have proved the more virulent. Our own experience is that in almost every instance the rough forms are the more virulent: it is these forms which are found, almost without exception, in strains freshly isolated from morbid conditions of the body—a fact which renders it difficult to prepare emulsions suitable for agglutination from freshly isolated strains—thus constituting a grievous drawback to prompt serological classification. As regards specificity,

while it seems broadly true that the rough forms are more sharply specific than the smooth, we have found exceptions to this rule.

The more one studies these cocci the more strongly is the impression conveyed that they are in a state of constant flux in which it is difficult to find any firm foundation for a permanent systematic classification. This is in harmony with the failure of most workers to correlate serological properties with the facts of nosology, though we are far from wishing to deny the importance of the chief scarlatinal types of Griffith—a matter which we propose to discuss in more detail later.

In the early days of our work we fell into hopeless confusion and error from lack of apprehension of the wide serological differences which might exist amongst the colonies on an agar plate. As a matter of fact, the sera which we made were almost wholly unspecific in character and brought down nearly all our strains indiscriminately, but to low titre.

Some of the techniques which have been advised for preparing good agglutinable emulsions are calculated to give rise to short-chained smooth forms, which indeed often give stable broth cultures but with no guarantee that the serological properties may not have undergone mutation as a result. Again, in maintaining a strain under prolonged cultivation, it is almost a routine to plate it out from time to time and start again from a single colony: if this is done casually, without regard to the characters of the colony thus selected, the way is opened for serious error.

On a previous page we have exhibited graphs showing the effects on two scarlatinal sera of heavy absorptions with a chance assortment of miscellaneous strains. One of these sera (Type II) showed itself extremely resistant to absorption, and was, therefore, put forward as an example of a highly specific serum, while the other (Type III) was so readily exhausted by most foreign strains as to pass essentially as a 'group' serum. The point which we desire here to emphasize is that while these conclusions are doubtless justified, they are true only of these particular examples of Type II and Type III serum. The conclusions do not necessarily apply to the cocci from which the sera were prepared, without certain reservations. We have worked with a Type II strain for a number of years, and on one single occasion we isolated from a plating one single colony which was typically smooth in appearance and behaved as such in a few experiments in which we used it as an absorbing strain. Thus this smooth Type II race completely removed all the agglutinin from our Smooth Dochez serum, whereas the ordinary rough Type II effected no absorption at all. We did not make a serum from the smooth Type II colony, but had we by accident happened on this colony as the source of our stock, there can be little doubt that we should have assigned eminently group characters to Griffith's Type II.

Curiously enough we had a similar experience with Griffith's Type IV—also an obstinately specific and rough strain. This on one occasion only furnished us with a single smooth colony presenting pure group properties.

Dr. Griffith, after many failures, eventually produced from this Type III (Lewis) strain a race which appeared to have more specific characters than those he had at first seen in the strain, and he kindly gave us a culture and some of the serum he had prepared from it. This race, which we tested by the absorption method as well as by simple agglutination, though it by no means presented the extreme specificity which characterized Types II and IV, was certainly in our hands definitely less unspecific than the races of the same strain with which we had previously worked.

We can justly apply the terms specific and group to the individual colonies on a plate and to sera made from such colonies, fixed by formol beyond the possibility of mutation. But we cannot, in quite such a restricted sense, apply these terms to entire strains. If we do so in the cases of Types II and III we mean that under cultivation the first is so obstinately specific that a colony with group properties is a rare freak—while in the latter it is extremely difficult to get a colony with really specific properties.

F. *Minor Variations in Serological Behaviour.*

Another sort of variation amongst haemolytic streptococci was detected, something much less striking than the differentiation into rough and smooth, specific and group variants. On many plates there occurred superficially identical colonies of which the organisms would agglutinate with the homologous serum to very different titres. The procedure for detecting this was as follows: legumin-agar plates were sown from a stock blood-broth culture; single colonies were picked from these into more blood-broth tubes and incubated. Next day, legumin-agar plates were sown from these tubes and the subsequent growth washed off and used for the agglutination test. Fig. 9 shows that the growths from eight plates of the Rough Shilling coccus so prepared agglutinated, some to a high titre (88 per cent. in the figure), one only to 9 per cent. of the full titre, and others to intermediate values. It appeared, however, that no stable variants had been produced; neither the good nor the bad agglutinators bred true. From the blood-broth tube which had produced organisms agglutinating to high titre, a plate was sown thinly; colonies were picked and the procedure carried on as before. Again (see Fig. 9, lower part) the emulsions from the different plates agglutinated to widely differing titres with the homologous serum. The same was seen when the blood-broth tube yielding poorly-agglutinable cocci was taken as a starting-point; the results with the two sets of emulsions were quite comparable.

It did not always happen that variation of the kind described could be detected. When colonies were picked from a plate sown with Griffith's Type I the emulsions derived from these all agglutinated to approximately the same titre, as shown in Fig. 10. These results suggest a possible explanation, apart from gross rough-smooth mutations, of the differing titres found on different days in experiments with one serum, and one strain of streptococci.

(ii) Graduated Absorptions

In considering the results of single absorptions—and we have done many hundreds—the question arises as to the degree of confidence

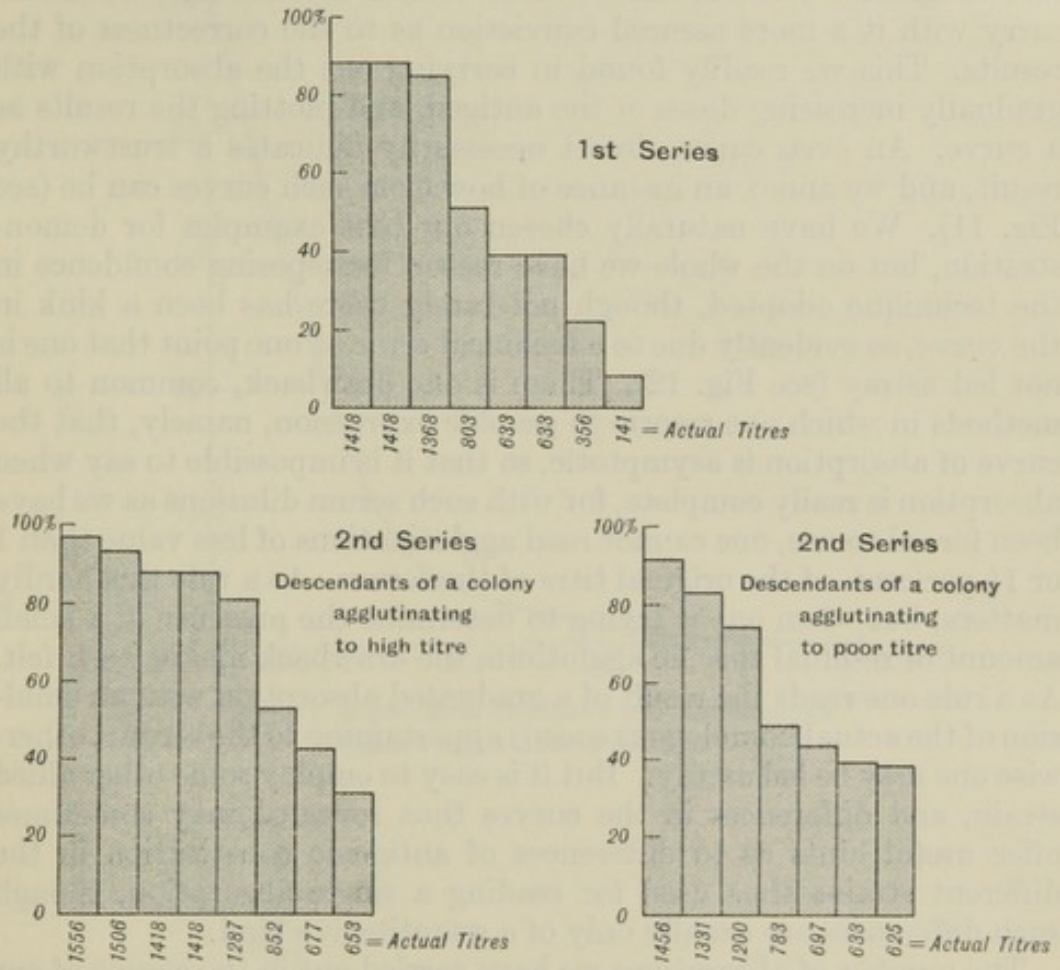

Fig. 9. ROUGH SHILLING
Titre of serum reckoned as 1 in 1600

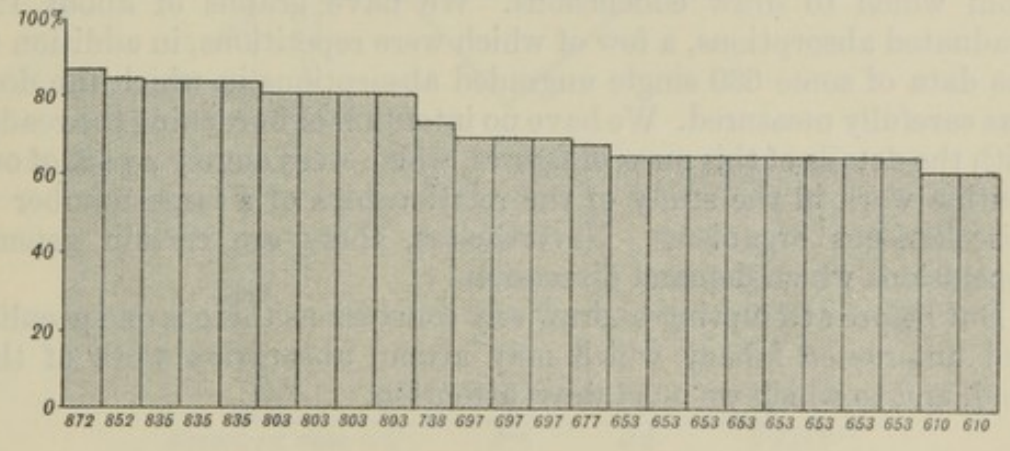

Fig. 10. Type I (Griffith)
Titre of serum reckoned as 1 in 1000

which one can repose on a single observation, however careful one has been in carrying it out. When a result has seemed to us improbable, we have, of course, repeated the observation, and sometimes with the same result; but it has not rarely happened that the second result has

differed from the first. It is almost inevitable, in a procedure which involves the use of small quantities of fluid and a somewhat complicated technique, that errors should creep in now and then. We therefore sought for some method which, even if more laborious, should carry with it a more assured conviction as to the correctness of the results. This we readily found in carrying out the absorption with gradually increasing doses of the antigen, and plotting the results as a curve. An even curve almost necessarily indicates a trustworthy result, and we annex an instance of how even such curves can be (see Fig. 11). We have naturally chosen our best examples for demonstration, but on the whole we have reason for reposing confidence in the technique adopted, though not rarely there has been a kink in the curve, so evidently due to a technical error at one point that one is not led astray (see Fig. 12). There is one drawback, common to all methods in which one resorts to graphic expression, namely, that the curve of absorption is asymptotic, so that it is impossible to say when absorption is really complete, for with such serum dilutions as we have been forced to use, one cannot read agglutinations of less value than 1 or $1\frac{1}{2}$ per cent. of the original titre of the serum. As a rule this hardly matters, but when one is trying to determine the presence of a small amount of residual specific agglutinin, the drawback makes itself felt. As a rule one reads the result of a graduated absorption with an emulsion of the actual homologous coccus appertaining to the serum; otherwise one may be led astray. But it is easy to employ some other allied strain, and differences in the curves thus revealed may sometimes offer useful hints as to differences of antigenic constitution in the different strains thus used for reading a single absorption, though such differences are usually only of a quantitative kind.

The number of absorptions we have carried out in the course of our investigations is quite considerable, so that we have a wide basis from which to draw conclusions. We have graphs of about 125 graduated absorptions, a few of which were repetitions, in addition to the data of some 630 single ungraded absorptions in which the dose was carefully measured. We have no intention of burdening the reader with the details of this mass of figures, which were merely a part of our routine work in the study of the relationships of a large number of miscellaneous organisms. Nevertheless, there are certain general conclusions which demand discussion.

But before attempting to draw any conclusions there is one peculiar and unforeseen fallacy which may attend absorption work of this kind, and to which we must draw attention.

