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Contributors

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AN OUTLINE OF IMMUNITY

LONDON (ROYAL FREE HOSPITAL)
SCHOOL OF MEDICINE FOR WOMEN
8, HUNTER STREET, W.C.1.

AN OUTLINE OF IMMUNITY

BY

W. W. C. TOPLEY, M.A., M.D., F.R.C.P., F.R.S.

PROFESSOR OF BACTERIOLOGY AND IMMUNOLOGY
IN THE UNIVERSITY OF LONDON

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PREFACE

It is probable that no subject in the medical curriculum is, at the moment, in a less satisfactory position than that to which we attach the label *Immunity*. It is a hybrid creature, not quite sure of its true affiliations. It wanders, a little uneasily, between Departments of Pathology, Bacteriology and Hygiene. At times it makes an attempt—which to some of us appears misguided—to set up house all by itself.

Whether because of this anomalous position or for some other reason the medical student's acquaintance with immunity tends to be transient and trifling. This is unfortunate. A high proportion of the total sickness with which the medical man has to deal, whether we think in terms of prevention or cure, results from infective disease; and it is a little strange that the branch of medical science that deals specifically with the events that happen in an infected host, and with the ways in which we can interfere in these events, should receive as little attention as it does.

It is of course quite true that much of the ground is covered under the label of pathology or bacteriology; but it is equally true that those aspects of immunity that are, at the same time, its most characteristic product and its most significant contribution to practical medicine are granted short shrift in hours under either head.

No sensible or humane person would suggest adding to the burden under which the medical student staggers. If he is to learn a little more about immunity he must learn a little less about something else. A personal view is that great benefit would result from teaching certain aspects of the subject during the pre-clinical years. Many of the fundamental phenomena of immunity are direct and natural extensions from chemistry, biology and physiology. They could well, perhaps best, be taught without any immediate and direct reference to the clinical manifestations of infective disease as it occurs in man. If these general principles could be mastered at an earlier stage, the student in the wards would, from the first, be in a position to apply them to his clinical problems in a way that is hardly possible to-day.

All this, perhaps, is a poor reason for adding to the long list of medical textbooks; but it may serve as an excuse for the way in which that task has been done. I have tried, in this book, to give in general outline our present views on immunity, with sufficient detail with regard to each particular problem to justify the conclusions set down. It has not been easy. Immunity is a vigorous infant among the sciences, just rising fifty years. Like most young and vigorous things, it is a little uncertain in its movements, a little

confused in its mind, and more than a little incoherent in its speech. The growth of the past ten years has changed its outlook widely ; and that growth cannot be neglected. It might be urged that a knowledge of established facts and reasonably stable theories is all, if not more than all, that can be expected from those for whom immunity is only one of many methods of approach to the problems of practical medicine. But the case is not so easily met. Ignorance of the present working hypotheses of immunity means inability to form a sound judgment on many severely practical problems. Immunological findings travel quickly, sometimes too quickly, from the laboratory to the ward and to the field. From the moment he is qualified, the student will have to act on his own views with regard to the efficacy of this or that immunological procedure. It is bad for him, and very bad for medical science, that his views should be uncritical and ill-informed. And so, with no escape from controversial matters, the didactic method becomes impossible. All that can be done is to give personal conclusions and the evidence on which they are based. Every medical man must learn the art of drawing the best conclusions he can from data that are often incomplete and sometimes highly confusing. He will find immunity an excellent training ground.

The evidence must in fairness be set down ; but no one who has tried to learn or teach will expect the student to remember more than a little of it. The rest will serve for reference. By the use of different types and spacing I have tried to indicate my own views as to the relative importance of the various parts of the text.

The references to original papers are more copious than is usual in a textbook of this kind, though even so they must needs be incomplete and arbitrary. I have included them so that any who are wise enough to prefer their own conclusions to those offered by others may find access to the records.

The substance of a great part of the material included here has already appeared in *The Principles of Bacteriology and Immunity* written in collaboration with my colleague, Professor Wilson, and I am indebted to him for permission to reproduce it in its present form. It has, however, been entirely rewritten. In some parts it has been compressed, in others extensive additions have been made.

I owe much to the help and advice of many of my colleagues in this School, including members of the Library Staff, and of the Bureau of Hygiene and Tropical Medicine. Their kindnesses have been so many that individual acknowledgment is impossible.

W. W. C. TOPLEY.

LONDON SCHOOL OF HYGIENE
AND TROPICAL MEDICINE.

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CHAPTER I

INTRODUCTORY

IMMUNITY and its unattractive variant *Immunology* are misleading names for an ill-defined subject of study.

They mislead in themselves. As plain English, *Immunity* does not mean what it says. This does not matter very much because, as Whitehead points out so aptly in his *Introduction to Mathematics*, the scientist shares with Humpty Dumpty the privilege of paying words extra and making them mean what he likes. But if we are to avoid confusion we must at least give our words definite orders and see that they are obeyed. It happens that the state suggested by the word that we have chosen as a generic label for our branch of science is one about which we know very little, except that it exists. We have, indeed, not bothered very much about it. Complete insusceptibility to infection removes the relationship between the particular host and parasite concerned from our field of interest, except in so far as we can learn from any instance of this kind something of the genetic laws that determine the transmission of this innate and complete resistance. Our main business is with those interactions between parasite and host that are characterized by a fluctuating equilibrium, and with the factors that shift this equilibrium, so that sometimes the parasite, sometimes the host, gains the upper hand. *Resistance* would be a better word than *Immunity*; but *Immunity* will serve well enough so long as we make it quite clear that we are using it to denote the resultant of two opposing systems, and that this resultant can assume any value from zero to infinity.

And our name misleads a little because of its associations. It happens to have been frequently applied to the data obtained by a particular kind of technique, and there has been a tendency to confuse technique with subject matter. The special methods devised by the immunologist—if he must accept the unpleasing name that custom has given him—are applicable to problems with which he has no concern; while the field proper to his study extends far beyond the limits that would be imposed by any definition in terms of the particular technique employed. The student of immunity is concerned with all data relating to the mechanisms involved in infective disease, by whatever means they are obtained. Any account given at the present time must stress unduly a particular set of phenomena, simply because they are the only pheno-

mena that have as yet been adequately studied ; but this is a historical accident that will be remedied by time.

The phenomena that present themselves for study are derived from the most diverse sources. Some come to us from epidemiology, using that term in its widest sense. Under natural conditions different animal species show a widely differing incidence of certain infections. Thus, anthrax is in the main a disease of herbivora. Of the animals dying of anthrax in this country in 1914, 733 were cattle, 5 were sheep, 32 were swine and 25 were horses. In Australia and South America sheep are more commonly affected ; but Algerian sheep are stated to be highly resistant. Tuberculosis is one of the commonest natural infections of man and cattle. It is common in pigs and in fowls. It is relatively uncommon in sheep, goats, horses and dogs. It is stated to be very uncommon as a natural disease in rabbits, guinea-pigs, rats and mice. Among mankind there are well-marked racial differences in its incidence and severity. In eight of the great cities of the United States in 1920 the mortality from tuberculosis among the white population varied from 0.794 to 1.216 per 1,000 living ; among the coloured population it varied from 2.855 to 4.205 per 1,000. Such instances could be multiplied *ad nauseam*.

The epidemiologist also records differences in the incidence and fatality of various infective diseases at different ages, suggesting in many cases an increase in resistance with age. He notes also that repeated attacks of the same infective diseases are in some instances very rare, as in measles, or smallpox, or diphtheria, or typhoid fever, while in others they are relatively common, as in influenza, or pneumonia, or the common cold.

Other data come to us from clinical medicine in the narrower sense. Infections that, in their usual course, progress slowly to death or recovery sometimes assume a fulminating form. Such a protean infection as tuberculosis shows the widest diversity in the varying prominence of its local and general manifestations. And, in any infective disease, why do some patients die and others recover ?

For the immunologist these are crude data that require analysis. Using his own methods he re-examines the phenomena presented by natural infection. In parts he transforms the picture they present. He finds, for instance, that the epidemiological data with regard to the natural incidence of tuberculosis tell a tale that is in places highly misleading. To begin with—even excluding as we have done tuberculosis in fish and reptiles—we must recognize not one type of infection but three ; since the human, bovine and avian types of tubercle bacillus behave very differently when tested by experimental injection into animals of different species. We cannot experiment on man, but we can determine the type of the bacilli isolated from human lesions. We find that he is susceptible to infection with either the human or bovine type, but apparently quite resistant to the avian type. Experiment confirms field observation as regards the susceptibility of cattle and pigs to tuberculosis ; but, while man is susceptible to both human and bovine types, the cow is very susceptible to experimental infection with the bovine type, but is relatively resistant to both

the human and avian types. The pig resembles the cow in its susceptibility to the bovine type, but is rather less resistant to the human and avian types, both of which give rise to a localized tuberculosis. Field observations and experiment tell very different stories about the goat. Natural infection is infrequent, but there is no evidence of resistance to the bovine type of tubercle bacillus as tested by direct inoculation. To experimental infection with the human and avian types the goat shows the same high resistance as cattle. The sheep is in much the same position as the goat. The suggestions offered by our field observations with regard to the horse and the dog did not mislead us. The horse is relatively resistant to experimental infection with the bovine type of bacillus, and quite resistant to the human and avian types. The dog develops localized lesions when inoculated with the human or bovine type, sometimes with a minor degree of dissemination; to the avian type it is highly resistant. But our records, such as they are, of natural infection in the rabbit and the guinea-pig are grossly misleading if we accept them as evidence of resistance to experimental infection. The rabbit develops a rapidly fatal tuberculosis when injected with bacilli of the bovine type. To the human type it is more resistant, developing a localized lesion with a minor degree of dissemination. To a moderate dose of avian bacilli, injected intraperitoneally or intravenously, it responds by developing a curious type of infection, described by Yersin and known by his name, in which the bacilli multiply widely in the tissues causing the death of the animal, but without giving rise to the characteristic lesions of tuberculosis. As for the guinea-pig, it is highly susceptible to both the bovine and the human types, and resembles the rabbit in response to the avian. In regard to rats, laboratory findings are in accord with statements as to the frequency of the natural disease. It develops only the Yersin type of infection, and that only in response to the injection of large doses of bacilli. With mice the position is less certain. The field mouse was found by Koch to be highly susceptible to experimental infection. It has been frequently stated that the white mice of the laboratory are highly resistant, resembling the rat in their response to massive experimental infection, but this view seems to require modification as the result of more recent observations. It may be added that birds are susceptible to infection with the avian type, but they are generally resistant to the bovine or human type—the parrot and the cockatoo affording exceptions.

That a clinical entity may correspond to an immunological diversity is true of many diseases other than tuberculosis. It is true, for instance, of lobar pneumonia. For the immunologist this is not one disease but several, each caused by a significantly different type of pneumococcus; and he notes that the statement that second attacks of pneumonia are not uncommon may belong to the Baconian category of a truth that has in it a mixture of a lie. Again he finds that enteric fever is not one but many; and his attempts at interference are planned accordingly.

Another important change that the immunologist makes in the clinical and epidemiological picture is in regard to the character and extent of the associa-

tion between any given parasite and the host species that it infects. He finds that the real range of interaction includes states of equilibrium in which the host shows no overt signs of disease. Whether we call all these conditions latent infections, or refer to many of the hosts as healthy carriers, matters little. No hair-splitting definition will help us much. The significant thing we have learned is that in some infections, such as measles, contact between a previously uninfected host and the virus of the disease usually results in a clinically characteristic attack, so that the epidemiological picture gives us a reasonably adequate description of the biological association, while in other diseases, such as cerebro-spinal meningitis or poliomyelitis, the clinically diagnosable cases form so small a fraction of the total number of infected persons that they might almost be regarded as occasional accidents in an association that, in its modal form, induces no such serious effects.

In re-examining the data that he receives from the field and from the ward, the immunologist relies in large part on experiments on animals. When he tries to interpret his data he is forced to rely almost entirely on this fundamental method of study. He cannot advance without some simplification of his problems, some control, at least, over the innumerable variables that determine the incidence and results of infective disease as it occurs in nature.

By such experiments he has found that he can increase the resistance of animals by infecting them with sublethal doses of a given pathogenic organism, or by injecting an organism that has lost its power to kill—though here he was forestalled by Jenner's experiment of vaccination in man—or, more safely and conveniently, by injecting dead organisms or their products. From analogy, and from the fact that he can demonstrate similar changes in the tissue fluids, he concludes that this artificial immunization in animals is essentially similar to the natural immunization that occurs during an attack of an infective disease. Since the tissues of the naturally infected or artificially immunized host play an active part in bringing about this increased resistance, he calls it *active immunity*. In some cases he finds that he can transmit this resistant state to a normally susceptible animal by injecting into it the serum of another animal that has been rendered immune. Since the tissues of the recipient appear to play a relatively passive part, he calls this *passive immunity*. He also finds that immunity of this kind is sometimes transmitted naturally from a mother to her young, either by the passage of the protective substances *via* the placental vessels, or by their ingestion during the first days or weeks of life in the colostrum. This he calls *congenital passive immunity*.

And so, if he has a taste for classification, the immunologist can draw up some such list as this :

1. Innate Immunity.

- (a) Complete.

- (b) Partial.

2. Acquired Immunity.

(a) Active.

(α) Naturally acquired.

(β) Artificially induced.

(b) Passive.

(α) Naturally acquired (congenital).

(β) Artificially induced.

Grades of Immunity.

We have noted that the kinds of immunity in which we are most interested are never absolute. To keep our ideas clear and precise it will be well to consider briefly the grades of immunity that may, in fact, be demonstrated, remembering always that they shade into one another by imperceptible degrees.

Fig. 1 gives, in diagrammatic form, a rough classification that will be sufficient for our immediate needs. It represents an infection of the invasive type, associated with a bacteræmia (an invasion of the blood stream by micro-organisms) as well as with local lesions; but it can easily be modified to meet the case of a toxæmic infection.

The width of the black wedge-shaped areas at any level may be taken to represent the chance of death, the degree of bacteræmia and the extent of the local lesions in an individual, or the frequency of these phenomena, or of latent infections, in a sample of individuals possessed of approximately the same grade of immunity.

Thus, starting with the completely susceptible, we may assume that all, or almost all, individuals infected will develop an acutely fatal bacteræmic infection. Local lesions will be infrequent, and minimal when they occur. There will be no latent infections.

Passing to our next arbitrary grade—partial immunity of low degree—we find fatal bacteræmic infections becoming less common, local lesions more frequent and more pronounced, and a small but increasing number of latent infections.

With partial immunity of a medium grade we find bacteræmia and death much less frequent, local lesions common and relatively extensive, and latent infections increasing in frequency.

With partial immunity of a high grade, death no longer occurs, bacteræmia is infrequent and, when it occurs, is slight and transient. Local lesions are becoming much less frequent and, when they occur, much less extensive. Latent infections reach a maximum frequency and then begin to decline.

Finally we reach the ideal—perhaps the dream ideal—of complete or solid immunity. The host is entirely impervious to all attacks of the parasite.

It will not have escaped attention that the grades of resistance that we have labelled as partial immunity are compatible with severe and often fatal infections, and that many infective diseases in their common clinical form might be regarded as occurring in partially immune persons. This view is almost certainly the right one. The syndromes that normally characterize such diseases as typhoid fever, or lobar pneumonia, are expressions of partial

immunity. They would not occur in a completely susceptible population attacked by a fully virulent parasite. The latter proviso is, of course, necessary. We may logically use the term immunity to express the relation of any given host to any given parasite, but events are determined by a balance—the balance between the virulence of the parasite and the resistance of the host.