A. *Paradoxical rise in Titre after Absorption.*

The inexperienced bacteriologist who carries out an absorption experiment might not unreasonably expect to find the titre of his serum untouched or reduced or wholly extinguished. But what conclusion is he to draw if he finds that the result of his saturation is to *raise* the initial titre of his serum? When we first encountered this

paradoxical result we put it down to some error in technique, though one of us had met with the same phenomenon in working with the

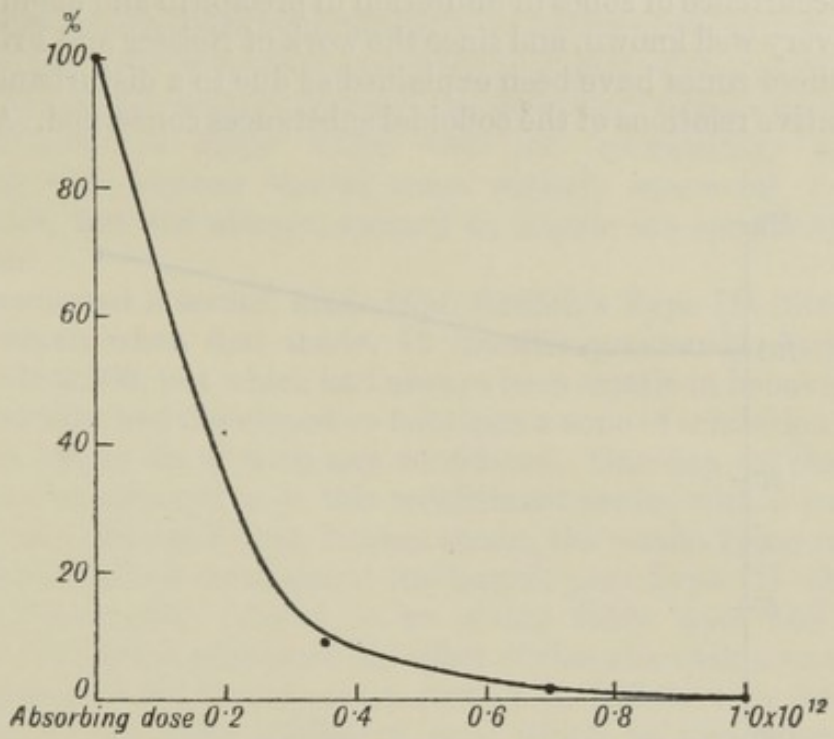

FIG. 11. Homologous Absorption with regular result
Griffith's Type I absorbed with Type I

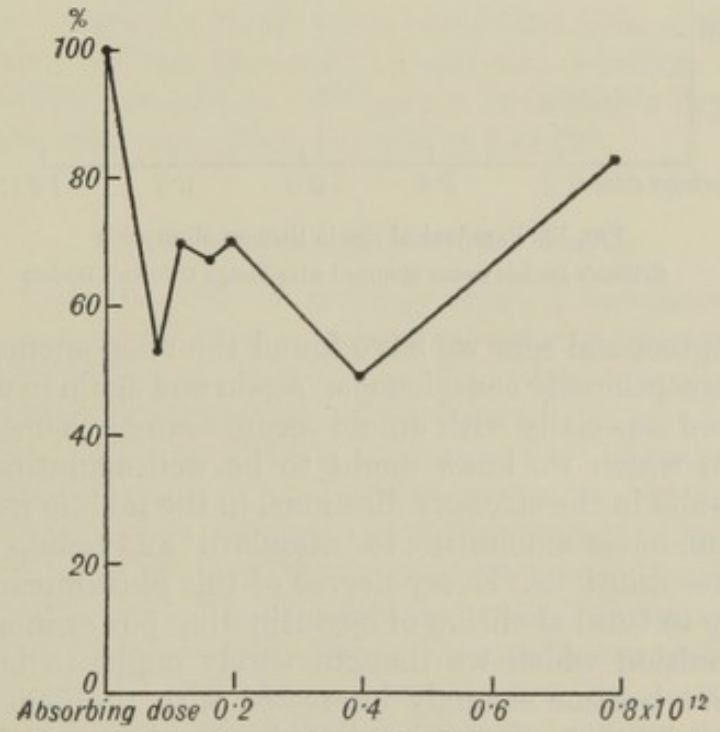

FIG. 12. Heterologous Absorption with incongruous result
Griffith's Type I absorbed with Smooth Cutbush

Salmonella group. But it was more frequent and striking amongst the haemolytic streptococci; indeed, we met with this rise of titre after saturation with a heterologous coccus in no less than 30 absorptions (see Fig. 13). We were, therefore, tempted to look into the matter,

and although we have by no means solved the problem we think we may have shed a little light on it.

The occurrence of zones of inhibition in precipitin and agglutination work is very well known, and since the work of Neisser and Friedmann (1904) these zones have been explained as due to a disturbance of the quantitative relations of the colloidal substances concerned. Amongst

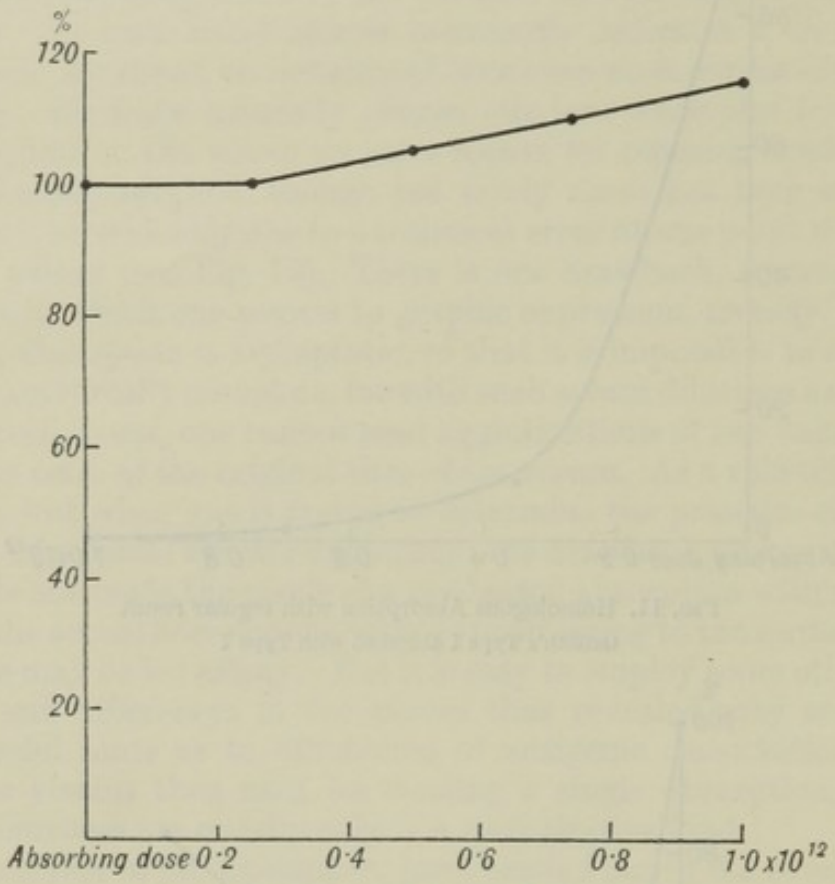

FIG. 13. Paradoxical rise in titre on absorption
Griffith's Dochez serum absorbed with Rough O'Brien's Dochez

our antistreptococcal sera we have found the phenomenon of inhibition zones exceptionally conspicuous. Again and again in dealing with a serum—and especially with an old serum—we have observed, with an emulsion which we knew ought to be well agglutinated by it, negative results in the stronger dilutions, in the middle reaches slight agglutination, never amounting to 'Standard' and fading away again in the weaker dilutions. Every degree of this phenomenon has been met with up to total abolition of agglutinating power in a serum and with an emulsion which we thought surely ought to have reacted together. We became strongly impressed with the belief that such sera contained some substantive inhibiting agent—as if the serum would like to have agglutinated the cocci if it could, and the cocci were quite prepared to be affected, but that some malevolent substance was hindering the reaction. It may well be that physical chemists will reject a hypothesis so crude as this, yet we have some experimental evidence which can be put forward in support of it. This evidence lies in the fact that under certain circumstances, which we must admit we

cannot altogether control, the supposed inhibiting substance can be removed by absorption with some heterologous coccus, or other finely divided foreign material.

Our first intimation of this fact was at a time when we were experimenting on washing our streptococcal emulsions with water, alcohol, or acetone in the hope of diminishing their tendency to spontaneous agglutination—an effect which was not infrequently achieved. Washing with acetone was at times entirely successful, though it sometimes, but not always, seemed to impair the specificity of the emulsion.

We possessed a serum, made from Griffith's Type III (Strain U.S. 55/4), which when first made, 13 months previously, had a titre of 2,000 to 3,000, but which had always been erratic in behaviour, and which latterly had developed so ferocious a zone of inhibition that we could no longer use it with any confidence. One day we chanced to carry out an absorption of this recalcitrant serum with a large dose (10^{12} per c.c.) of our Rough Dochez strain, the results being read with an acetone-washed emulsion of the homologous Type III which had been independently proved to be giving fairly good and specific results. To our astonishment the effect of this absorption was to raise the apparent titre of the serum from 1 in 250 to 1 in 2,500 with abolition of the lower inhibition zone which on this occasion was only a slight one. We then repeated the experiment twice, using *B. coli* as the absorbing agent in doses of 3×10^{12} and $0{\cdot}75 \times 10^{12}$ respectively. Again the result was a rise in the titre of the serum for its own coccus, but this time only a small one. Further, we absorbed the serum with a dose of $1{\cdot}3 \times 10^{12}$ per c.c. of Griffith's Type IV strain, and again the titre rose—from 1 in 250 to 1 in 390.

B. *Absorption of Sera with Kaolin.*

Next we tried the effect of a finely divided inorganic substance, namely kaolin, employing it in graduated doses ranging from 0·00009 to 0·9 g. kaolin per c.c. of neat serum. The titres were then read with the same emulsion as before. No effect whatever was produced by any dose of kaolin smaller than 0·9 g. per c.c., but at this point the agglutination became very good, and the apparent titre 1 in 2,500. Before this absorption the serum had possessed no readable titre; the treatment with kaolin did not seem to impair the specificity of the serum.

Similar results were obtained with a Rough Shilling serum, which had been behaving erratically, but in this case it was noticed that the effect of the kaolin was only apparent when the serum was well diluted (1 in 100), whereas a 1 in 10 dilution had sufficed with the Type III serum. It was also found that the Type III serum in question, after it had been kaolinized, was still further improved in titre by absorption with a heterologous coccus.

We only employed kaolinization on sera which had troubled us with inhibition zones, and the only emulsions which we washed in acetone were those which had become inagglutinable with their own

sera. But the results obtained were frequently irregular and anomalous, and we might well have exclaimed with Marmion:

'Oh what a tangled web we weave
When first we practise to deceive.'

The subject is, in fact, one which can only properly be studied in the light of physical chemistry by those better qualified than we in those sciences.

One thing, however, plainly emerged from our observations, namely, that, apart from any doctoring with kaolin or doping with acetone, a serum may unquestionably be raised in its apparent titre for its own coccus by heavy saturation with a totally heterologous organism.

It not infrequently happens that a strain of haemolytic streptococcus undergoes, while under artificial cultivation, some unexplained change in virtue of which it no longer agglutinates with its own serum. We have observed this repeatedly, and it is a remarkable thing that the change may be so marked that one of the most extreme examples of a rise in titre after absorption was in a serum absorbed with its own coccus after the latter had undergone this change.

From the observations just recorded it seems justifiable to conclude that in a heavy absorption of a streptococcal antiserum with a heterologous coccus the effect produced may be the resultant of two factors: (1) *the specific removal* of agglutinin, when there is any substance in common between the coccus used in making the serum and that used in absorbing it, such removal being total or partial according to the amount of the common substance, and (2) *the unspecific removal* from the serum of some unknown inhibiting substance which allows the agglutinin present to be more effective than it was before. Factor No. 2 seems erratic in its incidence and cannot be foretold, nor can an observed result necessarily, or even often, be repeated. It is probable that in an absorption test there is a very delicate colloidal balance readily upset by the slightest change in any one of the ingredients.

In setting forth the results of our experience with the graduated absorption test, we do not, therefore, offer any apology for the irregularity which is often apparent. We are careful merely to present the facts observed and to analyse them as well as we can.

C. *Method of Plotting Graduated Absorption Curves.*

In discussing our results it will be convenient to illustrate them graphically, and a clear form of presentation lies in plotting each absorption curve on squared paper using percentages in place of actual readings—the original titre of the unabsorbed serum counting as 100 per cent. The abscissae are the percentage titres. The ordinates represent the absorbing doses per c.c. of pure serum stated in English billions (10^{12}) or decimal fractions of the same.

The use of percentage figures in drawing our graphs has the drawback that the curves appear to be exaggerated above and minimized below. Thus a drop of 50 per cent. looks a great deal, but it only corresponds to a fall of one tube in the series, whereas a drop of one

tube when we are in the neighbourhood of 10 or 12 per cent. of the original titre entails very little movement of the curve.

Our graphs fall into certain rough groups, one of each of which has been chosen as an illustration.

D. *Groups of Graduated Absorption Curves.*

(a) *Group I. Complete absorptions.*

Group I includes all the complete absorptions—those, namely, in which at least the largest absorbing dose employed has removed the whole of the specific agglutinin (or group agglutinin in an unspecific serum) down to the limits imposed by the technique employed. This limit has usually been in the neighbourhood of 1 per cent. of the original titre of the serum.

Out of the 125 graduated absorption curves studied, 40 have conformed to this type, but one or two of these have been duplicates. The smallest absorbing dose which has been effective in extinguishing the titre of a serum has been $0{\cdot}14 \times 10^{12}$ and once $0{\cdot}07 \times 10^{12}$. The average effective dose in 42 absorptions was $0{\cdot}5 \times 10^{12}$ per c.c. of serum. In a few instances a dose of over $1{\cdot}0 \times 10^{12}$ was required to extinguish the titre of a serum. It must be assumed that the higher the initial titre of a serum, the larger will be the absorbing dose requisite to extinguish it. Inasmuch as the sera we employed varied in value from 1 in 325 to 1 in 9,000 such figures as those just given have only a rough value as an index of the order of the dose needed in a favourable case permitting of complete absorption. Further study of these complete absorptions shows that they fall into three groups:

(*a*) Some are autologous, the absorbing doses being of an organism identical with that with which the serum was prepared. Five curves come under this category.

(*b*) Others are merely homologous, the absorbing dose having by chance been prepared from a strain which was identical with the serum strain. Some twenty-six curves come under this heading—the evidence of substantial identity lying not in the single one-sided absorption, but in the 'mirror' absorptions carried out later. The question of absolute, as opposed to substantial identity, is a difficult one which we may leave for later discussion.

(*c*) Some sixteen of the curves fall under a different heading altogether; that, namely, in which a serum with eminently group properties is attacked by a coccus containing not only its own specific antigen, but also a large proportion of group antigen. This is a common occurrence and is only revealed by mirror absorption, which turns out a one-sided affair, as our graphs of reciprocal absorptions will indicate later.

We have already urged the necessity of reading the results of an absorption with an emulsion of the actual coccus used in making the serum. We have had in our hands some half-dozen strains presenting well-marked group characters and which we have been disposed to regard as so nearly identical that one might almost use them

interchangeably as indices of the extinction of a group titre in a serum, though, of course, one could never use a strain of the sort for determining the removal of a specific titre. Experience, however, taught us that extreme caution is needed in such a procedure. Only under the most solemn guarantees of absolute serological identity should one interchange strains used in reading results, for they must be regarded as reagents intended to prove the presence or absence of perhaps

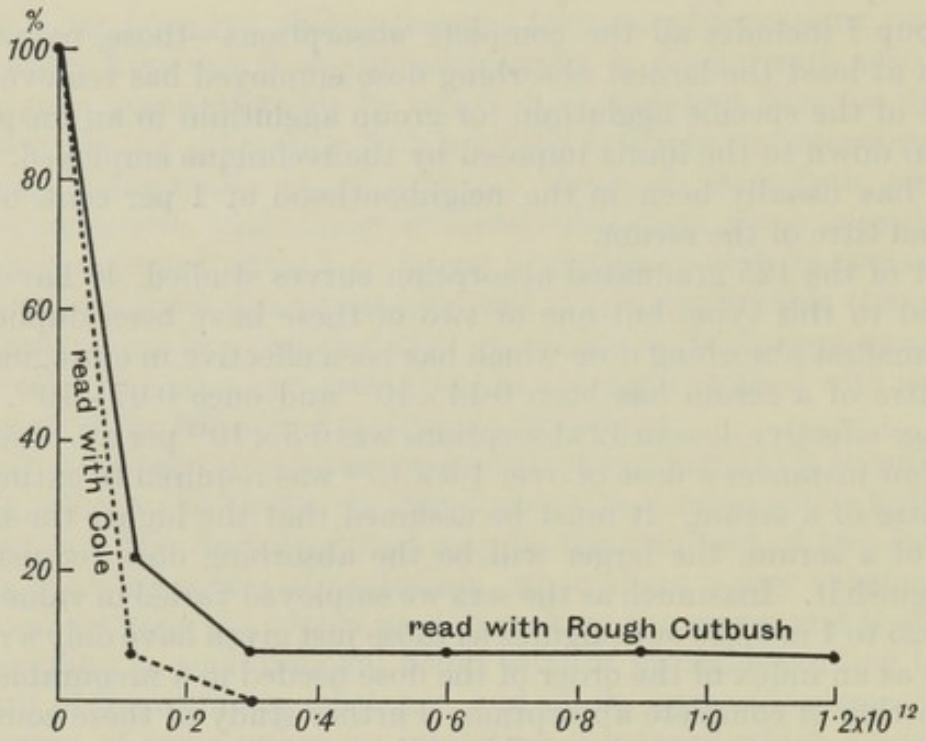

FIG. 14. A Group Serum absorbed with a heterologous Specific serum read with the actual serum strain (plain line) and with another group strain (dotted line)

Rough Cutbush serum absorbed with Type I

small traces of a given antibody. We possess a large number of graphs in which we have plotted the results of reading an absorption with more than one such indicator emulsion, and these results are sometimes different. We annex an example (Fig. 14): the serum here is our Rough Cutbush serum—one possessing group properties in high degree, and it has been absorbed with Griffith's Type I scarlatinal strain. The results have been read with two different group strains (*a*) with the original Rough Cutbush strain used in making the serum, and (*b*) with another eminently group strain. The curve yielded by the (*a*) readings is shown by a plain, and that yielded by the (*b*) readings by a dotted line. It is seen that Type I coccus removes from the Rough Cutbush serum in quite moderate dose ($0{\cdot}3 \times 10^{12}$) all the group element represented in the foreign group strain, but not all that in the Rough Cutbush strain—for the serum thenceforward presents a residuum of agglutinin which remains untouched by the largest dose of Type I coccus and which corresponds with an antigen not represented in the latter. Differences amongst these nearly identical group strains are perhaps better revealed by their use as indicator emulsions than in any other way.

(b) *Group II. Partial absorptions.*

Group II includes partial absorptions of every degree. We give two graphs illustrating this group—one in which rather more than

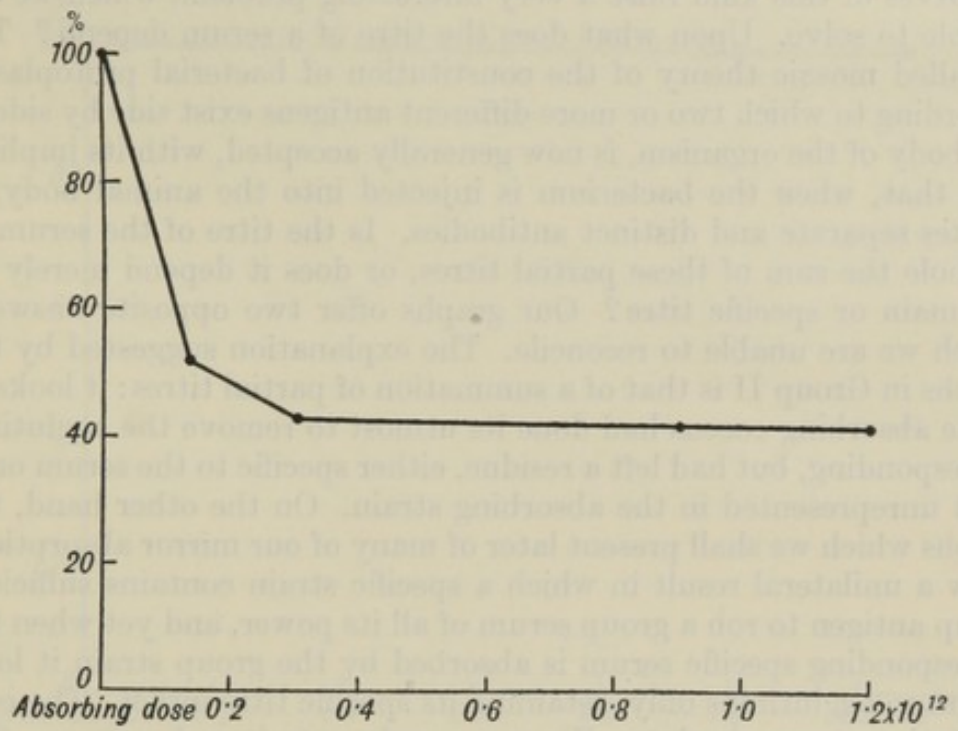

FIG. 15. Consistent curve of partial absorption
63 T serum absorbed with Type I

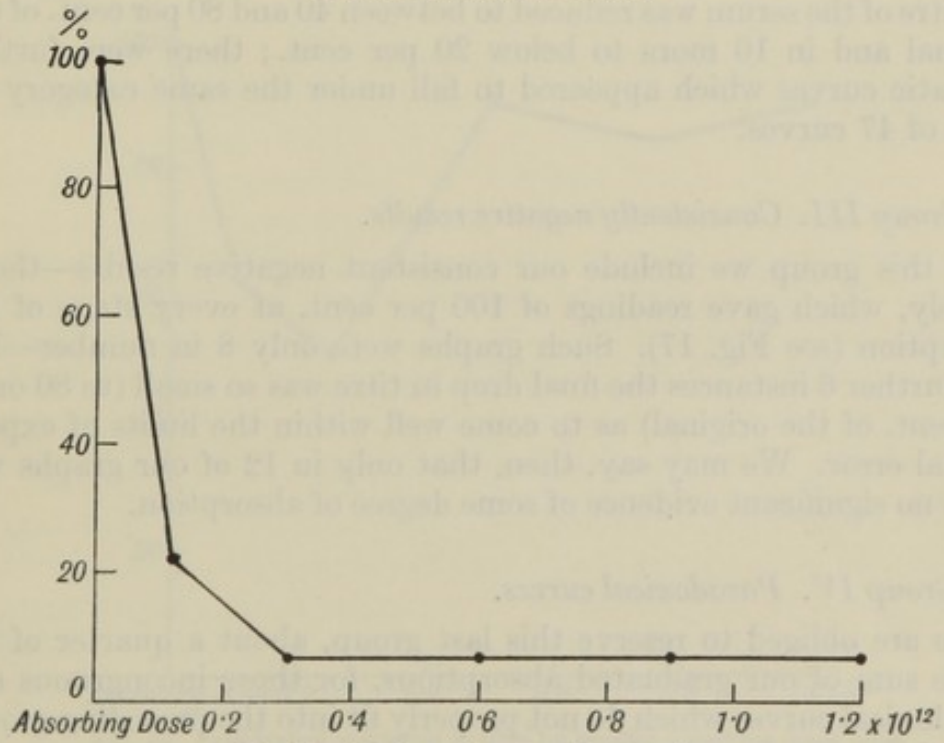

FIG. 16. An absorption not quite complete
Rough Cutbush serum absorbed with Type I

half the agglutinin is removed (Fig. 15), and one in which some 7 per cent. only is left behind (Fig. 16)—the latter being very similar to the true curve in Fig. 14. These are regular and convincing examples of

curves of this type; many of our graphs are much less regular than these. Such curves show a lowering of titre up to a certain point, after which no increase in the dose produces any further effect.

Curves of this kind raise a very interesting problem, which we are unable to solve. Upon what does the titre of a serum depend? The so-called mosaic theory of the constitution of bacterial protoplasm, according to which two or more different antigens exist side by side in the body of the organism, is now generally accepted, with its implication that, when the bacterium is injected into the animal body, it evokes separate and distinct antibodies. Is the titre of the serum as a whole the sum of these partial titres, or does it depend merely on the main or specific titre? Our graphs offer two opposite answers, which we are unable to reconcile. The explanation suggested by the graphs in Group II is that of a summation of partial titres: it looks as if the absorbing coccus had done its utmost to remove the agglutinin corresponding, but had left a residue, either specific to the serum or at least unrepresented in the absorbing strain. On the other hand, the graphs which we shall present later of many of our mirror absorptions show a unilateral result in which a specific strain contains sufficient group antigen to rob a group serum of all its power, and yet when the corresponding specific serum is absorbed by the group strain it loses its group agglutinins only, retaining its specific titre intact. The contradiction seems absolute. We can merely note it and pass on. Our absorption curves are numerous in this second group. In 30 instances the titre of the serum was reduced to between 40 and 80 per cent. of the original and in 10 more to below 20 per cent.; there were further 7 erratic curves which appeared to fall under the same category—a total of 47 curves.