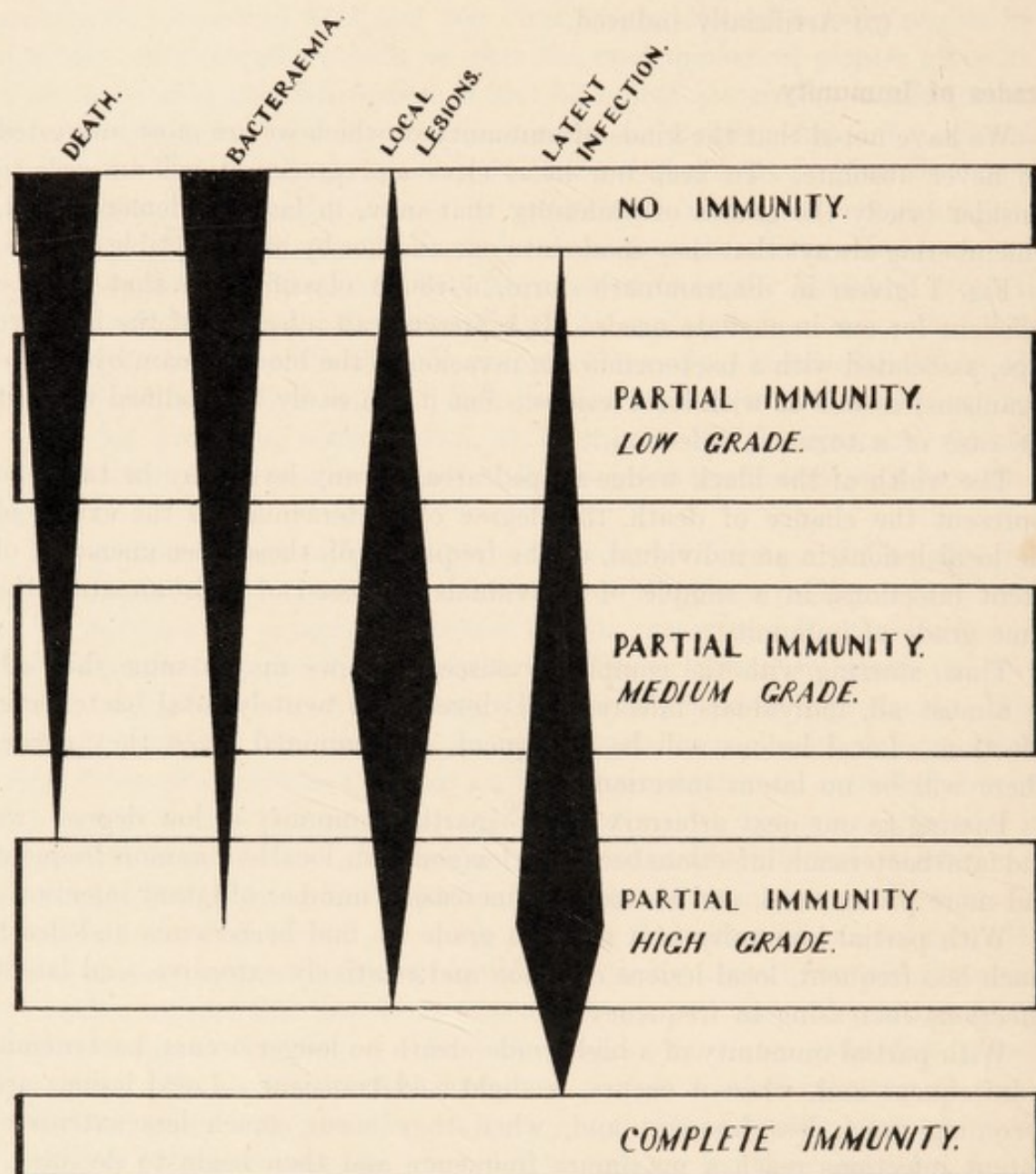


FIG. 1.

The conception that infective disease, as we usually see it, is an expression of partial immunity, and that minor increases in immunity tend to increase the frequency of milder infections, is of sufficient importance to justify a few illustrative examples, drawn from experimental data.

It was noted by Smith and Moore (1892) that normal and actively immunized rabbits respond very differently to the subcutaneous inoculation of *Pasteurella*

suisepitica. Normal rabbits develop a rapidly fatal septicæmia. Immunized rabbits may develop large local abscesses, but show much less tendency to succumb to an acute generalized infection. More recent studies by Jones (1924) afford a good illustration of active immunity in all its grades. The normal rabbit is highly susceptible to *Pasteurella avisepitica*, dying of a generalized septicæmia after the subcutaneous or intratracheal inoculation of 0.01 c.c. of a broth culture. In the latter case pulmonary lesions are slight or absent. In rabbits partially immunized by a single injection of a killed vaccine a similar intratracheal injection of living culture is followed, in most cases, by a severe and fatal pneumonia, usually associated with a suppurative pleurisy and pericarditis. In rabbits immunized by repeated doses of vaccine, intratracheal injections of living culture are usually without effect, while subcutaneous injections lead to the formation of localized abscesses followed by recovery.

Similar phenomena have been recorded in other bacterial infections. Thus Wadsworth (1904) found that the intratracheal injection of virulent pneumococci into normal rabbits was followed by a rapidly fatal septicæmia without local lesions, while similar injections in partially immunized rabbits were often followed by a characteristic pneumonic consolidation.

Differences of the same kind have been observed when strains of pneumococci of varying virulence have been injected intratracheally into normal rabbits. Thus Gaskell (1927) found that strains of high virulence gave rise to a rapidly fatal septicæmia without obvious pulmonary lesions; those of lower virulence to a fatal lobar pneumonia; those of still lower virulence to a patchy lobular consolidation, grading into an entire absence of reaction as the strains employed approached the region of entire avirulence.

The Relation of Immunity to Epidemiology and to Clinical Medicine.

Immunity is essentially an applied science. Its primary data are drawn from clinical medicine, and its outstanding achievement is that it enables us to do things, to interfere intelligently in the natural course of infective disease.

It must, therefore, remain in the closest touch with the field and with the ward. Its hypotheses, based largely on experiments in the laboratory, must stand their trial under field conditions. And here we are faced with a difficulty that is not shared by the more exact sciences. The relations of physics to the mechanical industries are superficially of the same kind as those of immunology to medicine, but actually they are very different. If a result obtained in the physical laboratory suggests a useful application to wireless reception or to aviation, or to any other practical and utilitarian end, it can be tried out with a reasonable certainty that its actual effect will be sufficiently obvious. It may succeed at once, or fail decisively at once, or succeed after adjustment; but in any case definite answers will come back from the field to the laboratory, and both parties to the transaction will learn something from their success or their failure. In medicine this is not always the case, simply because the

practice of medicine is a much more difficult thing than any kind of mechanical industry, or indeed than any industrial process at all.

It may be quite easy to tell when an immunological procedure is an unqualified practical success. It is much more difficult to tell whether it is a partial success, or an unqualified failure. And this cause of confusion, which in truth affects the immunologist in the laboratory almost as much as the physician in the ward, is so important that it deserves another chapter.

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CHAPTER II

THE MEASUREMENT OF IMMUNITY REACTIONS IN THE LIVING ANIMAL

It is impossible to understand the present position of immunology, with its mixture of established fact, half-knowledge, hopeful guessings and frank bewilderment, without an adequate grasp of the difficulties involved in measuring immunity reactions in the living animal, and in assessing the significance of such measurements when they have been obtained.

That these difficulties should have been overlooked by the pioneers is not altogether surprising. The effects demonstrated in many of the earlier experiments were so dramatic as to need no exact measurement to establish their significance. The need for standardizing antitoxic sera did, indeed, provide an obvious opportunity for developing adequate biometrical methods; but the need was not realized, for reasons that will become apparent later in this chapter, and the opportunity was allowed to pass. As new methods of immunization were developed and tested in the laboratory, in the ward and in the field, the publication of violently conflicting reports as to their efficacy showed that something was amiss; but the remedy was not obvious, at least to the immunologist. The barriers that separate the different departments of science are not easy to break down, especially when the roads by which these departments are entered diverge widely in their course and are hedged by very different intellectual disciplines. It was natural enough that the laboratory worker and the clinician should show little eagerness to learn and apply the methods devised by the statistician. But the result has been a quite unnecessary amount of confusion; and the confusion is likely to persist so long as the need for such methods is ignored. It will not, therefore, be extravagant to devote a chapter to a brief discussion of the ways in which some of our present difficulties may be overcome—by avoiding the need for statistical methods where this is possible, and by using them intelligently when they cannot be escaped. It will be necessary to refer to many of the reactions that will be discussed in detail in later chapters; but, for the moment, their exact nature and significance does not concern us—we are regarding them merely as things that can be measured.

It is obviously beyond the scope of this book to describe, even in outline, the technical details of statistical measurement; for these reference may be made to one of the excellent textbooks available, such as those of Yule, of Pearl (on the more purely medical side), of Fisher (on the laboratory and

experimental side), or the smaller primer of Woods and Russell. We must confine ourselves here to a brief discussion of general principles, with illustrative examples drawn from a few immunological problems.

The cause of our troubles is sufficiently obvious. It has been set out very clearly by Greenwood (1924) and by Yule (1924). The worker in the more exact sciences can reduce his uncontrollable variables to a minimum, or eliminate them altogether, and can so obtain constantly reproducible results. We cannot. In experiments on living animals, and still more in assessing the value of therapeutic or prophylactic procedures in man, we cannot exclude the interplay of factors about which we know little, except that they are certainly very numerous and may be very important. We cannot avoid an element of randomness in our observations; but, if we have planned our experiments wisely and fortune has been kind in eliminating major disturbing factors that we could not have foreseen, it will be only randomness of the kind that determines the number of heads and tails observed in a hundred tosses of a coin. The effect of such random variation can be calculated, and hence allowed for; it is the assessment of the necessary allowance in any particular case that is our main concern.

It will simplify discussion to consider in turn a series of problems of the kind that are continuously presenting themselves for solution.

THE MEASUREMENT OF RESISTANCE

To take the most obvious problem, though not the simplest, how can we measure the resistance of an animal to a particular parasite or to its toxin? Suppose first that we wish to measure directly the resistance of the animal as a whole, taking death or survival as our test. Then it is only under quite exceptional conditions that we can obtain *any* measurement on a single animal. If we are dealing with a very powerful toxin that kills rapidly, within a few minutes or hours, it is sometimes possible to give repeated and increasing doses at short intervals and so to measure approximately the amount which is necessary to cause death. But if our toxin acts more slowly, or if we are dealing with a living bacterium, this method cannot be employed. If we start with a small dose and the animal fails to die during the period—usually measured in days—within which we should expect death to occur, we cannot test it again by giving a larger dose, because our first dose will almost certainly have changed its resistance. It is not, from our present point of view, the same animal as before. If we start with a large dose and the animal dies, we do not know how it would have reacted to a smaller dose; if it lives, we know that it has resisted at that particular dose level, but we do not know and can never discover how it would have reacted to doses larger still. Moreover, we may justly be sceptical as to the complete and unique determination of the event—death or recovery—by the balance between the size of the inoculum and the animal's resistance, giving resistance its ordinary connotation of something intrinsic and relatively durable that distinguishes one animal from its

fellows. It is quite likely that many other factors, temporary, fluctuating, or altogether incidental, may influence the result in any particular case. It is clear that, if we take death or survival as our test, we must deal with groups not with individuals. This condition is, in truth, usually implicit in the question we want to ask. We are not often interested in the resistance of a particular mouse, or guinea-pig, or rabbit, as such. We are almost always regarding the individual as a representative of a class—normal, or treated in some way that may, we think, have altered resistance to a particular bacterium or toxin—and we are none of us naïve enough to believe in a standard guinea-pig, even if we confine ourselves to animals of a given breed, sex, age and weight. What we sometimes fail to realize is that we must, in most cases, test large numbers of animals before we can regard our result as representing truly the average behaviour of the class from which those animals are drawn.

There are occasions, however, on which we do want to measure the resistance of an individual man or animal; and, before discussing the methods to be adopted in measuring the resistance of groups, we may consider whether there is any way of solving this particular problem.

The Measurement of the Resistance of an Individual.

We cannot, as we have seen, obtain a direct measure of the resistance of any individual as an integral whole; but we can sometimes measure the resistance of a particular tissue, provided that it is possible so to localize the effect of any one inoculum that the reactions to several inocula can be observed simultaneously. This method is commonly exploited in the determination of skin sensitiveness to bacterial toxins. The immediately local effect of a small intradermic inoculation—0.2 c.c. is a convenient amount—is limited to the near neighbourhood of the site of injection. By spacing injections so as to leave a few centimetres between them it is easy to test several dilutions of a toxin on a comparatively small area of skin. In this way we can obtain an approximate measure of the smallest dose of toxin that will yield a characteristic reaction, and we can regard this as a measure of resistance—the smaller the dose that will produce such a result the less the resistance of the person or animal under test.

Sometimes we rely on a different kind of skin test—the allergic—in which a positive reaction indicates not susceptibility but resistance, showing an increased energy and rapidity of response to a particular bacterial product. In both cases it is necessary to establish, by extended trial, a correlation between the results of our skin tests and the effective resistance of the individual as a whole before we accept the one as a measure of the other.

There is another way in which we may attempt to estimate individual resistance—the demonstration and measurement of specific antibodies in the blood. In some cases we may inject the blood serum of the individual under test into a number of animals that we know to be normally susceptible to the toxin, bacterium or virus with which we are concerned. If we can show that a certain amount of serum will, on the average, protect an animal against a

certain dose of toxin, a certain number of living bacteria, or a certain amount of virus, we shall have obtained a measure of the resistance of the individual from whom the serum was obtained, in so far as his resistance depended on the presence of protective antibodies in his blood ; but such tests may, as we shall see in the following section, require the sacrifice of a considerable number of animals.

Alternatively, we may study *in vitro* the reaction between the serum of the individual whose resistance we wish to test and the bacteria or bacterial products with which we are concerned ; but our measurement of resistance is now becoming very indirect, and we must watch our assumptions with corresponding care. Suppose that our sample of serum has the power of killing a particular bacterium *in vitro* : does that mean that the person or animal from whom the serum came is resistant to infection ? Not necessarily, as we shall see. Suppose that the serum agglutinates the bacterium : does that indicate immunity ? In some cases, probably ; in others, perhaps ; in others, certainly not. In all such cases our only safe criterion is the actual demonstration of a high correlation between the possession of a particular kind of antibody and the resistance of its possessor. As our knowledge advances so will our ability to utilize *indirect* measures of immunity extend ; but in every case we must prove the existence of the correlation on which our method depends by trials carried out on adequate samples of experimental animals, or by the observation of adequate samples of human subjects, and so we come inevitably to the direct measurement of immunity in groups.

The Measurement of the Average Resistance of a Group.

Let us start with the simplest problem. How can we determine whether a given procedure has made any difference at all in the resistance of a group of animals, even without measuring how large that difference is ? If the increase in resistance is of a very high order, our task is easy. We have only to administer to our treated animals a large dose of bacteria or toxin, which experience has shown to be certainly fatal to all untreated animals of the same species, age, weight, etc., and note that the treated animals survive. But we shall be wise to make very sure about our "certainly fatal" dose, especially if our group of treated animals is small. We shall, indeed, always test a few untreated controls to guard against any gross experimental error.

But the increase in resistance may not be of this dramatic kind. Our treated animals may be unable to withstand a dose of bacteria or toxin that will kill *all* untreated controls though the mortality in treated and untreated groups may differ. Our problem is to establish the significance of this difference.