(c) *Group III. Consistently negative results.*

In this group we include our consistent negative results—those, namely, which gave readings of 100 per cent. at every stage of the absorption (see Fig. 17). Such graphs were only 8 in number—but in a further 6 instances the final drop in titre was so small (to 80 or 90 per cent. of the original) as to come well within the limits of experimental error. We may say, then, that only in 12 of our graphs was there no significant evidence of some degree of absorption.

(d) *Group IV. Paradoxical curves.*

We are obliged to reserve this last group, about a quarter of the whole sum of our graduated absorptions, for those incongruous and paradoxical curves which do not properly fit into the preceding groups and which may be thought, though we think usually unjustly, to discredit our technique. These odd results fall mostly under two headings.

(1) The majority is composed of those paradoxical cases in which the titre of a serum rises, instead of falling, as the result of saturation with a heterologous coccus. We have already sufficiently discussed such instances and we think that, as regards significance, they may be

added to those curves in which the absorption should be held negative in its results.

(2) In a smaller category come those curves in which the smaller

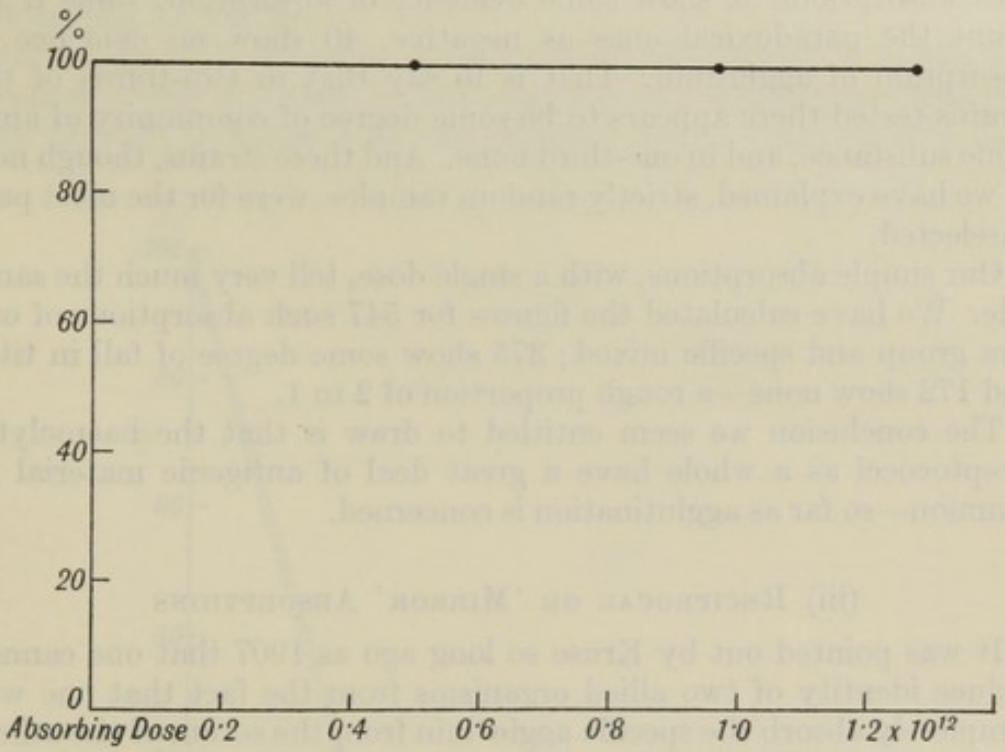

FIG. 17. An Absorption totally negative at all stages
Type I serum absorbed with Rough Napier

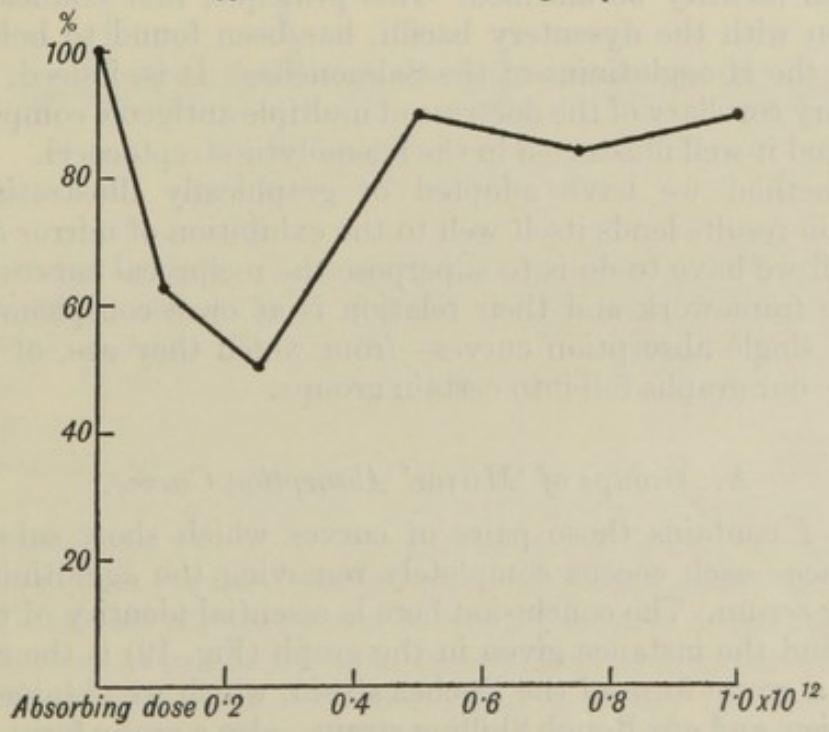

FIG. 18. Paradoxical reduction in titre by smaller absorbing doses only
63 T serum absorbed with Rough Napier

doses have seemed to produce a fall in titre which the larger doses have failed to confirm, the titre in the end proving to stand at 100 per cent. or little below it. We are unable to offer any rational explanation of these cases, of which we have only 8 (see Fig. 18).

E. *The Significance of these Absorption Curves.*

If we examine the figures more closely we see that out of 122 graduated absorptions 82 show some evidence of absorption, while if we count the paradoxical ones as negative, 40 show no evidence of absorption of agglutinin. That is to say that in two-thirds of the strains tested there appears to be some degree of community of antigenic substance, and in one-third none. And these strains, though not, as we have explained, strictly random samples, were for the most part unselected.

Our simple absorptions, with a single dose, tell very much the same tale. We have calculated the figures for 547 such absorptions of our sera group and specific mixed; 375 show some degree of fall in titre and 172 show none—a rough proportion of 2 to 1.

The conclusion we seem entitled to draw is that the haemolytic streptococci as a whole have a great deal of antigenic material in common—so far as agglutination is concerned.

(iii) Reciprocal or 'Mirror' Absorptions

It was pointed out by Kruse so long ago as 1907 that one cannot deduce identity of two allied organisms from the fact that one will completely absorb the specific agglutinin from the serum of the other. Only when each is found to absorb the serum of the other can true serological identity be affirmed. This principle, first enunciated in connexion with the dysentery bacilli, has been found to hold good amongst the H-agglutinins of the Salmonellas. It is, indeed, almost a necessary corollary of the doctrine of multiple antigenic components, and we find it well illustrated in the haemolytic streptococci.

The method we have adopted of graphically illustrating our absorption results lends itself well to the exhibition of mirror absorptions. All we have to do is to superpose the reciprocal curves within the same framework and their relation is at once conspicuous. As with the single absorption curves—from which they are, of course, selected—our graphs fall into certain groups.

A. *Groups of 'Mirror' Absorption Curves.*

Group 1 contains those pairs of curves which show substantial coincidence—each coccus completely removing the agglutinin from the other serum. The conclusion here is essential identity of the two strains, and the instance given in the graph (Fig. 19) is the relation between a group form of the Dochez strain, which we obtained from Dr. O'Brien, and our Rough Shilling strain—also a group form. There is no need for comment except to observe that it is not unusual for one strain to exhaust the serum of another completely, whereas in the reciprocal reaction there may be a small amount of specific agglutinin left, perhaps only some 1 or 2 per cent. of the initial titre, but nevertheless irremovable. This is not likely to happen except with strains so closely allied as to be practically identical.

We have met, amongst the mixed crew of haemolytic streptococci from the most varied pathological sources, with very few examples of the perfect serological identity implied by complete mirror absorption, not more than 6 in all.

Group 2. Here we place those pairs of absorptions in which one serum is wholly absorbed by the other coccus, but the other serum is only partially exhausted by the opposite coccus, there being a sub-

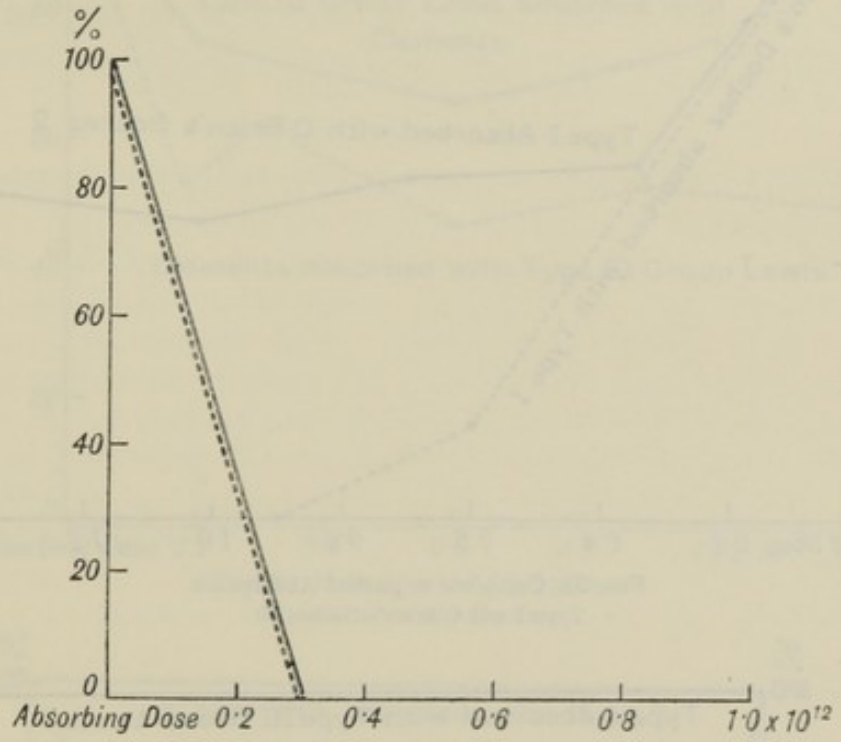

FIG. 19. Complete mutual Absorption
O'Brien's Dochez and Rough Shilling

stantial residuum left. We have in our series 6 pairs of cocci showing this absorptive relationship, which evidently betokens near relationship but not identity (see Fig. 20).

Group 3. Here comes a striking group of cases in which one member of a pair completely exhausts the serum of its fellow while its own serum remains wholly untouched in specific titre by saturation with the other coccus (see Fig. 21). The absorptive relationship appears wholly unilateral, but it is not quite so much so as it looks. If the results have been read with emulsions of the homologous cocci, as they should be, the curves are as stated; but if the titres of the apparently untouched serum are tested with an emulsion of a coccus possessing mainly group characters, it is found that it is only the specific titre which has weathered the storm, any group agglutinin which may have been present being found to have vanished. This is the fact upon which is based the practice of employing already absorbed sera for purposes of diagnosis by agglutination, and, indeed, of the original reaction described by Castellani (1902); and, in view of the variety of absorption relationships which we exhibit in this series, it is plain that such preliminary absorption should not be haphazard, but governed

by a careful study of the properties of the serum and coccus concerned. Five of our pairs of curves come into this group.