Let us take a concrete example. Thirty mice that had been repeatedly inoculated with a killed suspension of *Bact. aertrycke* and 30 uninoculated mice to serve as controls were injected intraperitoneally with a constant dose (1,000 bacilli) of living *Bact. aertrycke*. They were then observed over a period of 28 days. During this time 16 of the 30 vaccinated mice died of the infection,

and 29 of the 30 controls. The survivors were killed on the 28th day and cultures were taken from their spleens. *Bact. aertrycke* was recovered from 10 of the 14 surviving vaccinated mice, but not from the one surviving control. We may tabulate these results as follows.

Number of mice surviving on 28th day :

Vaccinated	14 = 46.67 per cent.
Controls	1 = 3.33 „ „
Difference	13 = 43.33 „ „

Number of mice showing no evidence of infection (completely resistant) :

Vaccinated	4 = 13.33 per cent.
Controls	1 = 3.33 „ „
Difference	3 = 10.00 „ „

It looks as though we had, by our vaccination, increased the resistance of our mice as judged by their ability to survive for 28 days. This is much longer than the time taken by unvaccinated mice to die of the disease—in this experiment the last control mouse died on the 8th day—so that we may take survival for this period as indicating resistance to the usual acutely fatal type of infection. But it is clearly uncertain whether or not we have produced any significant increase in the ability of our mice to resist infection altogether. The difference between 4 mice and 1, the numbers in the two groups that lived for 28 days and showed no sign of infection *post mortem*, is very small. We know quite well that if we repeated the experiment, taking all possible care to keep all our reagents and conditions unchanged, we should be very unlikely to get exactly the same result. If we repeated it ten times we should, perhaps, never get exactly the same result twice over, but we should be able to calculate an average result, and, if the different results did not vary very widely among themselves, we should place much more reliance on our average result than on the result of a single experiment. We should say that we had confirmed our finding in a large series of additional tests. But this proceeding would have sacrificed 600 mice. Is there any way in which we can tell how likely it is that our single trial has given us an approximation to our average result, without actually repeating the experiment over and over again ?

One thing is immediately apparent to our common sense. The larger the scale of our original trial the more likely it is to give a reliable answer. Clearly an experiment on two groups of 3 mice each could have told us nothing significant ; we used two groups of 30. If we had used two groups of 300 we should, whether we were able to explain the reason or not, have placed more confidence in our result.

It is here that simple statistical methods are most helpful to the immunologist, who is largely concerned with experiments of this particular kind. The statistician has evolved formulæ that allow us, on certain assumptions, to calculate the significance of the result obtained from a single experiment such as that we have described. For the mathematical considerations involved reference must be made to one of the standard textbooks on statistical methods ;

but we must get a clear understanding of the assumptions if we are to use the formulæ intelligently. If we assume that the only factors determining the differences in the number of deaths actually observed in several groups of animals, each of these groups being treated in exactly the same way, are the same kind of factors that determine the differences in the numbers of heads that we shall obtain when we toss a certain number of coins a certain number of times, then, given that we know the real chance of death, we can calculate the probability of obtaining at any one trial any particular number of deaths in a sample containing a particular number of animals. In the case of the coins, of course, the chance of obtaining a head is even, or 50 per cent., or 0.5—the last is the form the statistician uses; and we can calculate the probability of getting 3 heads and 17 tails, or 8 heads and 12 tails, or 14 heads and 6 tails, at any one tossing of 20 coins. Similarly, we can calculate the probability of getting any particular difference in the number of heads observed in two successive tossings of 20 coins. The statistician refers to these differences as “errors of random sampling.” The same principles hold when the chance of a particular event has some different value—say the chance of obtaining a six in throwing a die, or of a mouse dying when treated in a way that will, on the average, produce a mortality of approximately 17 per cent.

In applying this method to such an experiment as we have described we make the tentative assumption that our attempt at immunization has had no effect at all—that our two groups of mice have really exactly the same chance of survival, or of completely resisting the dose of living bacteria we have given them. Then we calculate the probability of obtaining the difference that we have actually observed. We can express this in the form of odds. If the probability works out at 0.1 the odds against such a difference are 9 to 1, if it works out at 0.002 the odds are 499 to 1. The higher the odds the more unlikely is our tentative assumption, the more unlikely it is that our two groups of mice were really equally resistant, and the more likely it is that it was our attempt at immunization that caused the difference we observed.

The necessary calculation involves a quantity known as the *standard deviation*. The only thing about the standard deviation that concerns us here is its use as a statistical measuring rod. Given a numerical value, representing an observed result of the kind we are considering, and its standard deviation, we can look up in the appropriate tables the probability of obtaining at any one trial a value differing from that observed by once or twice or n times the standard deviation.

The calculation of the standard deviation in such a case as this is very simple.

If of n animals x die and $n - x$ survive, then the chance of death, so far as we can tell from this single trial, is $\frac{x}{n}$. We call this p . The chance of

survival is $\frac{n - x}{n}$ or $1 - p$. We call this q . The standard deviation of x

(the *number* of deaths) is $\sqrt{npq}$. The standard deviation of $\frac{x}{n}$ (the *proportion*

of deaths or the probability of dying) is $\sqrt{\frac{pq}{n}}$. The standard deviation of $\frac{100x}{n}$ (the percentage of deaths) is $100\sqrt{\frac{pq}{n}}$.

In our experiments we are usually concerned with a difference, not with a single value. The standard deviation of the difference between two observed values is the square root of the sum of the squares of the standard deviations of the two values.

If we take two samples of animals and note that in one sample x of n animals die and in the other x' of n' animals die, our calculation proceeds as follows.

We are assuming that there is no significant difference between our two samples. Then our only available measure of the real chance of dying is the mean death-rate, expressed in this case as a decimal proportion. This is clearly $\frac{x + x'}{n + n'}$. We call it p^0 . The mean chance of survival is $1 - p^0$. We call it q^0 . The observed difference in death rate—it is convenient to express it as a percentage—is $\frac{100x}{n} - \frac{100x'}{n'}$ assuming that $\frac{x}{n}$ is larger than $\frac{x'}{n'}$. The standard deviation of this difference in percentage mortality is

$$100\sqrt{p^0q^0\left(\frac{1}{n} + \frac{1}{n'}\right)}.$$

In experiments of the kind we are considering it is a usual and a sound practice to take an equal number of animals for our test and control groups. In this case we can, if we choose, compare the actual numbers of deaths in the two groups, since the same number of animals will have been at risk in each. Our observed difference will be $x - x'$, and the standard deviation of this difference will be $\sqrt{p^0q^0(n + n')} = \sqrt{2np^0q^0}$.

Let us apply these methods of calculation to the figures on p. 13. We chose to record survivals, not deaths; from the statistical point of view it clearly makes no difference. The product of p^0q^0 will be the same.

Of 30 vaccinated mice 14 survived: of 30 control mice 1 survived.

$$q^0 = \frac{14 + 1}{30 + 30} = \frac{15}{60} = 0.25$$

$$p^0 = 1 - q^0 = 0.75$$

$$\begin{aligned}\sqrt{p^0q^0\left(\frac{1}{n} + \frac{1}{n'}\right)} &= \sqrt{0.75 \times 0.25 \times \left(\frac{1}{30} + \frac{1}{30}\right)} \\ &= \sqrt{\frac{0.75 \times 0.25}{15}} = 0.1118\end{aligned}$$

The observed difference in mortality is 43.33 per cent., and the standard deviation, expressed as a percentage value, is 11.18. So the difference is equal to 43.33/11.18 (3.87) times its standard deviation.

Of the 30 vaccinated mice 4 were completely resistant to the subsequent

inoculation: of the 30 control mice 1 was completely resistant. Taking q^0 to express resistance,

$$q^0 = \frac{4 + 1}{30 + 30} = \frac{5}{60} = 0.0833$$

$$p^0 = 1 - q^0 = 0.9167$$

$$\sqrt{p^0 q^0 \left(\frac{1}{n} + \frac{1}{n'} \right)} = \sqrt{\frac{0.0833 \times 0.9167}{15}} = 0.07136.$$

The observed difference in the proportion of mice completely resistant is 10.00 per cent., the standard deviation expressed as a percentage is 7.136. The observed difference is 10/7.136 (1.4) times its standard deviation.

And now we should turn to our tables. But we are not concerned with the exact probability of the difference we have observed being due to random chance; we want only to know whether the odds against this being so are very high, or high, or moderate, or low. We shall get all we need if we obtain the order of odds instead of their exact value. The values given in Table I will enable us to estimate them quite closely enough for our purpose.

TABLE I.

Deviation in Terms of the Standard Deviation; or Ratio between a Difference and its Standard Deviation.	Odds against this Deviation or Difference being the Result of Random Chance.
0.5	0.6 to 1
1.0	2.15 to 1
1.5	6.5 to 1
2.0	20.98 to 1
2.5	79.53 to 1
3.0	369.4 to 1
3.5	2,149 to 1
4.0	15,773 to 1
4.5	147,188 to 1

And so we get the answers we want. The odds are many thousand to one against the observed difference in survival rate having been due to errors of random sampling. So far as errors of this kind are concerned we can accept our result as significant, as showing that our attempt at immunization has increased the average resistance of our mice to a degree that protects many of them against an acutely fatal infection. But the odds against the observed difference between the proportion of fully resistant mice in each of the two groups being due to random chance are only about 5 to 1. Once in about every six trials we should get this difference even if our immunization had failed to render any mouse fully resistant. These odds are far too small to justify any definite conclusion. Our summing up would be that the injection of this vaccine induces in many mice a partial but not a complete immunity; while it is possible, but by no means certain, that a few mice are rendered fully immune.

The above is one selected example. It may be useful to note how the odds vary according to the number of deaths or survivals observed in two groups, each of 20 animals, one group being treated by some procedure under test, the other serving as untreated controls. Both groups, of course, will be subjected to the same dose of living bacteria, toxin or other agent against which the treatment is directed. These odds are shown in Table II, very short odds being neglected and odds of over 1,000 to 1 being no further specified. No odds are shown for less than 5 deaths in one group or more than 15 deaths in the other group. Where the number of animals in each group is as small as 20, and the number of deaths in each group is very small (say 4 or less) or very large (say 16 or more) the assumptions on which these calculations are based no longer hold. The example of the fully resistant mice among our groups of 30 was not above criticism in this respect.

It is of interest to inquire how far the expectations based on such a table are fulfilled in practice. Not many workers have taken the trouble to test their technique in this way, but Lockhart (1926) records observations bearing directly on the point at issue. In four experiments several groups, each of 20 mice, were injected with the same dose of the same culture of a pathogenic organism (*Bact. aertrycke*) and the number dying in each group during the succeeding 14 days was noted. In one experiment 9 groups were injected; the deaths were 16, 16, 17, 19, 18, 17, 15, 17 and 14. Table II shows that the difference between 14 and 19 would be expected once in 27 trials. It actually occurred once in 9. In a second series of 5 groups the deaths were 18, 17, 13, 16 and 13; the difference between 13 and 18 should have occurred only once in 17 trials. In a third series of 5 groups the deaths were 5, 2, 4, 3 and 5; there is here no difference that would not be expected on our assumption, but the death rate is very low in all groups. In a fourth series of 5 groups the deaths were 19, 20, 19, 20 and 19; there is no unexpected difference, but the death rate was very high in all groups. Such a series of tests may be regarded as a trial of the adequacy of our experimental methods. If the distribution of deaths actually observed in different groups that have, within the limits of experimental control, been subjected to exactly the same treatment agrees closely with that expected on the assumption that the only differences that occur will be those due to the error of random sampling, then our technique may be regarded as highly satisfactory. If our observed distribution differs widely from that expected, then we have not succeeded in eliminating other disturbing factors, of unknown nature, which may mislead us when we come to apply a known procedure to one group in order to test its effect. From this point of view Lockhart's figures may be regarded as reasonably satisfactory; though they suggest that the differences observed may not be due entirely to errors of random sampling. We shall clearly be wise to demand rather long odds before accepting a conclusion as statistically significant. Suppose we fix an arbitrary limit at a ratio of 3.0 between an observed difference and its standard deviation. This will give us calculated odds of approximately 369 to 1. Accepting such a limit, we should not in any of Lockhart's trials have been deceived into thinking that the behaviour of one group differed significantly from that of another.

And here we may emphasize a principle of the first importance. There is no sovereign efficacy in the application of statistical methods to such problems as these. They do not provide a final proof of any particular hypothesis. They simply tell us the odds against our result being due to chance—and to chance of a particular kind.

Suppose that we had treated 50 animals in some way that should, in the

TABLE II

SHOWING (IN THE FORM $x:1$, x ALONE BEING GIVEN) THE ODDS AGAINST DIFFERENT COMBINATIONS OF DEATHS IN TWO GROUPS, EACH OF 20 ANIMALS, ON THE ASSUMPTION THAT THE DIFFERENCE BETWEEN THE TWO GROUPS IS DUE TO ERRORS OF RANDOM SAMPLING.

Number of Deaths in First Group.		Number of Deaths in Second Group.														
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
5	58	12	—	—	—	—	—	—	—	—	9	18	39	89	228	633
6	127	26	8	—	—	—	—	—	—	—	—	8	17	37	87	228
7	276	55	16	6	—	—	—	—	—	—	—	—	8	17	37	89
8	633	122	34	12	—	—	—	—	—	—	—	—	—	8	17	39
9	>1000	285	75	25	10	—	—	—	—	—	—	—	—	—	8	18
10	—	702	172	54	20	9	—	—	—	—	—	—	—	—	—	9
11	—	>1000	422	122	44	18	8	—	—	—	—	—	—	—	—	—
12	—	—	1000	304	100	39	17	8	—	—	—	—	—	—	—	—
13	—	—	—	807	250	89	37	17	8	—	—	—	—	—	—	—
14	—	—	—	>1000	678	228	87	37	17	8	—	—	—	—	—	—
15	—	—	—	—	>1000	633	228	89	39	18	9	—	—	—	—	—
16	—	—	—	—	—	>1000	678	250	100	44	20	10	—	—	—	—
17	—	—	—	—	—	—	>1000	807	304	122	54	25	12	6	—	—
18	—	—	—	—	—	—	—	>1000	>1000	422	172	75	34	16	8	—
19	—	—	—	—	—	—	—	—	—	>1000	702	285	122	55	26	12
20	—	—	—	—	—	—	—	—	—	—	>1000	>1000	633	276	127	58

light of all our previous experience, have increased their resistance to a particular bacterium, but, when we injected them, together with 50 normal controls, with the bacterium in question, the death rate was much higher in the "immunized" animals than in the controls. Suppose that the ratio of the difference in mortality to its standard deviation was 3.23. This would mean that the odds against our observed difference being due to chance were 807 to 1. But we should not jettison all our previous experience and that of other workers. Things have happened with odds of 807 to 1 against them; and though we cannot calculate the odds against all previous experience having given us a wrong answer they are probably much higher. Nor need we adopt the view that the 1-in-808 chance has happened. It is far more likely that we have made some gross technical error, or that some unknown and unforeseen factor has intervened.

Our final conclusions, as Woods and Russell are careful to emphasize, must always be based on a balance of probabilities, only one of which is determined by our statistical measurement. Nor, of course, can such measurement ever *prove* a negative. If a result is not statistically significant it does not follow that the hypothesis on which the experiment was based is untrue. The most that can be inferred is that it is unlikely—unlikely to a specified degree—that the factor supposedly involved is sufficiently effective to produce a statistically significant result in samples of the size actually selected for our test.