Group 4. It sometimes happens that each of a pair of streptococcal

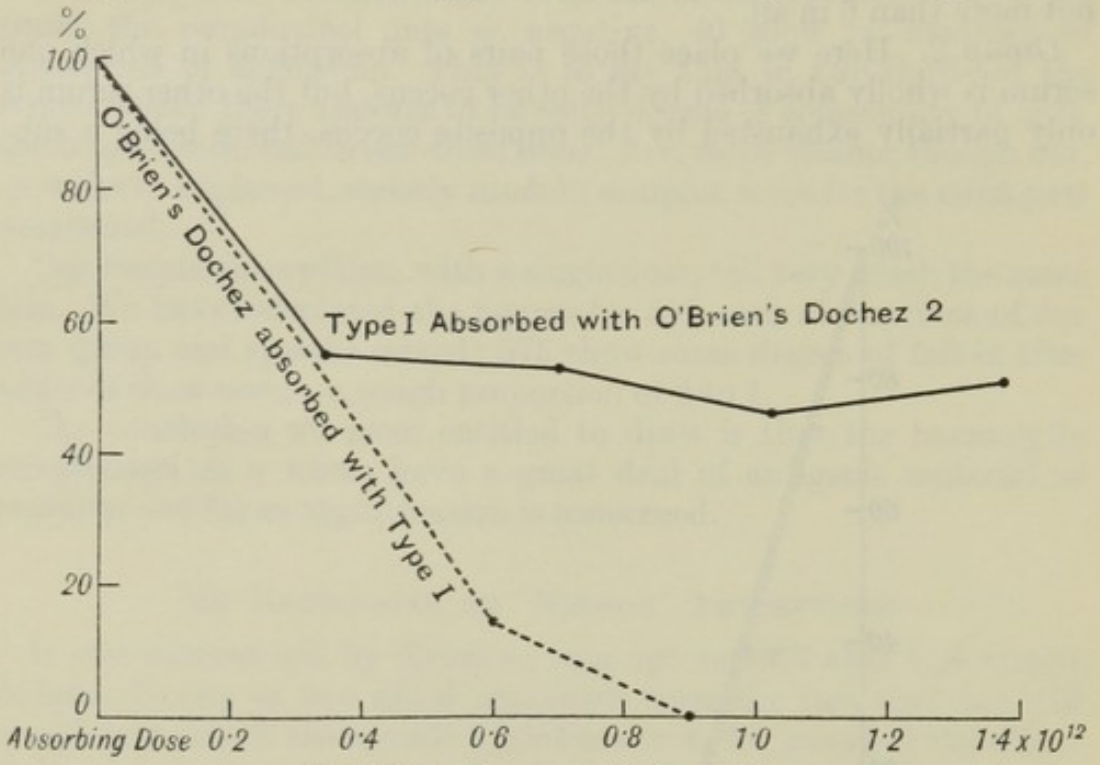

FIG. 20. Complete v. partial Absorption
Type I and O'Brien's Dochez 20

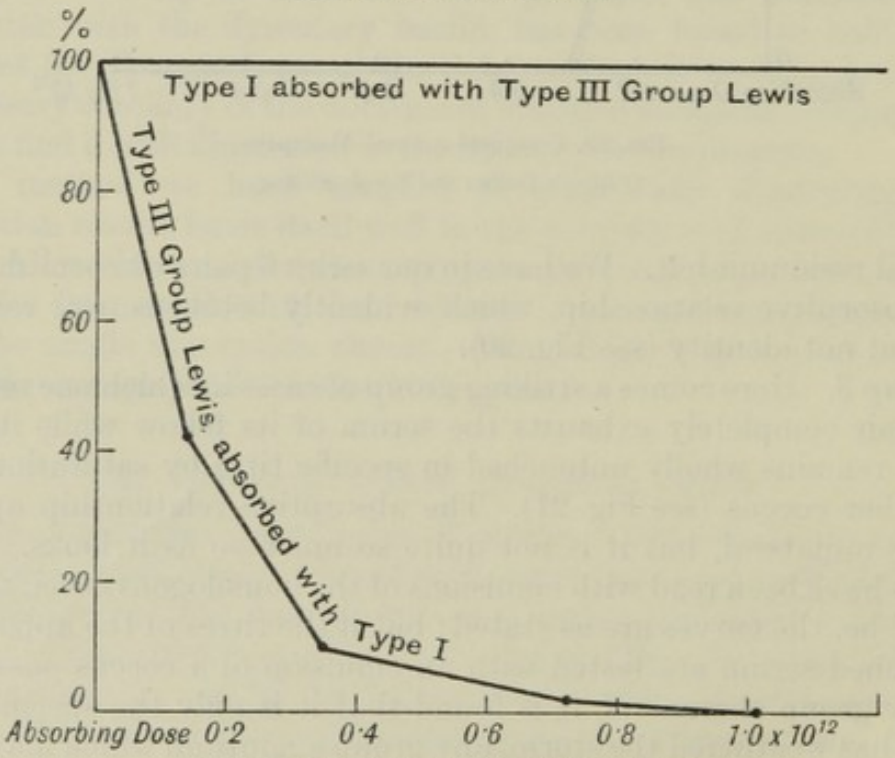

FIG. 21. Completely unilateral Absorption
Type I and Type III group Lewis

strains is capable of reducing the titre of the other's serum to a moderate degree, as shown in Fig. 22, while neither can extinguish the titre of the other's serum. This, in our experience, is not a common finding; we have only 2 pairs of curves showing this relationship.

Group 5. In this group, which we find the commonest, we place those pairs of absorptions in which one serum is totally unaffected in titre by saturation with its fellow coccus, while the converse absorp-

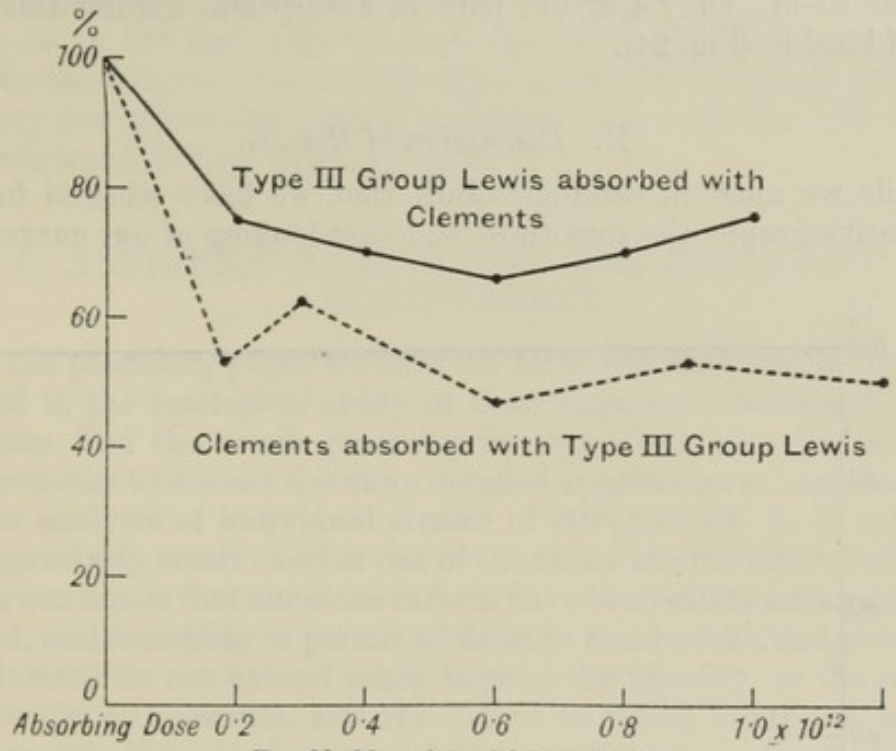

FIG. 22. Mutual partial Absorption
Type III Lewis and Clements

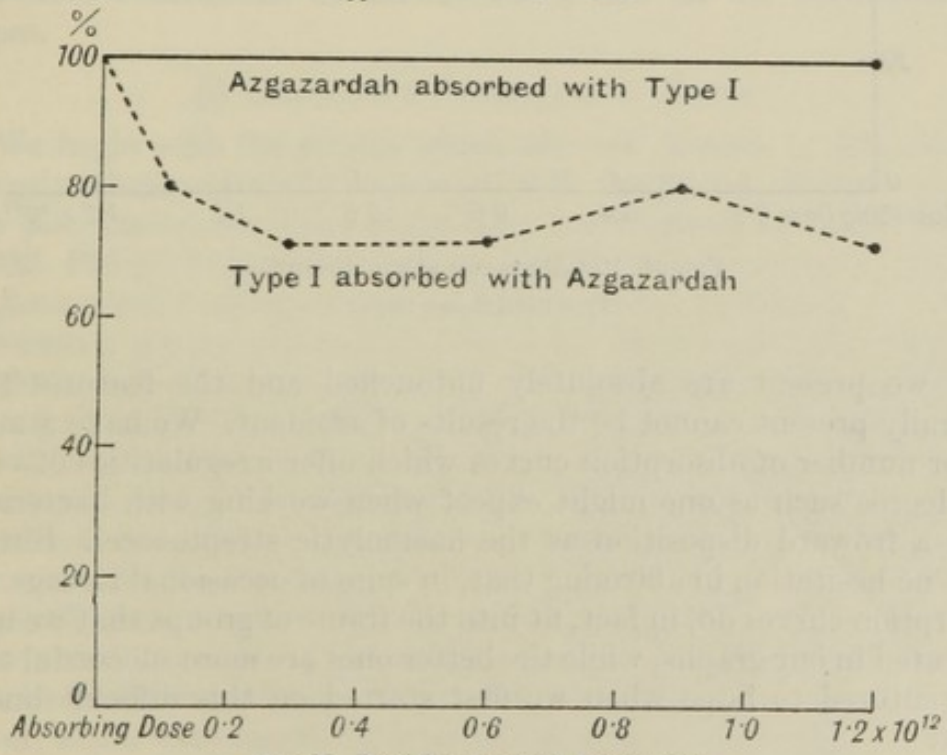

FIG. 23. Partial v. negative Absorption
Type I and Azgazardah

tion shows only a moderate reduction in the titre of the serum. We have 10 pairs of curves showing this absorptive relationship, which, like those in the preceding group, indicates only a remote connexion between the two strains concerned (see Fig. 23).

Group 6. Lastly come those instances in which neither coccus is able to effect any reduction at all in the titre of the other's serum—a finding which indicates the absence of any serological relation between them. Only 4 of our pairs of absorptions exhibit this total lack of kinship (Fig. 24).

B. *Discussion of Results.*

While we must in candour admit that we have selected for our illustrative graphs the smoothest and best looking of our curves, the

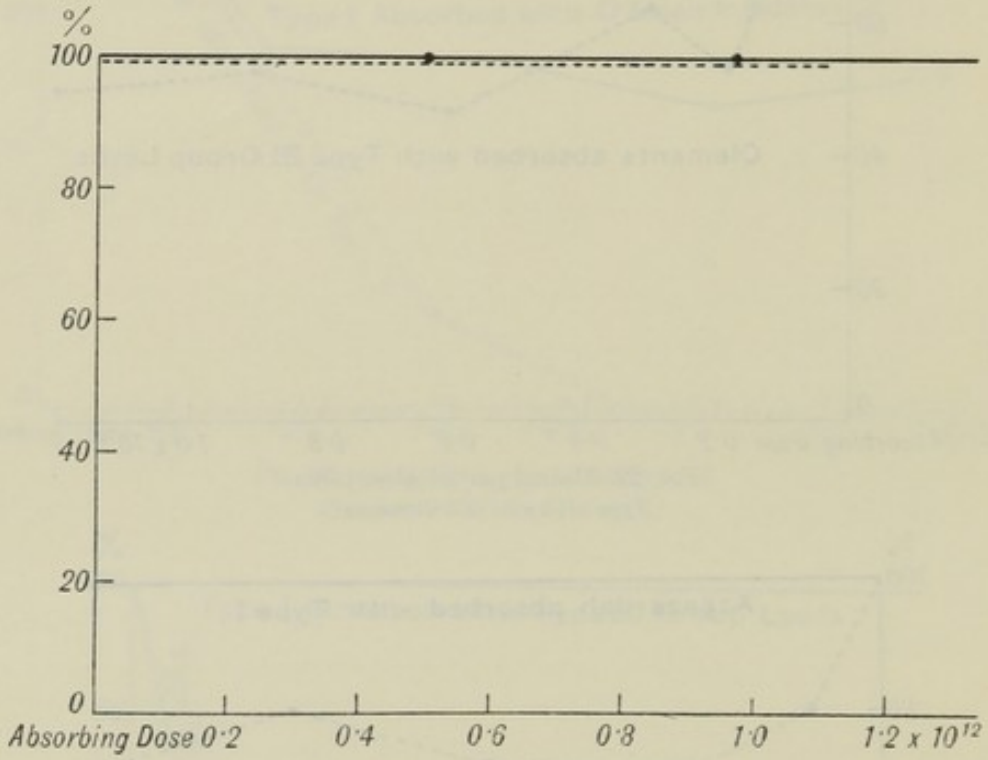

FIG. 24. Mutual absence of any Absorption
Type I and Rough Napier

ones we present are absolutely untouched and the features they severally present cannot be the results of accident. We have a much larger number of absorption curves which offer irregularities of varying degree such as one might expect when working with bacteria of such a froward disposition as the haemolytic streptococci. But we have no hesitation in affirming that, in spite of occasional zigzags, our absorption curves do, in fact, fit into the frame of groups that we have indicated in our graphs, while the better ones are more successful than we ventured to hope when we first started on this difficult line of work.

As regards the racial interrelationships suggested by the mirror absorptions, perhaps the most striking feature is the small size of the groups of pairs indicating complete serological identity, or disparity (Groups 1 and 6), 6 and 4 pairs respectively, whereas the remaining groups, which present evidence of some degree of serological affinity, number 22 pairs. This suggests a wide distribution of some form

of group antigen amongst these cocci, but we are not entitled to conclude that the mutual relation of the pair of curves in a reciprocal absorption is solely dependent on the relative amounts of group antigen contained in the respective cocci. Doubtless this may be so in many cases, but one must not lose sight of the possibility that there may be more than one kind of group antigen: indeed, when one comes to carry out mirror absorptions between a pair of group strains, there are definite hints that this may be so.

4. ANTIGENIC ANALYSIS OF THE HAEMOLYTIC STREPTOCOCCI

In the preceding pages we have set forth the methods we have employed in the serological study of these organisms as regards agglutinogens, and the rough general principles which have emerged. It now remains to discuss the more detailed application of these methods to the analysis of individual strains of streptococci. It is only in a comparatively small number out of the many strains which have been under our hands that our observations have been sufficiently numerous, varied, and searching to permit of definite conclusions, and even these conclusions do not extend much beyond the identity, or the reverse, of specific components, and the extent to which group components are present. Nevertheless, such data are sufficient to permit of certain tentative conclusions on classification, and on the permanence of types.

(i) Griffith's Scarlatinal Types

We begin with the strains which are well known in laboratories, namely, those especially associated with scarlatina. In this country the best known are those which have been defined by Dr. F. Griffith (1926, 1927). This writer, having found the more usual methods of agglutination technique too uncertain to be applicable to routine laboratory use on a large scale, devised a method of rapid naked-eye agglutination on a slide, preferably by already absorbed sera in fairly strong concentration (1 in 10). The technique, in his hands, proved a workable one, and in the course of 4 years he submitted a large number of strains from the throats of 375 cases of scarlet fever from the Park Hospital to classification by this test. He found that 68 per cent. of the streptococci obtained fell into one or other of four types (Type I, 10 per cent.; Type II, 25 per cent.; Type III, 22 per cent.; and Type IV, 11 per cent.). These constitute his major types, but in addition he found 32 per cent., equally from cases of scarlet fever, which were markedly heterogeneous in their serological relationships. One is reminded of the American classification of pneumococci in which there are three major types, while a residue of serologically miscellaneous varieties are lumped together as a fourth group. It has been our privilege to be in touch with Dr. Griffith during his work, and he has been most generous in supplying us with cultures, and, so far as his slender supplies allowed, with sera. We were so impressed with

the importance of his work in relation to the classification of the haemolytic streptococci that we have made his types an object of especial study, and we have done so, not by the aid of his agglutination technique, but to see if the results of that technique can be supported by more refined agglutination methods; we may say at once that we find that his results, with one possible exception, are so supported.

A. *Type I.*

The strain of Type I which we have used throughout was given us by Dr. Griffith in 1926, and was labelled S.F. 130, and has proved a remarkably stable strain, being after 5 years in the same condition as when we received it. Its colonies on trypagar are rough or moderately rough; nevertheless its emulsions stand up well in saline: only on one occasion have we found on plates what appeared to be smooth variants, but they soon died down. Emulsions of them only came down to about half the full titre of a Type I serum. Dr. Griffith once gave us a specimen of 'watery' colonies of this type; they failed to agglutinate with any of our standard smooth sera.

We have received Type I serum prepared by Dr. Griffith, and we have ourselves made sera on three different occasions—always from Strain S.F. 130. All have possessed similar properties, but we should not be surprised to find, in sera prepared from other strains of this type, slight differences, e.g. in the proportions of specific and group agglutinin present.

We have carried out graduated absorptions on our Type I sera some 35 times with other strains. The essential specificity of the type is shown by the fact that only 4 strains out of these 35 have proved able to remove the whole of its specific agglutinin. One of these 4 strains was from a case of scarlet fever in Moscow, two were from cases of otitis and may well have had a scarlatinal origin, and only one was from a case with no known connexion with scarlet fever—a blood culture from a fatal case of septicaemia in impetigo. In 14 instances amongst these absorptions the specific titre of the serum was lowered, but seldom to any great degree: the final titre was usually round about half the original. On the other hand, the initial titre was untouched, or even raised, by the absorption on 15 occasions. It occasionally happened that a partial lowering of titre was seen on one occasion and none on another. The story is much the same as that told by the much larger number of single, ungraduated absorptions with miscellaneous strains done on this serum. Here, out of a total of 70 absorptions, carried out with large doses, 36 showed no fall in titre or a fall so small (20 per cent. or less) as to be negligible, 28 showed a moderate fall, and 6 showed complete or nearly complete extinction of titre.

The conclusions that we draw from these attacks upon the titre of Type I serum are that it is in large part a specific serum, but that it further contains a considerable group element. This latter view is borne out by examining the powers of Type I coccus as an absorbing

agent upon alien sera. We have employed it thus on 35 occasions, 15 of which have been graduated absorptions; 8 sera have been untouched, and 4 have been reduced in their specific titres. But 7 have been completely exhausted and, of these, 5 were in an eminent degree group sera. It follows that Type I coccus, or at least the strain with which we have worked, contains group antigen to such a marked degree as to enable it to exhaust alien group sera of their characteristic agglutinins. This, however, does not affect the validity of the claim made for it by Griffith as a specific scarlatinal type.

B. *Type II.*

A culture of this type, labelled S.F. 22, was received by us from Dr. Griffith in 1926. Two other strains have been given us by Professor Topley (N.W. 25 and N.W. 29): these are essentially identical with S.F. 22, but all our sera have been prepared from the last-named strain.

We have absorbed these sera on 48 occasions with heavy doses of streptococci other than Type II, and we have also carried out 6 graduated absorptions. We have figured most of these absorptions on the composite chart (Fig. 7) given on p. 32, where we selected this Type as our best example of a highly specific one. This was owing to the extreme infrequency with which the specific titre of its serum was reduced by absorption with heterologous streptococci—only 5 times, out of 54, to any notable extent, and only once to complete extinction. One of the strains which effected a notable reduction was Type IV, which seems to have some serological relationship with Type II. The single strain which completely exhausted Type II serum was one from a case of erysipelas in Toronto (Eagles III in the National Collection of Type Cultures). The Type II serum was not absolutely exhausted on the first occasion on which we carried out a graduated absorption: there was a small residuum (1·8 per cent.) after absorption with a dose of $0{\cdot}5 \times 10^{12}$ cocci per c.c. serum, but on repeating the test we found a dose of $0{\cdot}6 \times 10^{12}$ to effect complete removal of specific agglutinin. The Eagles III serum is readily exhausted by Type II coccus. In spite, however, of this serological identity it is a curious fact that our Eagles III serum agglutinates Type I as well as Type II coccus—though not to more than 15 per cent. of its full titre; Type II serum will not agglutinate Type I coccus at all. Eagles III serum is absorbed by Type I only to a negligible extent, 70 per cent. of its agglutinin being untouched. Although, on the grounds of the difficulty of exhausting its sera, we must reckon Type II a more highly specific one than Type I, it is by no means devoid of a group element. We have used it as an absorbing strain on alien sera of 15 varieties, and it has proved capable of exhausting 4 of them—all sera with predominantly group properties—to complete extinction of titre, while it has lowered the titres of four more to some extent.

In our hands this has proved a remarkably stable strain, having

undergone no appreciable change in properties during the six years it has been in our possession. Its colonies on agar are moderately rough. Once, however, a few smooth colonies appeared amongst the rough, distinguished by their transparent look. A smooth strain was separated with some difficulty and was subjected to certain tests before it died out. It would not agglutinate with our stock Type II serum, but came down well with our Smooth Dochez serum, which it also absorbed completely, as it did a Smooth Shilling serum. It agglutinated well with a Smooth Napier, a Smooth Shilling, and a Smooth Cutbush serum, but not with the corresponding rough sera. In other words, it bore the same relation to Rough Type II that our other smooth strains did to their homologous rough forms, exhibiting marked group properties which strongly differentiated it from the parent strain. The appearance of such smooth strains in Type II seems an accident of some rarity.

C. *Type III.*

The discussion of this type presents considerable difficulty. Our experiences with the first strain given us by Dr. Griffith were by no means happy. This was one from the United States, called 55/4, and was at the time we first received it a typically rough strain, but smooth colonies were soon obtained and agar suspensions of the smooth strain agglutinated well with Type III serum, but also, to a much less degree, with sera of Type I and II—all sera being kindly furnished by Dr. Griffith.

Later in the year he gave us another strain of 55/4, ours having died out, but he told us that the strain had undergone some change, and would now barely agglutinate with its own serum. He also warned us that Type III was less well defined than Types I and II, and showed marked group properties.

At this time (November 1926) we made a serum for ourselves, using the same 55/4 strain, but without any attempt to separate rough and smooth colonies. We were at this time experimenting with the balanced phosphate broth technique, recommended by Dochez for improving agglutinability, and we found that, grown in this medium, the Type III strain came down with both ours and Griffith's Type III serum, but also equally well with the Type I and Type II serum. Agar suspensions of Type III, on the other hand, would not come down with this serum, nor with Griffith's Type III serum. Our experience with the serum we had made was unsatisfactory. It seemed devoid of any type specificity, and on the whole behaved merely as a group serum. It was the troublesome disposition of this serum which made us choose it for the experiments we have alluded to on a previous page, in which heavy absorption with a heterologous coccus, or even with kaolin, was employed to get rid of some inhibiting agent which appeared to be present. These procedures did, in fact, induce the serum to act more naturally, but we nevertheless were faced with the following astonishing facts.

Dr. Griffith had kindly given us two further strains, labelled N.W. 67 and 386, as examples of Type III. These strains, when tested according to Dreyer's technique, both agglutinated excellently with two specimens of Type IV serum (also given us by Dr. Griffith), and not at all with Type III serum. The previous strain, 55/4, had done exactly the same. In March 1928 we made a new serum, still from the same strain, but it would not agglutinate Type III strains, so in September 1928 the rabbit was given a second course of immunization. The serum now gave better results, and agglutinated 55/4 fairly well.

Dr. Griffith, hearing of our despair over such anomalies, now brought forth a new strain named Lewis which he had, meanwhile, adopted as his standard Type III. This proved a much more docile strain. We made a serum from it in November 1928, and had no further difficulties with inhibitions, but it had the properties of a pure group serum, and our efforts were now directed to ascertaining whether, behind these non-specific components, we could detect any evidence of type specificity.

Just as we chose Type II to be our ensample of a specific serum, so we have made this Lewis serum our model of an unspecific one, on the basis of the freedom with which its agglutinin is absorbed by alien strains, as exhibited in the graph on p. 32. We are very ready to admit that we may have chanced to choose, for making this serum, a Lewis strain which was exceptionally unspecific.

Now, of 40 occasions on which we absorbed this serum (Lewis) with alien strains or that made with 55/4, there were 17 of which the titre was wholly exhausted, or at least reduced to 2 per cent. of its initial value, and a further 18 in which the value was reduced to 10 per cent. or less. Only 4 of the alien strains tested failed to effect a considerable reduction.

We have carried out graduated absorptions on our Lewis serum with 9 alien strains, the observations being often repeated. Two of these strains proved ineffective: the rest led either to extinction or virtual extinction of titre. All the preceding facts can be paralleled by group variants which we have at many times split off from a number of strains in our possession. On the evidence so far forthcoming we could not assign any higher value to these Type III strains than to other group strains. True, there were 4 alien cocci which failed to exhaust the Lewis serum to any noteworthy extent, but that is liable to happen with any group serum and probably only means that these impotent strains do not happen to possess the particular group antigen in question. We have no right to assume that there is only one group antigen, in face of Bruce White's (1926) work in the Salmonella group. And just as this serum showed itself so easily exhausted, so in turn when we come to study the powers of the coccus as an absorbing agent upon alien sera, we find it unable to effect much, if any, reduction in titre except in the case of other group sera, and this whether we employ the coccus in its rough or smooth form. We have attempted the absorption of 13 alien sera

with this strain, and in the cases of only 3 sera did we secure complete exhaustion.

But despite our negative results with Type III, Dr. Griffith still maintained the presence of a certain type-specific component, and to convince us he presently produced a strain of Lewis which he affirmed to be much richer in this type-specific component than the form he had previously given us; with his usual kindness he presented us also with some serum which he had prepared from this strain. These we labelled 'specific Lewis' and proceeded to examine by our absorption methods.

In describing our observations on Types I and II it has been seen that the presence of a type-specific agglutinin is revealed with the greatest ease, and the same is true of Type IV and its serum. But with this 'specific' Lewis nothing could be found comparable with that seen in the other types. The most that we can affirm is that the 'specific' Lewis serum was not quite so easy to exhaust of its agglutinin as the serum we had made ourselves from the more unspecific strain—requiring a somewhat larger dose or showing, after absorption, a slightly higher percentage of residual agglutinin.