This leads us to turn our problem another way round. How large must our groups be in order that a given difference in behaviour may be detected as significant? Let us take a concrete case. Certain laboratory experiments have led to the production of a serum which, on *a priori* grounds, may be expected to have some curative influence on a particular infective disease. Like all new curative measures it *must* be subjected to a period of trial before it is adopted for routine use. Our expectations may not be realized. The serum may be quite useless in practice. It is not a question of trial or no trial, but of a well-planned trial that will give us a definite answer as quickly as possible, as against a planless, haphazard series of trials that will take a long while to tell us very little, perhaps at a considerable expense in lives. Let us suppose that the real average effect of the serum is to reduce the case mortality of the disease from 30 per cent. to 20 per cent.—not, perhaps, a dramatic result but one that will save a large number of patients if the disease is a prevalent one. The only way in which the existence of such an effect can be quickly and certainly established is to test the serum on an adequate group of cases and compare the mortality in this treated group with the mortality in an untreated group. The two groups must if possible be compared over the same period of time and in the same place, since the case mortality of almost all acute infective diseases fluctuates from time to time and from one locality to another. They must also be similar in all relevant respects other than the administration of the serum; for example, they must all be of the same race, and the age and sex distribution within the two groups must be approximately the same. Let us see what will happen if such a trial is carried

out on two groups, each containing 10, 50, 100, 150, 200 or 250 patients. The relevant figures are set out in Table III, the standard deviation being calculated on the percentage basis.

TABLE III

SHOWING THE RATIO DIFFERENCE IN MORTALITY/S.D. FOR TREATED AND UNTREATED GROUPS IN THE CASE OF AN ANTISERUM WHICH PRODUCES A DECREASE IN CASE MORTALITY FROM 30 PER CENT. TO 20 PER CENT.

Number in Each Group.	Deaths in Treated Group.	Deaths in Untreated Group.	Difference. Per cent.	S.D.	Difference in Mortality : S.D.
10	2	3	10	19.365	0.52
50	10	15	10	8.660	1.15
100	20	30	10	6.124	1.63
150	30	45	10	5.000	2.00
200	40	60	10	4.330	2.31
250	50	75	10	3.873	2.58
500	100	150	10	2.739	3.65

Quite clearly a trial with 10 treated and 10 untreated patients is useless. In different trials on this scale, one observer will record more deaths in the treated group, another in the untreated. There will be hopeless confusion. The position with groups of 50 treated and 50 untreated patients is not much better. In about one of every four trials a difference such as this would be recorded as the result of chance alone. There will be sharp differences of opinion as to whether the treatment does *any* good, and no possibility of assessing even approximately how much good it does. As the number of patients in each group increases the significance of the result recorded also increases—not proportionately to the number itself but in proportion to its square root. After a trial on two groups each of 100 patients we should begin to think it probable that the serum had done some good. After a trial on two groups each of 200 patients we should be inclined to adopt this view as a provisional conclusion pending further evidence. If trials on this scale were repeated we should be surprised if there were many unfavourable reports. After a trial on two groups of 500 patients we should have little doubt left. Moreover, with groups of this size we should feel fairly confident that the benefit to be expected from our serum was correctly represented by a decrease in case mortality from about 30 to about 20 per cent.—that is, by a saving of one patient in three who would otherwise have died—since the chance of the observed decrease differing from the real average by any given amount grows less and less as the number in the group increases.

It is of very real importance that trials of any new therapeutic measure should be planned and carried out on an adequate scale. Otherwise the period of so-called trial may be extended far beyond the necessary limits; and a useless measure may be employed sporadically for years, or—what is far more serious—a useful measure may fail to obtain general recognition because the

reports on inadequate trials are contradictory and confusing. Any student who cares to master the history of the use of anti-pneumococcal serum (see pp. 366-369) will find a case in point.

It should be noted that, in the case of a proposed method of prophylaxis, our field trials will often have to be carried out on a far larger scale, because the risk of contracting a particular infective disease is often far smaller than the risk of dying from it when contracted. If the attack rate of a disease is very low, our immunized and non-immunized groups must be greatly expanded—often to many thousands of persons—before the number of cases in our control group will be large enough for us to establish the significance of any difference in morbidity that may be noted.

If, however, we can select special groups that are for some reason exposed to a particularly high risk of infection—such for instance as the nurses or attendants in a fever hospital—we may be able to detect the effect of a given prophylactic method by the immunization of relatively small groups (see pp. 332, 336). The same will hold, of course, for the population at large in the case of infections with a very high attack rate, such as the common cold (see pp. 342, 343).

We have so far been concerned with problems in which each animal, or each patient, gives a qualitative result—death or survival, infection or no infection. There is obviously another kind of problem in which each animal may give a quantitative result: it may live so many days, or lose so much weight, or its temperature may rise so many degrees, and so on. It was in connection with observations of this kind, though not in the immunological field, that the use of the standard deviation was developed. It is, in such cases, calculated from the observed variations about the mean, or average, in any group; and it gives us a measure of the reliability of this mean, or of the significance of an observed difference between the means of two groups. But problems of this kind do not occur with sufficient frequency in the field with which we shall be mainly concerned to justify a description of the method of calculation. Reference may again be made to one of the statistical textbooks.

We may now turn to another common immunological problem, in which the statistical principles we have considered are applied in rather a different way.

THE MEASUREMENT OF TOXICITY OR VIRULENCE

We often want to determine the minimal lethal dose of a bacterial toxin or of a living bacterial culture. It is only quite recently that the work of Trevan (1927, 1929, 1930) has disclosed the magnitude of the errors inherent in determinations of this kind, and has indicated the steps that must be taken if they are to be avoided. As he points out the term *minimal lethal dose* defines a quantity which cannot in fact be measured. There is no standard animal. The smallest dose that will kill one animal will not kill another of

the same species and weight. Implicitly, of course, we have always recognized this, but explicitly we have not, and our negligence has led to woeful errors.

Trevan suggests that the term *minimal lethal dose* should be discarded and replaced by the term *average lethal dose*—defined as the dose which will kill 50 per cent. of a large group of test animals. The problem then is to determine how large the number of animals tested with each dose must be in order to allow us to find the true average lethal dose within any selected margin of error.

The considerations on which Trevan's solution is based will be clear from the diagrammatic curves in Fig. 2. Diagram A shows the kind of curve that relates dose to death

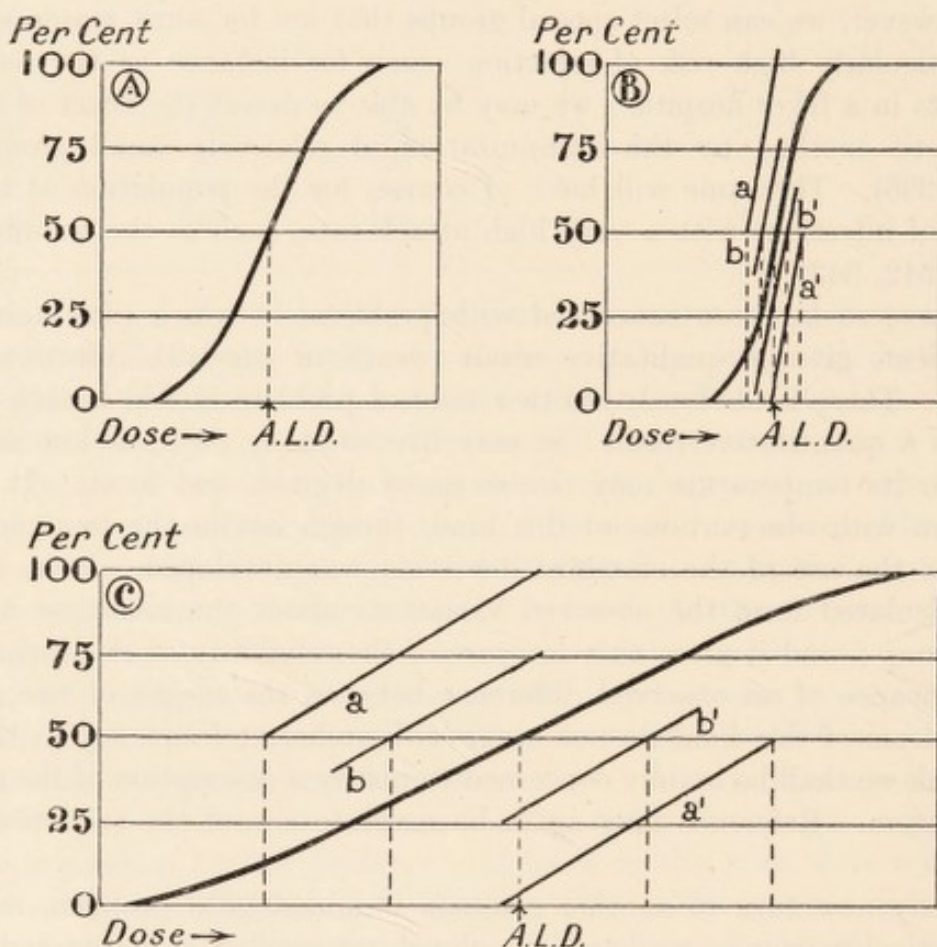


FIG. 2.

rate. As the dose increases from zero the percentage mortality at first rises slowly, until a dose is reached that kills some 10 per cent. of animals. It then rises more steeply. Between the doses corresponding to mortalities of about 25 per cent. and 75 per cent. it usually gives a close approximation to a straight line. It then rises less and less steeply until the certainly fatal dose is attained. This curve, which Trevan calls the *characteristic*, differs widely in its steepness according to the nature of the material administered. For many poisonous drugs it is very steep. For bacterial toxins it is less steep, much less steep for some than for others. For living bacterial cultures it is less steep still; sometimes the dose of living bacteria that kills all the animals of a group is more than a million times the dose that kills some of them. For our immediate purpose we may confine our attention to that part of the curve that lies between the points corresponding to mortalities

of 25 per cent. and 75 per cent. Diagram B shows this part of the curve for a characteristic that rises very steeply; diagram C for a characteristic that rises very gradually. We know that, if we inject the average lethal dose, the observed mortality will seldom be exactly 50 per cent., but will vary above and below this level in accordance with the principles we have described. Suppose we adopt three times the standard deviation as giving us the limits beyond which deviations will occur so seldom that we can ignore them. The standard deviation will differ according to the number of animals we test.

Let us calculate the standard deviations corresponding to different mortalities for groups of 10 animals, and again for groups of 50, and then mark off the value corresponding to three times these standard deviations above and below our characteristic curves, and draw other curves through them. These curves will not be strictly parallel to the characteristics, but over the part of the curve with which we are concerned their departure from parallelism will not be great. In each diagram let us drop a perpendicular from the point where these standard-deviation curves cut the ordinate level of 50 per cent. mortality to the base line along which we plot our dose. Now, within the arbitrary limits we have accepted, we may observe a 50 per cent. mortality when we inject a dose as small as that marked by the vertical from *a* or as large as that marked by the vertical from *a'* if we use groups of 10 animals. If we use groups of 50 animals, our margin of error will be marked by the verticals from *b* and *b'*. In the case of the steep characteristic B our error will not be large in either event. In the case of the very gradual characteristic C our error will be gross if we use the smaller number, considerable if we use the larger.

If we know the slope of the characteristic, and confine our attention to doses falling somewhere on the straight part of the curve, that is to doses not too far removed from the average lethal dose, we can calculate the number of animals that must be inoculated with a dose in this neighbourhood in order to determine the average lethal dose within any desired limits of accuracy allowing three times the standard deviation as our limit for sampling error (see Trevan 1927).

If *K* is the ratio between percentage increase in dose and percentage increase in mortality, in the neighbourhood of the average lethal dose, *D* the percentage deviation in dose that we are prepared to allow, and *N* the number of animals we must inject in order to keep within those limits of error, then

$$N = \frac{22,500 K^2}{D^2}$$

It will be noted that the validity of this formula depends on the assumption that the characteristic approximates to a straight line for a sufficient distance on either side of the point representing 50 per cent. mortality to include all errors of the order that concern us. In practice, this assumption does not hold in the case of many characteristics of the type depicted in C. The *form* of the curve as well as its *slope* may be greatly altered; the dose that causes a 75 per cent. mortality may, for instance, be ten or a hundred times the dose that causes a 50 per cent. In such cases the percentage error, and particularly the error in excess, may be a high multiple of that indicated by the formula.

A few examples, including some given by Trevan and others calculated by his method, will suffice as illustrations.

To determine the average lethal dose of a sample of diphtheria toxin within an error of ± 10 per cent., the animals employed being guinea-pigs, 23 animals must be inoculated with a dose in the neighbourhood of the average lethal dose.

To determine the average lethal dose of a moderately virulent culture of pneumococcus to within an error of ± 20 per cent., the animals employed being mice, 77 animals must be injected with a dose in the neighbourhood of the average lethal dose.

To determine the average lethal dose of a moderately virulent culture of

Bact. aertrycke to within an error of ± 20 per cent., the animals employed being mice, 287 animals must, according to our formula, be injected with a dose in the neighbourhood of the average lethal dose; but this is one of those cases in which the real error is very much larger than that indicated.

If we do not know the form of the characteristic, we must first determine it; and this may demand the use of large numbers of animals. Needless to say, we do not often determine the average lethal dose of toxins or cultures with anything like this degree of accuracy except in the case of reagents that are employed for purposes of standardization (see Chapter XXI).

The Comparison of Toxicity or Virulence.

A problem that presents itself to the experimentalist even more frequently than the determination of the average lethal dose of a single toxic filtrate, or a single bacterial culture, is the comparison of one toxin with another, or one culture with another. The number of animals required to perform such a comparative titration within any desired margin of error can be calculated by an extension of the method described above (see Trevan 1927). The number will clearly be greater than that required for a single titration, since we shall be concerned with the standard deviation of a difference or a ratio.

Thus, to compare the strength of two samples of diphtheria toxin to within an error of ± 10 per cent., using doses in the neighbourhood of the average lethal dose, we must inject 47 guinea-pigs with each of the two toxins.

The comparison of the virulence of two strains of a pathogenic bacterium to within any such margin of error is an almost impossible task. The slope of the characteristic, as well as the average lethal dose, alters with the virulence of the culture. We must, of necessity, be satisfied with far rougher estimates.

A concrete example will make our difficulties clear.

Lockhart (1926), in the case of two strains of *Bact. aertrycke*, gives the data for the results of intraperitoneal injections in mice set out in Table IV.

TABLE IV

Dose.	Number of Mice in Each Group.	Mortality per cent. Strain A52.	Mortality per cent. Strain Ellinger.
10,000,000	25	92	96
100,000	25	48	88
1,000	25	44	56
10	25	16	24

We can feel fairly confident that the Ellinger strain is more virulent than the A52 strain; at each dose level it has given a higher mortality. But we clearly cannot express our result as any numerical ratio. If this is the best we can do with 200 mice, we must be satisfied to detect gross differences in virulence, so far as this organism is concerned.

Another concrete example will be useful, since it concerns an organism that is less difficult to deal with than *Bact. aertrycke*, and illustrates the kind of error that may be involved by a blind faith in results obtained with small numbers of animals.

A series of six dilutions were prepared from a culture of pneumococcus of moderate virulence, and the same amount of each dilution was inoculated into 40 mice. The doses

administered, in terms of living pneumococci, were 10^6 , 10^5 , 10^4 , 10^3 , 10^2 and 10. Two of the mice that received each dose were then allotted at random to each of 20 numbered groups. Each group thus consisted of 12 mice, 2 for each dose tested. The deaths are recorded in Table V.

Adding across the rows, the percentage mortality for the whole series was, with the falling doses, 100, 95.5, 65.0, 12.5, 0 and 0. The average lethal dose was probably something between 10,000 and 1,000 pneumococci. But if we had used two mice for each dose—and it would have been a highly conventional procedure—we should have said in the case of groups 10 and 11 that the average lethal dose was about 1,000 pneumococci; in the case of groups 1, 2, 5, 6, 7, 12, 14 and 19 that it lay somewhere between 1,000 and 10,000 pneumococci; in the case of groups 3, 4, 8, 9, 13, 15, 16, 17 and 20 that it was

TABLE V

SHOWING THE NUMBER OF DEATHS IN 20 SAMPLES, EACH OF 12 MICE, INOCULATED WITH THE SAME SERIES OF GRADED DOSES OF THE SAME CULTURE OF PNEUMOCOCCUS.

Dose.	Group.																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
10^6	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
10^5	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	1	2	2
10^4	1	2	1	1	2	1	2	1	1	2	2	2	1	2	1	0	1	0	2	1
10^3	1	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0
10^2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

about 10,000 pneumococci; and in the case of group 18 that it was about 100,000 pneumococci. Had we been comparing 20 different strains of pneumococci, we might have been unwise enough to hazard the statement that strains 10 and 11 were about 100 times more virulent than strain 18; whereas, as this experiment showed, there would have been no adequate reason to assume any difference in virulence.

The Neutralization of Toxin by Antitoxin and the Comparison of Antitoxic Sera.

Measurements of this type are fortunately less seriously affected by errors of random sampling than are those we have been considering above. In such titrations we mix toxin and antitoxin in varying proportions and then determine which mixture contains an average lethal dose of unneutralized toxin. We always employ high multiples of the average lethal dose in making our mixtures, and the inevitable errors of random sampling affect only our estimate of the quota of toxin that is left unneutralized. Since our answer will be in terms of the total amount of toxin present in the mixture this error will be spread over the whole of that amount (see Trevan 1927).

Thus, as we have seen above, it requires 23 guinea-pigs to determine a dose of diphtheria toxin in the neighbourhood of the average lethal dose to within 10 per cent. of the real value, and 47 guinea-pigs in each group to compare the strength of two toxins to within the same range of error at the same dose level, but in comparing the neutralizing value of one antitoxic serum with that of another the use of 12 guinea-pigs in each group will, under the same

conditions, give a comparison accurate within ± 2 per cent., even after allowing for certain peculiarities of the toxin-antitoxin reaction which make the conditions less favourable than if we were dealing with a simple chemical neutralization. It is probable, as Trevan suggests, that the relatively high accuracy of this particular method of titration, which happened to be almost the first employed in immunological tests of this kind, was largely responsible for the failure to appreciate the extreme inaccuracy of other tests of an apparently similar nature.

It may be noted that the position is not always so favourable, even in the case of toxin-antitoxin titrations. Thus, using a high multiple of the average lethal dose of toxin, it requires 40 mice in each of two groups injected to compare two anti-dysentery (shiga) sera to within an error of ± 12 per cent. (Trevan 1929).

The Measurement of the Protective Power of an Antibacterial Serum, or the Comparison of Two Sera.

The mode of action of an antibacterial serum is quite different from that of an antitoxic serum, but the method of measurement is essentially the same; and that is all that concerns us here. Selecting some constant amount of bacterial culture, usually a high multiple of the average lethal dose, we mix with it varying amounts of the serum under test and inject the mixtures into groups of suitable laboratory animals, taking as the end-point of our titration the amount of serum that, with the dose of culture selected, reduces the mortality to the 50 per cent. level. If we wish to compare two sera, we determine this dose for each of them separately. For reasons that are discussed in Chapter XXI this comparative method has, for purposes of standardization, advantages over the single titration.

The number of animals that must be employed to obtain results within any required limits of accuracy can be calculated by Trevan's method when the form of the characteristic given by the serum-culture mixtures has been determined.

In the particular case of antipneumococcal serum, using a culture of high virulence and a dose of serum-culture mixture that will give a mortality in the neighbourhood of 50 per cent., 40 mice must be injected with the appropriate dose of each serum to obtain a measure of relative potency accurate to within ± 30 per cent. (Trevan 1930).

The Use of Skin Tests in the Determination of Toxicity, or of the Neutralizing Power of an Antitoxic Serum.—Just as in the measurement of resistance, the use of an appropriate skin test, when such is available, enables us to determine the strength of a bacterial toxin or the neutralizing power of an antitoxic serum to within any desired limits of accuracy at a smaller cost of animals than would be involved in any method depending on death or survival. This is particularly the case when we are dealing with neutralizing effects, or with comparisons between two toxins or two sera. It results naturally from the fact that we can carry out many tests simultaneously on the same animal,

and so eliminate those minor differences in resistance between one animal and another that are the most fertile source of sampling errors in our group experiments.

A NOTE ON THE RECORDING OF QUANTITATIVE RESULTS

It is a common habit among immunological workers to record their quantitative results in the form of curves or charts. The advantage is obvious. The meaning leaps to the eye. The danger is that it sometimes leaps before one looks. It is a very safe rule never to trust the tale told by a curve until the quantitative data on which it is based have been critically examined. If the points through which a curve is drawn are based on figures which are subject to errors of random sampling, and if the data permit the standard deviation to be calculated, it is a good plan to mark off three times the standard deviation above and below each point on the curve, join up these points, and, realizing that the curve might run anywhere within the broad band thus demarcated, reconsider its actual significance.

Tables are duller things than curves, and rather more trouble to master. But they tell much more illuminating tales. They should always state quite plainly the number of observations made, never percentages without this information. If a table has a column headed "per cent." always look first at the columns giving the figures on which the percentages are based. Occasionally it will be found that 33.33 per cent. in the last column corresponds to a 1 and a 3 in the columns preceding it. In such cases, of course, the 33.33 per cent. is quite meaningless.

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The student may, perhaps, feel that these biometrical difficulties have been laboured and elaborated *ad nauseam*; but if he will note how often, in the following pages, it is necessary to record that A. has reported such-and-such a series of observations, while B. on repeating the experiment has obtained completely divergent results, and C. attacking the same problem from a slightly different angle has obtained results that agree neither with A.'s nor with B.'s, he will realize that the point at issue is not without importance.

The crux of the matter is this: simple statistical principles of the kind we have been considering are not rules to be applied in drawing conclusions from an experiment that has been done—they are part of the experiment itself, an essential factor in its proper planning.

If an experiment on a particular scale is quite unlikely to give a significant answer, then it is better either to enlarge its scale to the extent required, or to abandon it.

The voyager who ignores tidal streams and compass deviations will arrive somewhere in the long run; but he is less likely to identify correctly the place

at which he has arrived than is another who allows for these variables by the accepted methods of elementary navigation.

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CHAPTER III

THE MECHANISMS OF BACTERIAL INFECTION

BEFORE we can discuss the mechanisms by which the host resists infection, it is necessary to build up as clear a picture as we can of the ways in which the parasite injures the host. This is a little unfortunate, since the best picture we can produce at the moment is sadly confused in some places and extremely sketchy in others. But parts of the main outline are quite clear and definite, and it is with these that we shall be mainly concerned.

Specific Infection. Koch's Postulates.

Although not immediately pertinent to our main problem, a brief discussion of the kind of evidence which will justify the conclusion that a particular disease is caused by a particular parasite forms a convenient introduction to the consideration of infective processes in general, and raises points of some interest and importance.

A series of conditions, which must be fulfilled in order to establish such a causal relationship, are set out in most bacteriological textbooks under the title of "Koch's Postulates," though there is no clear evidence that Koch ever enunciated them in the categorical form in which they are usually quoted. They run in general terms as follows :

(1) The organism should be found in all cases of the disease in question, and its distribution in the body should be in accordance with the lesions observed.

(2) The organism should be cultivated outside the body of the host, in pure culture, for several generations.

(3) The organism so isolated should reproduce the disease in other susceptible animals.

We should to-day recognize additional ancillary criteria which help us to form an accurate judgment as to the relationship of a suspected organism to a given disease, as, for instance, the demonstration of specific antibodies in the blood of an infected man or animal.

It is obvious that technical difficulties may prevent us from fulfilling all Koch's postulates in every case. We may, for instance, be unable to cultivate the causative parasite although we can demonstrate its constant presence in the lesions by suitable staining methods ; this has hitherto been the case with regard to the bacillus of leprosy. Or the organism may be so small, and its morphology so doubtful, that we are unable to recognize its presence with any certainty in stained preparations, or even to distinguish it by the more delicate method of examining or photographing unstained preparations with the aid

of dark-ground illumination and light of short wave lengths. When to this difficulty is added an inability to cultivate it *in vitro*—as is still the case with most of the filtrable viruses—we may have to take a short cut, neglect the first two postulates, and rely on the reproduction of the disease in a suitable animal by the inoculation of filtrates from infected blood or tissue extracts, which are at least free from intact cells or living bacteria. In other cases the third postulate may be our stumbling-block. We may be able to demonstrate a characteristic bacterium in such a relation to the lesions of a particular disease that we can have no reasonable doubt that it is playing a causative rôle, and we may have learned how to propagate it in pure culture outside the body; but we may be quite unable to produce in any experimental animal lesions which bear any close resemblance to those which characterize the disease in man.

It is often possible, in the absence of complete proof, to establish the causative rôle of a particular organism with so high a degree of probability that there need be no hesitation in accepting this association as a reasonable provisional hypothesis. At the same time it must be recognized that any omission in the complete chain of evidence involves a risk of error; and much confusion has been caused by uncritical attempts to support the claims advanced on behalf of numberless bacteria isolated from different parts of the body in various diseases.

A little more may usefully be said with regard to the first and third postulates. In the early days of bacteriology the proposition that the causative organism of a disease should be present in every case adequately examined was held to imply its converse—that the organism should not be present in persons not suffering from the disease. And in some instances, such as the investigations that followed Loeffler's demonstration of the diphtheria bacillus, serious doubts were thrown on the claim of an authentic causal organism when it became clear that it could be isolated from the throats of persons who were quite certainly not suffering from diphtheria. Nowadays we have learnt to adopt a different standpoint. Our present conception of the probable distribution of any pathogenic parasite is that it will be found in every case of the specific infection that is clinically obvious, and is adequately investigated under satisfactory conditions; in many cases of minor infection that show little resemblance to the fully developed disease; and in a certain proportion of apparently healthy persons, especially in contacts.

With regard to the third postulate, we should not expect complete identity between the clinical picture in man and the lesions produced in an experimental animal. The picture presented by any infective process depends as much upon the reactions of the host's tissues as upon the activities of the infecting parasite; and in this, as in other respects, one animal species differs from another. Sometimes this is a small matter. No one who had any knowledge of the morbid anatomy of tuberculosis in man or cattle would hesitate in identifying the experimental disease in the rabbit or guinea-pig, in spite of minor superficial differences. But the lesions produced in experimental animals by the typhoid bacillus, or by the scarlatinal streptococcus, bear little obvious resemblance to the corresponding human diseases.

The student should note very carefully that there are two distinct stages

in the use of experimental animals in the study of infective disease. In the first we are trying to reproduce a particular infection in a form which is clinically or pathologically recognizable as identical with the natural disease. This is the kind of reproduction demanded by Koch's third postulate. In the second we are utilizing our susceptible animal for an intensive study of the particular infective process with which we are concerned. This is really the more significant and interesting part of our investigations. The lesions produced in the guinea-pig by the subcutaneous inoculation of cultures or filtrates of the diphtheria bacillus bear little resemblance to diphtheria as it appears in man; but almost the whole of our present knowledge of that disease, which has at last placed us in a position to exert effective control, has been built up on knowledge gained from experiments on that small laboratory animal.

It should be noted, also, that there is a shifting of emphasis from the clinical to the bacteriological side as our knowledge progresses. At the start of our inquiry we are presented with a clinical or epidemiological entity, and our task is to discover the microbial parasite which is the essential cause. We may, as in the case of diphtheria, or syphilis, or tetanus, find that a single specific parasite is involved; indeed this is far the most frequent solution if we allow for minor racial variations within the causative microbial species. We may, however, find that a disease which is a clinical entity involves several distinct bacterial species as alternative infecting agents. Secondary pneumonia affords one example, bacillary dysentery another. Once the connection between a particular parasite and a particular disease has been firmly established, the tendency of the bacteriologist is to focus his attention on the organism, rather than on the clinical condition to which it most frequently gives rise, and to try to build up a picture of the reactions between that organism and a susceptible host species in general. Thus we should think in terms of pneumococcal infection, rather than in terms of pneumonia; and regard that particular condition merely as one example of the reaction between the pneumococcus and the human tissues, including in our generalized picture such lesions as middle-ear disease, sinusitis, meningitis, peritonitis, suppurative arthritis, etc., and keeping constantly in mind the part played by bacteræmic spread and selective localization.

It will be convenient, in this and the immediately succeeding chapters, to deal exclusively with bacterial infections, since most of our knowledge has been acquired in studying infections of this type. In a later chapter we shall discuss how far our conceptions have to be modified in the case of infections with the filtrable viruses. Protozoal and helminthic infections are beyond the scope of this book; though there is a growing tendency for the methods that have been elaborated by the student of bacterial disease to be applied in this extended field.

Invasion of the Tissues by Bacteria.

With very few exceptions, bacteria and other microbial parasites must gain access to the host's tissues and multiply in them before they can produce any

harmful effect; and we may for the moment regard the capacity to pass through some protective covering layer—most commonly through one of the mucous surfaces of the body—and to multiply in the subjacent tissues, as an essential attribute of a pathogenic organism. The “passing through” must not, of course, be interpreted too literally, or as necessarily implying any active motion on the part of the parasite. Apart from mechanical injuries to the skin or mucous membranes, micro-organisms probably gain access to the tissues by the same routes and by the same methods as inert particles of non-living matter, particularly in those areas where lymphatic tissue lies near the surface and lymphatic drainage is abundant, as in the nasopharyngeal region, the tonsils, and the lymphoid follicles of the intestine. In other cases they appear to reach the crypts of mucous glands, and then, after local multiplication, to penetrate to the subepithelial tissue. In any event it is probable that the capacity for invasion depends in considerable part on a capacity to withstand the conditions existing on the particular surface where lodgment is first made, or in the channels or viscera through which the parasite must pass before it gains contact with a penetrable surface. It happens that the logical point of departure in a description of the reaction between a pathogenic parasite and its host—the transit from the outside world to the host’s tissues—is the part of the picture which is most sketchy and unfinished. Only a few details have as yet been filled in, and their significance is not always obvious. It will be more convenient to deal with this aspect of the question in a subsequent chapter (see pp. 45–54) and to pass at once to the events which follow the passage of the parasite into the tissues.

A broad distinction can be made between those parasites which tend to remain localized at or about their point of entry, and those which are rapidly disseminated throughout the body and produce a generalized infection associated with a bacteraemia. It is unlikely, as we shall see later, that any bacterial parasite multiplies freely in the blood-stream, and the presence of large numbers of bacteria in the circulating blood would seem, in most cases, to be the expression of a loss of equilibrium, in which a rapid overflow of organisms from extensive or numerous foci of infection cannot be effectively balanced by the mechanism which removes them, or in which a less rapid overflow results in a similar accumulation because the clearing mechanism is in some way put out of action.

The property of invasiveness is one of the characters which distinguish one bacterial species from another, or one strain from another within the same species. Since, in many cases, the pathogenicity of a particular bacterial parasite appears to be mainly determined by its relative rapidity of spread within the tissues, it is logical enough to refer to those species which spread rapidly as *virulent* and to those which spread slowly, or not at all, as *avirulent*; and this usage is generally followed. The term *virulent* is sometimes used as though it were completely synonymous with *invasive*; but this usage is unjustified by derivation and singularly inconvenient in practice. If rigidly adhered to it would involve the exclusion from the class of virulent bacteria of all

those organisms that exert their lethal effect by the production, in localized foci, of powerful toxins. We could, for instance, no longer speak of virulent and avirulent diphtheria bacilli. It is better practice to retain the term *virulent* in its correct sense of *poisonous*, without any implication as to how the poisonous effect is produced, and to apply it to any organism which gives rise to a rapidly fatal infection. The particular attributes on which virulence depends can be described in other terms.