We possess a number of strains of streptococci exhibiting well-marked group properties. They are of various origins, scarlatinal and non-scarlatinal, and when we come to compare them amongst themselves by such refined methods as mirror absorption, we can detect small differences amongst them. More especially when we have used them as index emulsions for reading the results of other absorptions do we find that they are apt to record differing results. The facts suggest that there may be two or more group antigens commingled in these group strains. For practical purposes this may doubtless be neglected, unless we need for some specific purpose to pursue antigenic analysis to its utmost refinements.

When we come to examine into the relations between the group Lewis and the 'specific' Lewis strains by the test of mirror absorption, we get curves which show apparent identity, as we might expect with two strains which, at most, differ only in their relative proportions of group antigen. However, on searching amongst our data for further evidence we have found a couple of mirror absorptions which are worth discussion. We have already referred to a streptococcus named Cutbush—one of the four strains mentioned on p. 36 as having been split, at an early stage of our work, into rough and smooth varieties. The Rough Cutbush, like its companion, the Rough Shilling strain, was found from the first to have eminently group properties, and when the Lewis strain came into our possession we were unable to find any serological difference between the two. When we gave a culture of Rough Cutbush to Dr. Griffith he pronounced it without hesitation to be a Type III. So confident did we feel as to this identity that we never hesitated to use the Rough Cutbush strain for reading absorption results on Type III serum.

We have carried out reciprocal absorptions between Rough Cutbush and both group and specific forms of Lewis and have plotted

graphs which show that whereas the two Lewis strains readily and almost equally absorb the Cutbush serum, the reverse is not true. The Rough Cutbush strain completely absorbs the agglutinin from the group Lewis serum, but it fails to remove much more than half of the agglutinin from the specific Lewis serum. It must be conceded that there is a definite difference between the group and specific Lewis strains in so far as the specific serum still has more than 40 per cent. of its agglutinin remaining after heavy absorption with Rough Cutbush coccus, while the group serum is wholly exhausted by a smaller dose of the same organism, and we possess other graphs which show a similar happening in lesser degree. Thus our Rough Shilling serum is easily exhausted by specific Lewis coccus, but the specific Lewis serum still has 18 per cent. of its agglutinin left after saturation with a dose of $0{\cdot}72 \times 10^{12}$ per c.c. of Rough Shilling coccus.

It may be argued that such residual titres after saturation indicate a type-specific element in the specific Lewis serum, but we should be disposed to answer that a type-specific element which is of such elusiveness as to require the oblique methods just described for its demonstration can hardly be on all fours with the striking and conspicuous type components of Types I and II (and, as we shall next show, of Type IV). We regret that, after all the kindness and patience which Dr. Griffith has shown in supplying us with material, the serological methods we have adopted scarcely enable us to see eye to eye with him in this matter of Type III, which does not seem to us comparable with his other Types, but rather an unspecific affair difficult of certain identification. It is perhaps not without significance that in the important epidemiological work recently published on school epidemics by Glover and Griffith (1930), while Types I and II play a conspicuous part, we hear nothing of the almost equally common 'Type III'.

D. *Type IV.*

This type was not determined by Dr. Griffith till later than the other three, and according to his account is of feebler pathogenic power than they are.

We first received it from him in November 1927 (the strain being named 433 T); later he gave us another strain labelled N.W. 28. He was kind enough to give us also 2 specimens of Type IV serum, and we prepared for ourselves a stock of extremely potent serum (the rabbit was first immunized with 433 T and some months later was given a second course of injections, using N.W. 28).

Judged by ordinary agglutination the strains of Type IV were highly specific, though it showed some coagglutination with Type II serum. But the serum itself sometimes brought down Types I, II, and III in an unexpected manner though only to a fraction of its true titre. Once, as we have just mentioned, 2 specimens of Type IV serum furnished by Dr. Griffith agglutinated 3 Type III strains

when Type III serum did not: curiously enough our own Type IV serum did not do this.

For a long time our Type IV strains proved exceedingly rough, and it was then difficult or impossible to prepare an emulsion which would stand up for purposes of agglutination. After a couple of years, however, it became more tractable.

We were prepared, from what we had seen in simple agglutination tests, to find a group component, common to the other types, in this type also. The essential specificity of the type was made more apparent when it was subjected to absorption tests.

Our Type IV sera have been absorbed with 36 strains of alien cocci and never once have they been exhausted of its specific agglutinin, though large absorbing doses were used. One strain reduced the titre by rather more than half, and 7 others by small amounts, down to 70 or 80 per cent. of the original titre, but 27 alien strains failed to reduce the titre of Type IV sera by a hair's breadth. The only graduated absorptions of Type IV sera are comprised in the 5 curves which we possess of reciprocal absorptions involving Type IV. In these the specific titre of the Type IV serum is quite untouched. Of the titres of the opposing strains, 2 are entirely wiped out by saturation with Type IV coccus, while 3 are reduced in varying degree.

It was a surprise to us, in view of the difficulty with which the specific agglutinin was removed from Type IV serum by alien cocci, to find that Type IV coccus was a rather potent absorbing agent on foreign sera. The coccus must contain, in addition to its sharply defined specific component, a considerable proportion of one or more group elements. We have subjected 16 alien sera to saturation with Type IV coccus and only 2 have failed to undergo some reduction in titre. Three eminently group sera have been completely exhausted, including our group Lewis serum, representing Type III. The remaining sera have suffered a reduction in titre of round about 50 per cent.; amongst these are sera with claims to a considerable degree of specificity—such as Griffith's Types I and II and our Rough Napier serum.

In view of these facts we have no hesitation in supporting Griffith's claim that Type IV is a specific entity. While its type-specific component is very sharply defined and easily demonstrated, it seems, in view of its absorbing powers, to possess a somewhat larger group element than Types I and II. Once we chanced to obtain a colony with completely group properties, but as a rule Type IV is a highly specific strain.

(ii) The Dochez Strain

The Dochez streptococcus deserves study for more than one reason. It has historical interest as the earliest recognized scarlatinal strain now extant. Its high toxigenic power has led to its being employed in many laboratories for the preparation of scarlatinal toxin and antitoxin, and its widespread cultivation has afforded the opportunity for serological mutation. It is not a strain of common occurrence in England, but it is well known in the United States, and out of 8 strains

received from an epidemic in Moscow we found no less than 3 serologically referable to the Dochez strain.

It was first isolated in 1922 from the throat of an early case of scarlet fever in New York and has been carried on in Professor Dochez's laboratory ever since, chiefly in blood-broth subculture, with mouse passage from time to time. In America it was also maintained by Dr. Wadsworth, who kept up its virulence by weekly mouse passage; Wadsworth's strain, known as E. 14, was brought over to England by Dr. E. W. Todd, who kindly gave us a culture still highly virulent in June 1930.

Dr. O'Brien, of the laboratories of the Wellcome Foundation, received the strain direct from Professor Dochez at an early stage of its career, and has employed it for many years for making toxin and antitoxin, being unable to find any strain superior to it in toxigenic power. Dr. O'Brien gave us a culture in March 1925, and he also furnished a culture to Dr. Griffith. We made serum from the crude strain, and, as later studies proved, this was a group serum of no selective value. At that time we were groping in the dark, with no knowledge of specific and group mutants and no efficient absorption technique, but by February 1926 we had realized the existence of rough and smooth forms and had separated the Dochez strain into rough and smooth varieties, serologically distinct.

With the rough form we made a new serum, which proved to be highly specific for the Rough Dochez strain, but at this stage our notes were confused. Deliberate attempts to prepare rough and smooth Dochez sera were not made till March 1927. The rough strain and serum which we now accepted as genuine proved to be serologically identical with Griffith's Type I, and we are forced to believe that some error must have occurred, probably in the labelling of a tube. Although we have repeatedly tried to split off from our original Dochez strain one having Type I characters we have never succeeded. Moreover, two further cultures kindly furnished by Dr. O'Brien, also Wadsworth's E. 14 and cultures sent us direct from Professor Dochez in New York, all conformed to the Dochez strain in the possession of Dr. Griffith and bore little relation to Type I beyond being sometimes agglutinated by Type I serum. Moreover, since Type I is one of the commoner scarlatinal types in England it would be odd that it should be a derivative of a type so uncommon in this country as the Dochez type.

Abandoning therefore our early observations on the original Dochez strain, given us by O'Brien, as probably tainted with error, we will confine ourselves to the strains we obtained later from Dr. O'Brien, Dr. Griffith, and Professor Dochez, which are quite sufficient to exhibit the changes which may be undergone in 9 or 10 years of laboratory culture by a single known strain.

At the time Dr. Griffith gave us his standard Dochez strain in November 1928, he gave us 3 other strains from Anna Williams in America, labelled A.W. 56, A.W. 57, and A.W. 17B, affirming them to be the same as the Dochez strain. We also could find no differences

of any kind amongst them. Wadsworth's E. 14 was also similar, and also our 3 Russian strains (Moscow 52, 69, and 81). All the above we judged to belong to one type, identical with the cultures later sent us from New York by Professor Dochez himself, labelled New York 5. We have carefully compared by serological means these various strains so diverse in their laboratory history.

Dr. Griffith was good enough to furnish us with a supply of more than one serum made from his Dochez strain and we made a serum of our own from one of the later cultures sent us by Dr. O'Brien. Griffith's serum appeared to possess marked group properties: it clumped our known group strains with impartiality, but produced no effect upon the Type I coccus. Conversely, Griffith's Dochez coccus was agglutinated by our current group sera practically to their full titre, but not at all by Type I serum.

When, however, we turn from the evidence afforded by agglutination alone to that yielded by absorption, we find a different state of affairs. We have saturated the Griffith-Dochez serum 26 times with other cocci and we find that its specific agglutinin is removed with great difficulty. The only strains which truly exhaust this serum are those of its own group—Wadsworth's E. 14 and our Moscow strains. The alien group strains so readily brought down by this serum fail, when put to the proof by the absorption test, to remove its agglutinin. Even such strains as Group Lewis and Rough Cutbush effect nothing. Only Smooth Napier, amongst the group strains tested, brings the titre of Griffith's Dochez serum down below 10 per cent. of its initial value.

When we examine the absorbing powers of this strain we find that it is inert as regards such power upon all our specific sera save one, while readily exhausting our group sera—the relation with the latter being of the one-sided character shown in Fig. 21. Amongst the specific sera we were astonished to find Rough Napier serum completely exhausted by Griffith's Dochez coccus, though the reverse was not the case. The unilateral relation was here the reverse of that seen with other specific strains. We are unable to explain this.

The general conclusion we have formed as regards the Dochez strain maintained by Dr. Griffith is that it is entitled to rank as a specific type equally with Types I, II, and IV, but that, like these last, it contains a large group element.

When we procured from Dr. O'Brien, in May 1929, a new culture of his stock Dochez strain, and compared it with Griffith's strain (also originally from O'Brien's stock) we found certain striking differences. In the first place, we found that the new O'Brien II, as we called it, was agglutinated practically to full titre by our Type I serum, whereas the Griffith strain was untouched by that serum. The Griffith Dochez serum agglutinated the O'Brien II strain to full titre, but to our surprise was not absorbed by it in any degree worth mentioning (once to 80 per cent. of the initial value), though we tested two different sera from Dr. Griffith and used very heavy absorbing doses (up to $1{\cdot}4 \times 10^{12}$ per c.c.).

Tested against our stock sera this second culture from O'Brien was found readily agglutinable and to fairly high titre by nearly every serum tried, even by all the sera of Griffith's major types. This had by no means been the case with Griffith's own Dochez strain, which had responded only to our group sera (see p. 66). One noticeable fact was that this O'Brien II strain failed to agglutinate with Griffith's Dochez serum.

When we later made a serum with the O'Brien II strain, it turned out almost a pure group serum, bringing down our various group strains with impartiality. We have only a few records of absorptions of this serum, but they indicate that it was readily exhausted by such strains as had been proved to exhaust our other group sera. As an absorbing strain it was by no means effectual; we used it on 13 sera and only 4 of them, all with group properties, were exhausted.

A third strain sent us by Dr. O'Brien in March 1931, also from his standard culture, proved much the same as the second strain he had given us. It was a group strain, and it failed, like its predecessor, to agglutinate with Griffith's Dochez serum. It agglutinated with Type I serum, but not to its full titre; it failed to absorb that serum even in a colossal dose ($1{\cdot}7 \times 10^{12}$ per c.c.).

Finally we come to the Dochez strain received in February 1931 from Dochez himself, who informed us that during the 9 years it had been cultivated in his laboratory he himself had made no extensive study of the serological variations arising, but that he had noted occasional variations in strains from other laboratories derived from his original strain. As we received it this culture was inagglutinable by Griffith's Dochez serum and only to a feeble degree by Type I serum. It was, however, agglutinated by our current smooth sera, but not, curiously enough, by sera of the Type III complexion such as Rough Cutbush. This suggests that there may be more than one kind of group in what, in our present state of knowledge, we lump together as 'Group sera'. We made no serum from this strain, but it was found to absorb our 'O'Brien II' serum completely. The identity of this strain with that carried on by O'Brien seems beyond dispute and its relation with that held by Griffith is probably that of a group and a specific variant.