It may, then, be stated that multiplication of bacterial cells within the tissues is one of the essential factors on which infection depends. Some species can produce a fatal infection without any wide dissemination within the body, others only cause death when such widespread dissemination occurs. Some part of the mechanism of resistance will clearly be concerned with the killing and removal of the bacterial cells as such.

Bacterial Localization.

Apart from the generalized dissemination that characterizes many rapidly fatal infections, or the last stages of infections that tend to run a longer course, most bacteria tend to lodge and multiply in tissues remote from their point of entry to the body; and certain species, or groups of species, differ sharply from one another in regard to the particular tissues or organs in which such localization occurs.

The site of the primary lesion and the direction of the initial spread of infection are largely determined by the portal of entry to the body, and by the local anatomy, particularly by the direction of the lymph flow. But the portal of entry in its turn is determined by the natural habitat of the parasite, and this by its potentialities for life under different environmental conditions. Thus, staphylococci are normal inhabitants of the skin. Those types, or races, that are potentially pathogenic, may gain access to the underlying tissues by the hair follicles, or by the ducts of the sebaceous glands, and cause the characteristic pustules or boils. But the ability of staphylococci to survive on the skin appears to depend on some inherent property shared by few other bacterial species (see p. 45).

Many of those bacteria that lead a parasitic existence in the nasopharynx, and are spread from host to host by droplet infection, tend in consequence of this common portal of entry to show a rough similarity in the distribution of the lesions they produce; but the similarity is very incomplete, and breaks down altogether on a close analysis. Thus, pneumococci, hæmolytic streptococci, influenza bacilli, diphtheria bacilli and meningococci are all spread by droplet infection. Diphtheria bacilli and hæmolytic streptococci both tend to lodge and multiply in the tonsils, the former giving rise to the characteristic lesions of faucial diphtheria, the latter to tonsillitis. Both produce soluble toxins, which diffuse widely from the initial lesion. But the tendency to invasive spread is much greater in the case of the streptococcus than in that of the diphtheria bacillus. Pneumococci, hæmolytic streptococci and influenza bacilli may all spread to the lungs and cause pneumonic lesions, but their rôle

in this regard is very different. The pneumococcus fully merits its name by its pre-eminence as an invader of the pulmonary tissue. It is the sole important bacterial agent in the primary pneumonia of adolescence and adult life. In the secondary pneumonias associated with such infections as measles, whooping cough and influenza, it shares its honours with the influenza bacillus and the hæmolytic streptococcus. The meningococcus, although it is a nasopharyngeal parasite and spreads by droplet infection, produces its characteristic lesion in the meninges. Pneumococci, hæmolytic streptococci and influenza bacilli may also give rise to meningitis; but whereas this is the modal reaction of the meningococcus, when it produces clinically obvious infection, it is a relative rarity among the host of pathogenic reactions caused by the three other bacterial parasites.

The common sites of the primary lesions of syphilis are obviously determined by the usual mode of infection; but the secondary and later stages are the same whether the primary chancre was genital or extragenital, and the tissues affected and the lesions produced are highly characteristic of the disease.

Instances could be multiplied *ad nauseam*. The point that needs emphasis is that the distribution of infective lesions depends in large part on the biological characters of the infecting parasite. It is a very safe prophecy that these differences in selective localization will in time be traced to differences in the biochemical behaviour of the pathogenic bacteria. Such differences must, of course, have their counterpart in variations in the local metabolic processes in the different tissues of the host. We are, at the moment, almost blankly ignorant of the factors concerned.

The Stimulation of Specific Cellular Reactions.

The differences between the infections produced by the various pathogenic micro-organisms are not confined to the distribution of lesions within the body. The lesions themselves are different and may be highly characteristic. The tendency of certain bacterial species to stimulate a localized polymorphonuclear reaction, associated with pus formation, has earned for them the title "pyogenic." Such experimental evidence as is available indicates that the migration of polymorphonuclear leucocytes to the focus of infection is the direct result of an attractive (chemotactic) effect, exerted by some diffusible bacterial product. Equally characteristic lesions, which differ among themselves, are produced by the various non-pyogenic organisms. The diphtheria bacillus, the typhoid bacillus and the *Treponema pallidum* give rise to tissue reactions which are so distinct that any competent histologist could differentiate them without bacteriological assistance.

The special case of tuberculosis is of particular interest in that a beginning has been made in tracing the connection between the various chemical products of the bacterial cell and the type of tissue reaction to which they give rise (Anderson 1927, 1929, 1932, Anderson and Chargaff 1929, Sabin 1932, Sabin, Doan and Forkner 1930, Smithburn and Sabin 1932). It would appear that the characteristic reaction of large mononuclear cells of the epithelioid type, and their fusion to form giant cells, is determined by the phos-

phatides and waxy substances present in the bacillary bodies, and particularly by an optically active saturated fatty acid—isomeric with cerotic acid and named “phthioic acid”—which may be separated from these fractions. Some constituent of the phosphatide fraction would seem to be responsible for the characteristic caseative necrosis. A polysaccharide fraction, which has been isolated from tubercle bacilli and tested in the same way, fails to provoke any characteristic reaction; while a protein fraction, in which the toxicity of the bacterial cells appears mainly to reside, provokes a leucocytic response, but not the epithelioid-cell proliferation or the giant-cell formation.

It is not, of course, the case that every pathogenic bacterium provokes a distinct and characteristic cellular response, or that the pathologist unaided can arrive at a correct bacteriological diagnosis from a histological examination of the infected tissue; but, as Kettle (1927) has pointed out so clearly, there is a broad correlation between certain types of lesion and certain groups of bacteria, and the groups which the pathologist would recognize on the basis of the distribution and type of the lesions produced are, in many cases at least, those which the bacteriologist would recognize as containing closely related bacterial species.

All this may seem very obvious and elementary; but it is of the first importance to realize that different pathogenic parasites have different points of attack, and different methods of attacking. We shall here be concerned almost entirely with a few types of reaction of general significance in the interplay of parasite and host, for the sufficient reason that our ignorance with regard to other possible or probable factors prevents any useful discussion. But we cannot doubt that such factors exist, and the immunologist of the future may be more concerned with them than with the particular protective mechanisms that occupy so large a share of our attention to-day.

Bacterial Toxins.

There is no apparent reason why the mere presence in the tissues, or blood stream, of any reasonable number of cells of an order of size expressed in low multiples of μ should exert any harmful effect on the host that harbours them. No mechanical theory of the pathogenic action of bacteria is compatible with our knowledge of the way in which the tissues deal with inert particles which have gained access to them. The basis of all harmful effects of bacterial infection is quite certainly chemical; and only when the chemist has replaced the immunologist shall we be able to give an intellectually satisfying account of what happens when a particular parasite invades a particular host. In the meantime we must do what we can with the crude data at our disposal.

It has long been known that certain bacterial species produce highly poisonous substances which give rise to characteristic lesions or symptoms when injected into susceptible animals. These are the bacterial toxins. A convention has become established according to which those toxins which diffuse readily from the bacteria that produce them are referred to as *exotoxins*, while poisonous substances which remain attached to the protoplasm of the bacterial cells are referred to as *endotoxins*. As knowledge has accumulated, it has been

possible to correlate this character with others, and a broad distinction may be made between characteristic members of the two groups.

A typical exotoxin is readily separated from a bacterial culture, in a suitable fluid medium, by simple filtration through a candle of porcelain or diatomaceous earth. It shows a characteristic affinity for certain cells of a susceptible host, producing easily recognizable symptoms during life, or lesions which can be detected after death. Thus one exotoxin can usually be distinguished from another by what we may term its pharmacological action. Instances of this specific effect are afforded by the action of tetanus toxin on the motor nerve cells, by the ocular and pharyngeal paralyses caused by the toxin of *Cl. botulinum*, and by the acute fatty degeneration of the cardiac muscle, the hæmorrhagic lesions of the adrenals, and the late paralysis in animals that survive, which are a characteristic of experimental diphtheritic toxæmia.

A particularly good example of this specific pharmacological action is provided by recent studies on the toxin produced by the *Staphylococcus aureus* (Kellaway, Burnet and Williams 1930). This toxin is peculiarly suitable for investigation along these lines in consequence of the extreme rapidity of its action. While maximal doses of diphtheria toxin fail to kill a guinea-pig in less than ten hours, 0.05 c.c. of staphylococcal toxin will kill a rabbit in less than three minutes, and in cats doses of 0.1 c.c., or more, per kilo usually cause death in less than twenty minutes. When the crude staphylococcal toxin is inoculated intravenously into a cat there is at first a transitory fall in blood pressure, vasomotor in origin, and traceable to some pharmacologically active substance derived from the medium. This is followed by a rise in pressure, and then a rapid terminal fall, almost to zero. The temporary rise may be due in part to an increased output of adrenalin. The final fall is due principally to obstruction to the pulmonary circulation following constriction of the pulmonary arterioles, and resulting in right-heart failure; there is also a direct action on the heart itself due to constriction of the coronary vessels. (For other examples of the pharmacological action of toxins see Trevan 1931.)

An exotoxin is usually fatal in a very small dose. The *Minimal Lethal Dose* of a potent diphtheria toxin for a guinea-pig is about 0.002 c.c., of a potent tetanus toxin about 0.0005 c.c., and of a potent botulinum toxin about 0.0001 c.c. Such toxins are usually relatively thermolabile. Diphtheria toxin is inactivated by heating at 58°–60° C. for 1–2 hours; tetanus toxin by heating at 60° C. for 20 minutes. Lastly—and this is a matter which will be considered more fully in a subsequent chapter—exotoxins are usually highly antitoxinogenic; that is, on inoculation into a suitable animal they stimulate the production of antitoxins which neutralize them specifically and in accordance with the law of multiple proportions.

The typical endotoxin is not liberated into the fluid medium in which the bacterium producing it is growing, and hence cannot be separated from the bacterial cells by simple filtration. The usual method of preparing a solution, or suspension, of an endotoxin is to break up the bacterial cells by prolonged grinding, with or without salt, by alternate freezing and thawing, or by merely allowing them to autolyse in a fluid medium. Toxins of this type may in most cases be demonstrated by the injection into a susceptible animal of the unaltered bacterial cells. In this case it is assumed that the liberation of the toxin is brought about by the lysis of the cells *in vivo*. The endotoxins do not, in general, give rise to diagnostic symptoms or lesions in experimental animals, so that it is not usually possible to recognize any particular endotoxin

by its pharmacological action. The Minimal Lethal Dose of a typical endotoxin is in general far larger than that of a typical exotoxin, sometimes the ratio is of the order of 1,000,000 to 1. Thus the M.L.D. of a killed culture of the cholera vibrio is about 0.5 c.c. for the guinea-pig, the M.L.D. of meningococcus for the mouse is about 2 mg. of bacillary substance, and the M.L.D. of the gonococcus for the same animal is about 10 mg. of bacillary substance. Endotoxins are usually relatively thermostable. Cholera endotoxin requires 1 hour's heating at 80°–100° C. for its inactivation; the endotoxin of the meningococcus requires 1 hour's heating at 120° C.; while the same treatment has failed to inactivate the endotoxin of the gonococcus. Finally, the endotoxins are seldom actively antitoxinogenic. In some cases it has proved impossible to prepare anti-endotoxic sera. A more common experience is that the prolonged immunization of an animal results in the production of an antiserum which is able to neutralize a few M.L.D.'s of the endotoxin; but the law of neutralization in multiple proportions usually fails: x c.c. of serum may protect an animal against y M.L.D. of toxin, where x is some such amount as 0.1–0.5 c.c. and y is a low number such as 1–5; but nx c.c. of serum will not protect against ny M.L.D. of toxin where n is any large number. Indeed, in many cases, a relatively small increase in y brings us to a limit at which no amount of anti-endotoxic serum will afford protection. It would seem that the anti-endotoxins must differ qualitatively from the anti-exotoxins—their mode of action must be different.

Although this broad distinction can be made by comparing typical exotoxins with typical endotoxins, our difficulties become very great if we attempt to assign each toxic bacterial product to its correct group, and we soon find it necessary to jettison several of the differential criteria which we have mentioned above. Thus, the toxin of *Cl. botulinum* is a typical exotoxin, filtrable, with a characteristic pharmacological action, and antitoxinogenic; but it is relatively heat-resistant (75°–80° C. for 10 mins.). The toxin of *Cl. welchii* is filtrable, has a characteristic local action on the tissues, and is antitoxinogenic; but its M.L.D. is relatively large (0.02–0.1 c.c. for the pigeon), and it is relatively heat-resistant (70° C. for 30 mins.). The erythema-producing toxin of the hæmolytic streptococcus—the toxin which is responsible for the rash in scarlet fever, and is employed in the Dick reaction—is filtrable, has a characteristic action on the skin of a susceptible person, and is antitoxinogenic; but its M.L.D. is very large (5–10 c.c. or more of an unconcentrated filtrate for the rabbit) and it is very resistant to heat (96° C. for 45 mins.). The hæmolytic streptococcus also produces another filtrable toxic constituent which differs from the Dick toxin in being thermolabile (55° C. for 30 mins.), in causing lysis of red blood cells, and perhaps of leucocytes, though the identity of streptococcal hæmolysin and leucocidin is not accepted by all observers. Its M.L.D. is large, of about the same order as the thermostable toxin, but in this dose it kills susceptible rabbits within 24–36 hours with an associated hæmoglobinuria and characteristic evidence of intravascular hæmolysis at necropsy (McLeod and McNee 1913, Channon and McLeod 1929). Until recently it appeared to be non-antitoxinogenic; but Todd (1932) has shown

that, by the use of suitable reagents, antihæmolysin can be demonstrated to high titre in the serum of immunized animals. The toxin of Shiga's dysentery bacillus has a selective action on the mucosa of the large intestine and on the central nervous system in the rabbit, and it is antitoxinogenic. But its M.L.D. is relatively large as compared with the more typical exotoxins (0.1–1 c.c. of filtrate or fluid culture for the rabbit; 0.002 mg. of dried bacterial substance for the mouse) and it is relatively heat-resistant (75°–90° C. for 1 hr.). Its distribution between the bacterial cells and the culture fluid is curious. It is present in filtrates from an autolysed broth culture; but it is also present in the bacterial cells themselves and the toxin is commonly preserved in the form of dried bacterial substance.

One implication which has sometimes—though without justification—been attached to the term “exotoxin” is very probably misleading. Toxins of this type have been described in words which suggest that they are of the nature of active secretions of the bacterial cells. There is little definite evidence that this is so. Though some exotoxins, as that of *Cl. welchii*, are produced in high concentration during the phase of active bacterial growth, others, such as diphtheria, tetanus and Shiga toxins, only attain a high concentration in a fluid culture when the phase of active growth has passed, and the majority of the bacteria present are dead or dying. How far autolytic processes may be concerned in toxin-production is quite uncertain. We have, in fact, no real knowledge of what toxins are, or how they are produced. We can detect them only by their toxic action.

In our studies of bacterial toxins we have to be content with crude reagents—filtrates, autolysates or extracts which quite certainly contain a multitude of substances besides those which we desire to investigate. We have not yet succeeded in isolating any bacterial toxin in a chemically pure state, although some degree of concentration has been attained by various methods of fractional precipitation.

In view of all these facts it is obvious that the terms “exotoxin” and “endotoxin”—and especially the latter—have no very clear significance, and may easily be misused. A verbal cloak for ignorance is a garment that often hinders progress.

Other Immunologically Active Bacterial Constituents.—It is, perhaps, somewhat illogical to place first among such constituents the bacterial enzymes, of whose immunological significance we are as yet entirely ignorant. It is, however, convenient to consider their possible importance before passing to substances of the second group.

It is a little surprising that these enzymes, on whose activity we rely so largely for purposes of identification and classification, have received little or no attention from the immunological point of view. It is reasonable to suppose that the occurrence in the tissues and body-fluids of catalysed chemical changes, proceeding independently of the normal body metabolism, might seriously interfere with the host's nutritional economy. It is probable that the relatively minute amount of bacterial substance present in

the tissues as a whole, even in a heavy bacterial infection, has tended to divert attention from this particular form of bacterial activity. It happens, however, that recent studies (Quastel 1924, 1926, Quastel and Wooldridge 1925, 1927, 1928, 1929, Quastel and Whetham 1925, Quastel and Woolf 1926, Quastel, Stephenson and Whetham 1925, Cook and Stephenson 1928) have placed our knowledge of the mode of action of these bacterial enzymes on an entirely new footing. The effect of various bacterial suspensions upon a variety of substrates has been investigated, using methylene blue as an indicator of reduction and carrying out the tests in the Thunberg vacuum tube. The results obtained have given us a far clearer picture than we possessed before of the range of activations of which the bacterial species are capable. But even more interesting, from our present point of view, is the demonstration that many of these specific enzyme activations are quite independent of the normal co-ordinated activities of the bacterial cells. It is hardly possible to hazard a guess at the actual conditions existing in the bacterial suspensions employed, though we may be reasonably sure that the majority of the cells are dead or dying. The important point is that some of these studies have shown quite clearly that certain enzymic activations may be effected by dead bacteria, and that the treatment of the bacterial suspensions by various physical and chemical agents will inactivate some enzyme systems without affecting others.

It seems quite possible that, whenever there is formed in the tissues a nidus of bacterial cells, alive or dead, there may result in the immediate neighbourhood a diversion of the normal metabolic processes sufficiently serious to derange the local cell-activities. It is not impossible that the diffusion of bacterial enzymes might convert such a local effect into a more general one.

Should such factors be found to be concerned in the reaction between parasite and host they would consort well with our knowledge of certain aspects of infection and resistance. Thus, it seems to be fairly definitely established that ferments are relatively ineffective antigens—that is, they fail to stimulate the formation of neutralizing antibodies when injected into the animal tissues, or do so only to a minimal degree. In this they share one of the characters of many so-called endotoxins. In another respect they appear on the evidence at present available to differ fundamentally. All those bacterial ferments whose heat resistance has been studied are relatively thermolabile. Exposure to temperatures above 37° C. may considerably reduce the activity of some of them, and heating to temperatures in the neighbourhood of 80° C. results in complete inactivation. Many of the endotoxins, on the other hand, will withstand heating at 100° C. or even at 120° C. for considerable periods. This property of heat resistance is shared by the group of active substances next to be considered.

If it is curious that the bacterial enzymes should have rested so long in the immunological limbo of neglected possibilities, it is still more curious that those who have studied the fluctuations in certain chemical constituents of the blood should have concerned themselves so largely with metabolic diseases, and have paid so little attention to the changes associated with bacterial infections.

Such changes have of course been reported, both in natural infections in

man (Andresen and Schmidt 1927) and in experimental infections and toxæmias in animals (Menten 1926, Menten and Manning 1925, Levine and Kolars 1926, Zeckwer and Goodell 1925); but the available evidence is meagre and difficult to interpret. Quite recently Delafield (1931) has made a more systematic attempt to explore this particular field with results which indicate the desirability of further investigations along these lines. Suspensions of certain Gram-negative bacilli, when injected intravenously into rabbits, provoke a characteristic response. There is at first a marked hyperglycæmia, which reaches its height in a few hours. The blood sugar then returns to the normal and in most cases falls below it, so that the animal becomes definitely hypoglycæmic. Occasionally this hypoglycæmia may reach the convulsive level. During the hyperglycæmic phase there is a fall in the inorganic blood phosphorus; and this blood constituent rises again to the normal level, or above it, during the succeeding phase of hypoglycæmia. This action of the bacterial cells is not inhibited by steaming, by short periods in the autoclave at 120° C., or by extraction with alcohol or acetone.

In a more recent paper Delafield (1932) has shown that the particular character of bacterial structure that determines the reaction to Gram's stain is in some way associated with the chemical changes induced in the tissues. Several different species of Gram-negative bacteria give rise, on intravenous injection, to the succession of changes summarized above; though one species may differ in activity from another. Suspensions of Gram-positive bacteria on the other hand seldom, if ever, induce this particular chemical syndrome.

Of the nature of the substances concerned or of how they act we have as yet no certain knowledge. It is possible (Evans and Zeckwer 1927) that the hyperglycæmic phase may be the result of adrenal stimulation, since it has been found that hyperglycæmia does not occur in rabbits which are injected with dead bacteria after one adrenal has been removed and the other denervated. The whole problem, however, awaits further inquiry.

A third group of bacterial constituents are known to be immunologically active, though pharmacologically and biochemically inert. They are antigenic components of the bacterial cell, which react to the detriment of the host by combining specifically with the protective antibodies. They will be considered in greater detail in later chapters.

Hypersensitiveness and Allergy.

There are reasons, which will be discussed more fully elsewhere, for believing that the tissues may become sensitized during the course of a bacterial infection, so that a bacterial constituent which before called forth little or no response may act as a powerful stimulus. In some cases the vigorous response evoked is injurious to the host, so that a bacterial product which possesses little or no toxicity for a normal animal may be highly toxic for an animal which has become sensitized by infection. The well-known tuberculin reaction in the guinea-pig is a case in point.

Some Illustrative Examples.

The above are the various bacterial factors which, so far as we know at the

moment, are certainly, probably, or possibly concerned in the pathogenesis of infective diseases.

A few illustrative examples may serve to emphasize the more important points.

Botulism may be placed at one end of the scale, as an example of a pure toxæmia. It is not a true infection, but is closely analagous to the effects that may follow the consumption of such vegetable poisons as those produced by certain fungi or plants. There is no certain evidence that *Cl. botulinum* ever invades the tissues, or multiplies in them. Man is affected by eating food in which the organism has grown and produced its toxin.

Tetanus is one step removed, in that the bacillus must gain access to the tissues and multiply in them in order to produce its toxic effects; but its invasive powers are very slight. It usually gains access to the body as the result of some mechanical injury, and the local multiplication of the organism is in many cases assisted by gross injury to the tissues, by the injurious effects of some associated infection, or by the presence of some chemical agent, such as a calcium salt, which alters the local conditions to the advantage of the parasite.

Diphtheria takes us a little further along the scale. The bacillus is capable of implanting itself in the faucial region, and of spreading from host to host, by ordinary droplet infection or in similar ways. It has relatively slight but quite definite invasive powers. It causes a characteristic local lesion, and may occasionally invade other tissues and organs. Its lethal effect, however, depends almost entirely on its activity as a toxin-producer.

The hæmolytic streptococcus combines the activities of a typically toxigenic organism with those of the highly invasive group of bacteria. It produces a relatively heat-stable toxin, with a characteristic action on the skin, which is the cause of the typical toxic rash in scarlet fever; but it also invades the tissues freely. Indeed, highly virulent strains of the organism are among the most rapid and vigorous tissue-invaders with which we are acquainted in human pathology. It is very probable that the thermolabile toxin produced by this streptococcus, which is actively lytic for both red cells and leucocytes, plays some part in the rapid generalization of infection (Channon and M'Leod 1929).

As we move along the scale we meet a variety of organisms whose pathogenicity seems to depend entirely on their power of spread and multiplication in the tissues, and whose more important effects seem to be localized to the areas in which such multiplication occurs. These differ among themselves in selective localization, and in the tissue reactions to which they give rise. The staphylococci most commonly cause localized suppurative lesions, with a varying degree of lymphatic spread, which may sometimes be rapid and extensive. More rarely they give rise to a generalized infection of the pyæmic type, in which the cocci are carried from the original focus of infection in detached fragments of an infected thrombus. A heterogeneous group, including the tubercle bacillus, the leprosy bacillus, the *Treponema pallidum*, the *Actinomyces bovis*,

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the actinobacillus, and the glanders bacillus give rise to the group of diseases known as the infective granulomata. Certain organisms, such as the pneumococcus, are particularly apt to invade the lungs; others, such as the organisms of the typhoid-paratyphoid group, give rise mainly to intestinal lesions, and so on. In every case bacteræmic spread may occur at some period of the infection; in many it is an essential feature of the evolution of the disease. In those infections which are liable to run an acute and fatal course, or in which a fatal exacerbation may occur at some later stage of the disease, the degree and persistence of bacteræmia is in direct relation to the severity of the condition. Thus the case-fatality in pneumonia is far higher in those patients whose blood contains pneumococci in detectable numbers, than in those who yield negative blood cultures.

At the extreme end of the scale are those bacteria which tend to spread rapidly throughout the tissues, causing an acute and fatal septicæmic infection with a minimum of local lesions. The septicæmic forms of plague or anthrax and acute streptococcal septicæmia afford examples.

The defence mechanisms brought into play and their relative importance will clearly differ according to the type of infection. It is with these mechanisms that we shall be concerned in the following chapters.

SUMMARY

(1) An ability to gain access to the tissues, and to multiply in them to a lesser or greater degree is an essential attribute of a pathogenic organism. Some organisms produce their effects by the local production of a powerful toxin which passes into the tissues, and produces its toxic effects on cells remote from its site of origin. Others produce their main pathological effects at the site, or sites, in which they are multiplying. Of these latter, some give rise mainly to lesions limited to the near neighbourhood of their point of entry; others to lesions remote from this point, but characteristically localized in certain tissues or organs; others, again, to a generalized bacteræmic infection. These different modes of attack are often combined in the case of a single infecting bacterium.

(2) The selective localization of bacteria in particular organs or tissues of the body is determined in part by characters inherent in the bacteria themselves. The anatomy of the infected host, the biochemical reactions of different tissues, and the defence mechanisms brought into play, combine with these inherent bacterial characters in determining the exact distribution of lesions encountered in each particular case.

(3) Pathogenic bacteria differ in the cellular reactions to which they give rise. These differences may be highly characteristic.

(4) Among the bacterial toxins are some which are characterized by the ease with which they may be separated from the bacterial cells which produce them. Under suitable conditions they diffuse into a fluid medium and may be obtained from the cells by simple filtration. Toxins of this class are com-

monly referred to as *exotoxins*. They are usually characterized by possessing a specific pharmacological action, and by being actively antitoxinogenic. Their Minimal Lethal Dose is generally small, sometimes very small. They are in many cases thermolabile, being inactivated by temperatures in the neighbourhood of 55° C.

Apart from these toxins there is another class of toxic bacterial products which are characterized by remaining incorporated in the substance of the bacterial cells. They can be obtained in a state of solution, or perhaps suspension, only by methods which result in the disintegration of the cells. They do not, in general, exert any characteristic pharmacological action by which they can be differentiated from one another. They are feebly antitoxinogenic, and the antitoxins to which they give rise do not obey the law of combination in multiple proportions. Their Minimal Lethal Dose is usually large. They are relatively heat-stable, withstanding temperatures of 80°–120° C. for an hour or more. They are often referred to as *endotoxins*—a term which has little to recommend it.

(5) Other constituents of the bacterial cells—such as the ordinary bacterial enzymes, and the thermostable substances which produce changes in the concentration of reducing substances and phosphates when injected intravenously into rabbits—may play a part in the phenomena of infection; but we have at the moment no definite evidence on this point.

(6) Certain other bacterial constituents, which are antigenic but not primarily toxic, may exert an important influence on the course of infection by neutralizing antibodies which act on the bacterial cells.

(7) As the result of infection the tissues of the host may become hypersensitive to the infecting bacteria or to their products. Such sensitization may play an essential part in some of the phenomena of infective disease.

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CHAPTER IV

THE MECHANISMS THAT HINDER OR PREVENT THE ACCESS OF BACTERIA TO THE TISSUES

As noted in the previous chapter, an ability to gain access to the tissues and to multiply in them to a lesser or greater degree is an essential attribute of a pathogenic organism. It is logical to commence our study of the defence mechanisms of the host by considering the barriers that are opposed to the ingress of bacterial parasites from the outside world. Unfortunately our knowledge is here at its sketchiest. It has, naturally enough, been our custom to eliminate this initial step in the natural process of infection by injecting bacteria directly into the tissues of our experimental animals—our uncontrollable variables are formidable enough, without adding to them by including in any single study the events that happen on a body surface as well as the reactions in the host's tissues. It is only within recent years, and in the hands as yet of relatively few workers, that any serious attempt has been made to study systematically the mechanisms involved in the first line of defence.

It will be convenient to take in turn the various body surfaces and portals of entry, and to summarize very briefly our scanty knowledge of their behaviour towards bacteria that establish contact with them.

The Skin.—It needs only the most casual acquaintance with the ordinary chances of life to establish the value of an intact skin as a barrier against bacterial infection. The inability of the deeper tissues to protect themselves against the free access of bacteria from the outside world set a sharp limit to major surgical interference in pre-Listerian days. There can be little doubt that the skin functions in the main as a simple mechanical barrier, but it has recently become clear that the efficacy of this barrier is enhanced by a complex biological mechanism which enables the skin surface to free itself rapidly from the majority of those bacteria that are continuously impinging upon it.

Our knowledge of this mechanism has been derived from two independent sources: the observations of Arnold, Gustafson and others (1930), which form part of a systematic study of the self-disinfecting mechanisms of the body surfaces and those of Colebrook (1930) on the sterilization of the hands. Colebrook found that hæmolytic streptococci were rapidly killed on the skin of the normal hand. Three minutes after swabbing a finger lightly with a broth culture—by which time the skin was apparently dry—30,000,000 cocci could be recovered by thorough swabbing with sterile broth. One hour later 1,722,000 viable cocci were recoverable; two hours later 7,000 cocci. Similar observations were made with *Proteus vulgaris*, Friedländer's pneumobacillus and *Bact. coli*. Simple control experiments, in which glass surfaces were swabbed, allowed to dry and sampled

at intervals thereafter, showed that the bactericidal effect could not be attributed to simple desiccation. While the skin of the normal hand shows this striking capacity for freeing itself—at least partially—from bacteria which form no part of its normal flora, its reactions are entirely different towards those species that habitually live upon it. Thus, Colebrook found that no amount of washing, nor any of the chemical disinfectants commonly employed for sterilizing the hands in obstetrical practice, would remove from the skin the numerous staphylococci and less numerous diphtheroid bacilli that normally vegetate thereon.

Arnold, Gustafson and others (1930) (see also Karns and Arnold 1931) record a more detailed series of observations along similar lines. Taking *Erythrobacillus prodigiosus* as the test organism, they found that 90 per cent. of the bacteria placed on the skin of the hands or back were killed within 10 minutes, about 99 per cent. within 20 minutes, and that none could be recovered in most cases after 30 minutes. Many other bacterial species behaved in the same way. Using the palmar surface of the clean hand *Bact. coli*, *Bact. typhosum*, and *Bact. enteritidis* could not be recovered 10 minutes after application. Certain regions, such as the lateral margins of the dorsal surface of the finger-nails and the region under the nail tip, freed themselves far more slowly from the bacteria applied to them. Dirty skin in any situation had relatively little power of autosterilization. Using *Bact. enteritidis* as a test organism on the hands of workmen (electricians and plumbers) before and after washing the hands at the end of the day, it was found that the dirty hands gave no reduction in the number of recoverable bacteria in 10 minutes, a 5 per cent. reduction in 20 minutes, and a 15 per cent. reduction in 30 minutes. After washing there was an 85 per cent. reduction in 10 minutes, and a complete disappearance after 20 minutes. The dead skin, tested on cadavers within 15 minutes of death, had lost most of its bactericidal action. With *Bact. coli* there was a 12 per cent. reduction in 10 minutes, a 16 per cent. reduction in 20 minutes, and a 50 per cent. reduction in 30 minutes. As in Colebrook's experience it was noted that staphylococci could constantly be recovered from the normal human skin. When *Staph. aureus* was applied experimentally to the clean palmar skin, there was an initial rapid reduction (82 per cent. in 10 min.), but the organism was still recoverable after much longer periods.