We have gone with considerable detail into this Dochez strain because it appears to present in a very definite form the difficulties attending serological diagnosis amongst the haemolytic streptococci. It shows, in the first place, how weak a reed to lean on is mere agglutination. Of the various branches of what is undoubtedly a single primitive strain we see one entirely unaffected by sera of Types I, II, III, and IV, while other strains are brought down by these readily. Again, we see a serum made from the Griffith branch of the strain at one time agglutinating and at other times failing to agglutinate the later strains we received from O'Brien and entirely failing to agglutinate the main stem sent us by Dochez. And all this is not the result of rough and ready agglutinations on a slide, but of the most refined methods which we have been able to employ, confirmed by frequent repetition.

The absorption method helps us to some extent, for although some strains may be agglutinated by an unauthorized serum, such as Type I, they consistently fail to absorb it. But the real breakdown in the absorption test is evident when we see the Griffith serum practically unreduced in titre by saturation with our later O'Brien strains.

The explanation, of course, is to be sought in the facts of dissociation. As we have already recorded, we discovered at an early stage of our work that a given strain of haemolytic streptococcus can dissociate itself, or be artificially dissociated, into forms with almost no serological relation one with another, though what brings about the dissociation, or how permanent it is, we do not know. It would seem that, in the case of this Dochez coccus we have a strain which is at heart a group one and in Dochez's laboratory has remained so, but that in O'Brien's laboratory it has had at least one lucid interval of specificity during which he handed a subculture to Griffith, in whose laboratory it has maintained itself in the more or less specific state, while in O'Brien's laboratory it has again lapsed into the unspecific form. Who, then, can wonder that the serological classification of the haemolytic streptococci is in a confused state? It so happens that the exceptional toxicity of the Dochez strain has caused it to be preserved in many laboratories for a long term of years. This has afforded us the opportunity of tracing the serological vicissitudes of the strain in its various branches. The opportunity has been a unique one, but there is no reason for doubting that the same sort of thing may occur with other strains.

(iii) Heterogeneous Scarlet Fever Strains

In his studies of the scarlet fever throat Dr. Griffith found that only some two-thirds of the haemolytic streptococci which he isolated could be assigned to his four major types. The residue were a mixed lot of heterogeneous forms not only unlike the major types, but dissimilar amongst themselves.

He was so kind as to give us examples of some 10 of these types, together with sera which he had made from them. We were not, however, able to make any more of them than he had, or to correlate them with any of our own strains. They were what might be called 'serological singletons' such as made up the vast majority of our own non-scarlatinal strains.

In a group of 8 strains, which we obtained from an outbreak of scarlet fever in Russia, 4 out of the 8 could not be determined. Three were in all respects referable to the Dochez type; another was an excellent example of Griffith's Type I.

(iv) Summary of Observations on Scarlet Fever Strains of Haemolytic Streptococci

It will be seen that the work we have carried out by Dreyer's method on strains from the scarlet fever throat, mainly on material supplied to us by Dr. Griffith, confirms in the main the conclusions he

had reached by other methods. Only in one particular do we find ourselves at variance with him, namely in the claim of his Type III to rank as a specific entity with his other major types. The conclusion seems to follow that there is no one serological form of streptococcus which can be credited with the causation of this disease, though there are three or four recognizable serological types which seem exceptionally commonly found in it. Opinion seems to be more and more inclined to the belief that the property which determines the power of causing scarlet fever is rather the intensity of toxin production than any power discoverable by agglutination.

(v) Puerperal Fever

We have had under our hands more than 40 streptococcal strains from puerperal sepsis or from the throats of persons suspected of having been concerned in its causation. Many of these have been derived from the maternity wards at St. Bartholomew's Hospital, but the great majority have emanated from Queen Charlotte's Hospital, having been given by Dr. Colebrook to Dr. Griffith and handed on by the latter to us. Dr. Griffith had made sera from a large number of them and he kindly supplied us with such a sufficiency of these sera that we had no need to make specifically 'puerperal' sera of our own, though in one case, for special reasons, we did so.

We examined these strains side by side with our ordinary surgical strains—first by orienting simple agglutination tests, followed up, if any clue suggested itself, by the more exacting absorption test. We possessed a good range of sera—16 of our own and 14 given us by Dr. Griffith, and our practice was to put up a new strain, when it had been found sufficiently stable in saline, against all 30 sera at once. The readings obtained were then reduced to percentages of the known full titres of the serum for their own strains, and these percentage figures were then plotted as columns on a chart in which the individual sera were always arranged in a definite order. Charts thus prepared were strictly comparable with one another, and showed, at a glance, similarities and differences in the strains under survey. Some strains proved so highly specific that they reacted with only one of the 30 sera, others so devoid of specificity that they reacted in some degree with nearly all. The slighter degrees of agglutination—20 per cent. or less of the full titre of the serum—were not deemed of serious significance, but many group forms were found to agglutinate up to 40 or 50 per cent. of a number of sera at once. It was extremely rare to find a strain agglutinating to the full titre of any serum but its own —but this we did see on two or three occasions. This or anything approaching this was the signal for an absorption test, but it was seldom, indeed, that hopes were realized.

The number of our strains examined in this laborious manner was necessarily limited—not more than 38 in all, including puerperal, surgical, and erysipelas strains. The remainder were examined on a more modest scale.

The importance of serological differentiation of streptococci associated with puerperal fever lies largely in the possible epidemiological application of the results. We have made a few attempts, as have others, to track down the source of infection of a patient with puerperal fever by isolating and studying serologically the haemolytic streptococci in the throats of attendants and other contacts. Our efforts have been unsuccessful; we have not succeeded in isolating streptococci identical with the infecting strain from swabs from any of the throats examined. We have been hampered by two practical difficulties. First, many of the organisms isolated have been at first very rough and difficult to study; secondly, in certain instances we did find an apparently close relationship between the 'puerperal' strain and a strain from a contact, but that was only due to the fact that the organisms concerned were of predominantly 'group' complexion.

One patient with puerperal fever does, however, require special mention. Haemolytic streptococci were isolated from her uterus, from her throat, and from blood-culture. The throat strain fell by agglutination and absorption tests into Griffith's scarlatinal Type II. The organism from the uterus was too unstable for agglutination tests; but as it completely absorbed the agglutinin from a Type II antiserum it can probably be safely classed as Type II also. The complete conviction which mirror absorptions could provide is, however, lacking, as no antiserum was made against this puerperal strain. The relationship of the blood strain to the others is obscure; it also was too 'rough' to use in agglutination tests, but unlike the uterus strain it absorbed no agglutinin from a Type II serum.

(vi) Erysipelatous and Surgical Strains

We have had the opportunity of studying 11 strains from cases of erysipelas, including 5 of Birkhaug's and 3 of Eagles's strains from the National Collection of Type Cultures. Agglutination tests carried out with many of these streptococci against the sera available did not reveal any close relationship with any of the organisms—one of them an erysipelas strain—against which we had prepared sera. At most they agglutinated to a low titre with Griffith's Type I and Type III scarlet fever sera, as do many other strains with a considerable group element. One exception to this generalization has already been mentioned: Eagles's Type III erysipelas strain was found to be identical with Griffith's scarlet fever Type II when mirror absorptions were carried out. Dr. Griffith has also examined this Eagles's III strain and confirms our finding. As far as our limited observations went there were no obvious relationships amongst the erysipelas strains except in so far as they contained the group elements common to them and other streptococci.

The findings with the surgical strains were much the same; many of the results have been portrayed in the figures illustrating the various types of absorption curves. No definite types of organisms stood revealed. Three surgical strains, however, as mentioned earlier, fell

into Griffith's Type I; two of these were from cases of otitis media and may have had a scarlatinal origin; the third was from a patient with septicaemia following impetigo.

5. SUMMARY

The main object of the research has been to ascertain, by using the more refined methods of antigenic analysis, whether it was possible to construct a grouping of haemolytic streptococci likely to be of practical value in clinical work, and especially to see whether serological varieties could be correlated with apparently distinct diseases.

It was soon clear that agglutination tests by themselves would prove a weak reed. For accurate antigenic analysis, quantitative agglutinin-absorption tests were imperative. Much work was necessary to devise the best method of preparing suitable emulsions of streptococci for agglutination tests. The attack on this difficulty led to a study of the acid-agglutination of streptococci; and astonishing facts were brought to light concerning the effect of the final—and even of the penultimate—culture medium upon the acid-agglutinability and serological behaviour of the organisms.

In work of this sort, many serious pitfalls can be avoided only by a recognition of the extreme variability of *Str. pyogenes*. It appears that more or less rough and smooth colonies may be picked from a plate; but these must not be supposed to correspond with the rough and smooth forms of *B. coli* and Salmonella. Commonly the rough variants are the more virulent, but the reverse may be true. Commonly, also, the rough form is the more specific and the smooth the group form, but there are exceptions to this rule too.

Single strains of cocci such as the Dochez strain may change greatly on cultivation; and sera prepared against one strain at different times may be found to have quite different properties. Organisms and sera with pronounced 'group' properties may lead the unwary astray. Some of the techniques advised for preparing good agglutinable emulsions actually tend to the production of 'group' forms. Thus it may have happened that a worker who has, as he imagines, recognized a serological type of streptococcus associated with a certain disease, has actually been deceived through working with a serum which is largely 'group' in character.

Something about these group and specific races was learnt by means of absorptions with single adequate doses, but far more was revealed by absorptions with graduated doses of organisms and by mirror absorptions. From these it appeared that only exceptionally are two strains of streptococci serologically identical and very rarely are they entirely dissimilar. Almost always there is more or less group antigen in common between them; there are even definite hints that more than one group antigen exists. There is a wide basis from which such conclusions can be drawn; for about 125 graduated absorptions and about 630 ungraded absorptions have been carried out.

This general study of the racial interrelationships of haemolytic

streptococci led on to a more detailed antigenic analysis of certain strains, particularly Griffith's four major scarlet fever types. The individuality of his Types I, II, and IV was readily confirmed. It was otherwise with his Type III, which did not seem entitled to rank beside the others as a distinct entity, but was rather a predominantly group form. The Dochez strain appeared also to be a distinct variety, though it seems to be an uncommon one in this country. Other strains from scarlet fever were 'serological singletons'. Apparently no one serological form of streptococcus can be credited with the causation of scarlet fever, though three or four recognizable serological types seem exceptionally commonly found in it.

No distinct types stood out from a miscellaneous assortment of puerperal, surgical, and erysipelas strains of streptococci, though an occasional representative of Griffith's scarlet fever Types I and II was found amongst them.

The literature concerning the serological classification of haemolytic streptococci is most confusing. Each worker has used a different technique and has made no attempt to correlate his results with those of his forerunners. Others may yet attempt the Herculean task. It is hoped that the work here recorded may be of some service to them and that some of the many pitfalls have been revealed. A serological study by methods more accurate and quantitative than most workers have used has failed to bring order into the chaos. 'The more one studies haemolytic streptococci the more strongly is the impression gained that they are in a state of constant flux in which it is difficult to find any firm foundation for a permanent systematic classification.'

6. PERSONAL ACKNOWLEDGEMENT

The work which is recorded in the preceding pages was rendered possible by the unfettered gift to me of the sum of £1,000, in furtherance of research work, by Messrs. Wallace Bros. & Co., Ltd., of Crosby Square, E.C. 3. This generous gift was made in 1924 and I eventually decided to spend it in paying a suitable salary to a trained laboratory worker to assist me. I was so fortunate as to secure the services of Miss Lettice Digby, and with her help the work on haemolytic streptococci was started early in 1925. Unfortunately Miss Digby's health broke down in the summer of 1927; her place was then taken by Mrs. E. M. Christie. By the autumn of 1928 the kind gift of Messrs. Wallace had become exhausted and at my request the Medical Research Council was good enough to take over the payment of Mrs. Christie's salary, and this they have continued to do ever since.

I have therefore a double debt of acknowledgement to record, first to Messrs. Wallace and secondly to the Medical Research Council. To both of these bodies I offer my sincere thanks. But no words are adequate to express my debt to the two ladies whose unremitting assistance has enabled the laboratory work to be carried on over so many years. Mrs. Christie's name naturally appears with mine on the

title-page, though I have had the responsibility of the actual writing of the report.

We must leave to others to assess the value of what we have done. The results may seem somewhat meagre for seven years' work, but they represent the survey of a very large field and it is hoped that they may, at least, spare others a similar labour.

F. W. ANDREWES.

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