The mechanism of this autosterilizing action is as yet quite uncertain. It seems probable that the chemical properties of the sweat are the determining factors. Arnold and his colleagues note that the pH of the normal skin is markedly acid (5.8–5.22, see Schade and Marchionini 1928) and are inclined to attach considerable importance to this factor; but they found it impossible to test this thesis experimentally, since they were not able to alter the pH of the superficial layer of the skin by any method that did not involve injury to the epidermis with exposure of the deeper layers, or gross interference with the vascular supply.

Fleming (1922, 1929, 1932) has recorded the presence in tears, in nasal secretion, and in many tissues including the skin, of a relatively thermolabile substance which, in high dilution, causes the lysis of certain non-pathogenic bacteria, and in particular of an organism to which he gave the name of *Micrococcus lysodeikticus*. This substance, *lysozyme*, is also active against some strains of staphylococci and intestinal streptococci of human origin. In high concentration it acts, though less energetically, on other pathogenic bacteria. It is natural to inquire whether it plays any part in the self-disinfecting action of the human skin. If so, it would seem that its rôle is relatively unimportant; since Colebrook (1930) records that many of the bacteria that are rapidly removed are not susceptible to its action, while *Micrococcus lysodeikticus*

itself disappears less rapidly than such species as *Str. hæmolyticus*, *Bact. coli* or *Proteus vulgaris*.

The Mouth, Stomach and Intestinal Tract.—When bacteria gain access to the mouth, provided that they are not introduced as constituents of some food or drink that is immediately swallowed, they adhere to the mucus with which the lining epithelium is plentifully covered.

A series of careful studies by Bloomfield (1922*a, b, c*) have enabled him to trace in some detail their subsequent fate. In an initial series of experiments carbon particles were placed on the tongue, on the palate, in the sublingual space, on the mucosa of the cheek, on the posterior pharyngeal wall, or on the tonsil, and their subsequent movement was carefully observed. It was found that there was little or no tendency for the particles to spread at large over the surface of the mouth, but that they were removed from the various sites of implantation in an orderly manner. The general path of removal was directly backwards. There was no forward dissemination nor was there any tendency to wide lateral spread—carbon placed well to one side of the tongue near its tip did not, for instance, cross the mid-line. The paths of transit from the tongue, sublingual space and palate all converge at the base of the tongue. The tonsils, protected by the faucial pillars, occupy a position that is out of the way of these lines of passage so that under normal conditions particles that are deposited on the tongue are swept past them. The currents that bear the particles backwards are almost certainly the result of suction, set up by the lips, cheek, tongue and palate; and particles are removed with particular rapidity from the sublingual space and from the lateral buccal mucosa, where the flow of saliva is maximal and the local formation of the mucosa is least favourable to retention. Particles placed directly on the tonsils are in part removed rapidly by the general flushing action of the saliva; but those that once gain lodgment remain relatively stagnant for a considerable period of time. Having reached the base of the tongue the particles are periodically swallowed. In general, carbon particles are almost completely removed from the mouth within 15 to 30 minutes. Subsequent experiments with *Sarcina lutea* showed that bacteria introduced into the mouth were removed in the same fashion as carbon particles. Attempts were made to alter the flora of the tongue by thorough and repeated washing. It was found that the greater part of any bacteria artificially implanted could be rapidly removed in this way, but no amount of washing would produce any significant change in the character or number of the normal lingual flora. It would appear that bacteria entering the mouth are unable to persist on any of the mucous surfaces for more than a few hours unless their biological characters are such as to allow of successful colonization. Once colonized they become part of the local flora, and can no longer be dislodged by any simple mechanical means, nor probably by any ordinary disinfectant.

Once bacteria have been swallowed they are subjected in the stomach and upper part of the duodenum to the action of the gastric juice, with its high hydrogen-ion concentration. It has long been recognized that the gastric secretion imposes a barrier to the passage of bacteria from the mouth to the intestine, but the recent studies of Arnold and his colleagues (Arnold 1926,

1927, 1928, 1929, Arnold and Brody 1926a, b) have placed our knowledge on a more detailed basis. By fixing various segments of the gastro-intestinal tract of the dog to the skin of the abdomen they were able to withdraw samples of the contents by sterile puncture as desired, and thus to determine the nature of the normal flora in relation to the acidity or alkalinity of the intestinal contents, and also the fate of other bacteria when administered by the mouth.

Table VI shows the relation between the hydrogen-ion concentration of the intestinal contents and the prevailing bacterial flora at different levels.

Using as test organisms *Erythrobacillus prodigiosus* and *Ps. pyocyanea*, it was found that these bacteria, when introduced in watery suspensions into the empty stomach of a dog 12 to 18 hours after a meal, failed to reach the cæcum. Introduced in an alkaline buffered watery suspension or in alkaline buffered milk they reached the cæcum in large numbers.

TABLE VI

Portion of Small Intestine.	pH of Contents.	Bacterial Flora.
Duodenum	5.2-6.0	Few Gram-positive cocci.
Jejunum, upper half . . .	5.5-6.5	" " " "
Jejunum, lower half . . .	6.0-7.0	Gram-positive cocci, few Gram-positive and Gram-negative bacilli.
Ileum	6.8-8.0	Rich and varied bacterial flora.

Whether the bactericidal action of the duodenal contents is entirely determined by its hydrogen-ion concentration, is, perhaps, open to question (see Meyer and Löwenberg 1928).

With regard to the possible effect of Fleming's lysozyme in the stomach and intestine, Goldsworthy and Florey (1930) have studied the distribution of this substance in mucus and mucosal extracts from several animal species, using as test organisms *Micrococcus lysodeikticus* and a series of unidentified susceptible bacteria isolated from the air of the laboratory. In the cat, no lysozyme could be detected in mucus from the stomach or colon, though small amounts could be demonstrated in saline extracts of the dried mucosæ. In the dog, detectable amounts were present in the mucosa of the intestinal tract, more in the colon than in the stomach. In the rabbit, the mucosal extracts were actively lytic. The colon was more active than the stomach, the small intestine falling between the two. In the guinea-pig, on the other hand, the mucosa from the stomach was more active than that from the intestine. What part this substance plays in eliminating susceptible species from the intestinal flora it is however impossible to say.

It is of obvious interest to inquire whether those procedures that alter the normal conditions in such a way as to allow bacteria to pass alive through the stomach and duodenum to the lower segments of the intestine have any effect in promoting their passage through the intestinal mucosa to the lymphatics and blood-stream. Arnold (1928) has studied this problem experimentally. *Bact. coli* and *Erythrobacillus prodigiosus* were injected with minimal injury into a dog's duodenum, and counts were made of the bacteria in the lymph

collected from a canula inserted into the thoracic duct. The results are summarized in Table VII. It will be noted that alkalization alone does not suffice to induce the transit of bacteria from the intestinal lumen to the lymphatics, but that addition of bile, or still better of egg white, so alters the conditions at the mucosal surface that such passage occurs. The negative findings with the other suspending fluids employed are in accord with those of Neisser (1896) and of Teale and Embleton (1914). It would appear that only under quite exceptional conditions is the intestinal mucosa permeable to living bacteria; and it would seem (Nedzel and Arnold 1931) that raw egg albumin is peculiarly potent in inducing the passage of bacteria not only from the intestinal lumen to the lymphatics and the blood, but in the reverse direction from the bloodstream to the intestinal lumen.

TABLE VII

Solution injected with Bacteria.	Average No. of Bacteria in Lymph per c.c.	Time of Appearance after Injections.
Alkaline solution and egg white .	500 to 1,000	First 5 mins. and lasting for 30 mins.
Neutral solution and egg white .	None	—
Alkaline solution	None	—
Neutral solution	None	—
Alkaline solution and bile . . .	50 to 100	First 5 mins. and lasting for 20 mins.
Neutral solution and bile	3 to 5	During first 30 mins.
Alkaline solution and dog's serum	None	—
Neutral solution and dog's serum	None	—

The Nose, Nasopharynx and Respiratory Tract.—The nasal cavities are in part protected from air-borne bacteria by the anatomy of the anterior nares. The greater part of the inhaled bacteria appear to be arrested at or near the nasal orifices (see Thomson and Hewlett 1896). Those that pass beyond this point adhere to the film of mucus that covers the nasal mucosa, and are then swept back—in this case by the current set up by the ciliated epithelium—towards the naso- and oro-pharynx, where they join the bacteria being swept back by suction currents from the mouth (see Bloomfield 1919, 1922*d*).

Whether this mechanical clearance is the only factor concerned is again uncertain. Arnold, Ostrom and Singer (1928) introduced *Erythrobacillus prodigiosus* and *Bact. coli*, by spraying, into the nose of men, rabbits, dogs and guinea-pigs, and noted their rapid disappearance. They state that swabs taken from the posterior pharyngeal wall gave no indication that there was any extensive passage of the bacteria to the throat; but they were unable to detect in nasal washings or secretions any substance that was bactericidal to the test organisms employed. It may be noted that the rate of disappearance recorded by Arnold and his colleagues—a diminution in numbers during the first 5 minutes, and total disappearance within half an hour—was more rapid than that recorded by Bloomfield—little change in 2 hours but almost

complete disappearance in 24. This slower rate of elimination was also noted by Bloomfield in the case of particles of Kieselguhr placed on the nasal septum.

Lysozyme is known to be present in high concentration in the nasal secretions, and it is altogether probable that it plays a part in freeing the nasal cavities from those species of bacteria against which it is active.

Whatever may be the nature of the complex of mechanisms involved there is every reason to suppose that the same result is attained in the nose as in the mouth. The majority of bacteria that enter at the external nares are removed or destroyed, and fail to obtain any but the most transient footing among the normal nasal flora; those that survive and multiply do so because they are in some way adapted for successful colonization, and once such colonization has occurred they may persist for an indefinite period of time.

The conditions in the nasopharynx have been sufficiently dealt with in considering the elimination of bacteria from the mouth.

It would appear (Calamida and Bartarelli 1902) that the accessory nasal sinuses are normally sterile.

There is little doubt that in health the trachea and bronchi contain few if any living bacteria (see Thomson and Hewlett 1896, Bloomfield 1922*d*); and it is probable that the main protective mechanism consists in the filtering action of the nasal passages combined with the adherence of bacteria which pass the posterior nares, or are drawn in *via* the mouth, to the pharyngeal or upper laryngeal mucosa. It is however quite certain, from the ordinary experience of the *post-mortem* room, that dust particles, and hence presumably bacteria, reach the lungs in numbers that, if minimal in any short period of time, attain a considerable total in the course of months or years.

Under exceptional conditions (see Neuninger 1901, Paul 1902, Quesnil 1902, Bloomfield 1922*d*) bacteria may reach the lungs in relatively large numbers, though those that penetrate so deeply never form more than a small fraction of the total number inspired. Once past the vocal cords, the inspired organisms are probably dealt with mechanically in three ways. They may adhere to mucus and be expectorated; they may perhaps be driven upwards by the ciliated epithelium; they may reach the deeper bronchi or lung alveoli and be removed, probably by phagocytic cells, to the regional lymph glands. Whether any immediately bactericidal mechanism is at work we do not know.

The Conjunctivæ.—It is convenient to consider the conjunctivæ in connection with the upper respiratory tract. The mechanical flushing action of the tears is an obvious phenomenon, and there is little doubt that the main route of removal is *via* the lachrymal duct.

Maxcy (1919) was able to recover *Erythrobacillus prodigiosus* from the nose within 5 minutes after its introduction into the conjunctival sac. Stort (1891) failed to recover *Bact. coli* from the rabbit's conjunctiva 1 hour after its implantation; but found that it persisted for a much longer time if the lachrymal duct were previously tied.

Tears have the highest lysozyme content of any secretion yet examined. At a dilution of 1:40,000 they may cause complete lysis of a suspension of

Micrococcus lysodeikticus. There can be little doubt that this agent suffices to rid the conjunctiva of many of the organisms that come in contact with it, and that it plays a part in the local defence of this region against certain pathogenic bacteria (see Ridley 1928).

The Genital Tract.—It is probable, though there is no definite evidence on the point, that the external genitals, so far as they are covered with a complete dermal layer, share the bactericidal action of the skin elsewhere. There are probably additional selective factors—favourable or inhibitory for particular bacterial species—resulting from localized glandular secretions, or from differences in the amount of moisture in the skin.

The urethra in the male and female is normally sterile, or contains in the neighbourhood of the meatus a few staphylococci and diphtheroid bacilli. The mechanical flushing action of the urine, combined perhaps with its slightly acid reaction (pH 6·0), is probably an important factor.

The vagina has a highly characteristic flora, consisting largely of aciduric bacilli of the Döderlein type (see Küster 1929). The normal vaginal secretion has a marked bactericidal action on many species of bacteria. Thus Menge (1894) found that *Ps. pyocyanea*, staphylococci and streptococci introduced experimentally into the vagina could not be recovered after 21 to 26 hours.

The Normal Bacterial Flora.

It will be obvious from the above that the surfaces and portals of entry of the animal body are in no sense virgin soil, on which any invading bacterium can easily obtain a foothold. The various skin and mucous surfaces are differentiated from one another by the nature of the epithelium that covers them, by the acidity or alkalinity of the fluids in which they are bathed, by the frequency, rate and direction of the currents in the thin layer of supernatant fluid, probably by the presence and nature of lytic or bactericidal substances of which we as yet know little, and in many other ways. As a result each region of the body has developed a highly distinctive flora—on the *skin*, staphylococci and diphtheroid bacilli; in the *mouth*, *pharynx* and *tonsillar region*, a wide variety of bacteria including streptococci of the viridans or indifferent type, Gram-negative cocci of the *Neisseria pharyngis* type, frequently hæmophilic bacilli of the influenza type and certain types of diphtheroid bacilli, less frequently pneumococci, and sometimes hæmolytic streptococci, together with a variety of other organisms of which our knowledge is very limited, because they fail to grow on our usual culture media; in the *stomach*, owing to the high acidity of the gastric juice, there appears to be no normal bacterial flora; in the *duodenum*, mainly Gram-positive cocci, giving place to more numerous cocci and coliform bacilli in the *jejunum*, and to a rich and varied flora of aerobic and anaerobic species in the *lower intestinal segments*; in the *nasal cavities*, a relatively scanty and restricted flora, consisting mainly of diphtheroid bacilli and white staphylococci, with infrequent streptococci, Gram-negative cocci and hæmophilic bacilli; in the *vagina*, a highly distinctive flora consisting almost entirely of aciduric bacilli of the Döderlein type; and so on.

The intestinal flora varies rather widely with food, and so with age (see Tissier 1900, Cruickshank 1925, 1928, Dudgeon 1926, Rettger and Cheplin 1921). But with this exception the characteristic bacterial flora of the body appears to be established soon after birth. Döderlein's bacillus becomes established in the vagina within a day or so. Streptococci of the viridans type appear in the mouth and throat, and staphylococci on the skin. There is little doubt that these bacteria are passed to the infant directly from the mother, or from other persons in its immediate entourage, and take up their characteristic positions in virtue of their adaptation to the particular environment that they there encounter.

Gundel and Schwarz (1932) have recently carried out an interesting study into this problem. They examined 426 nasopharyngeal swabs from 51 mothers, 645 swabs from their new-born infants, and 66 swabs from nurses in attendance. In accord with earlier observers they found that the nasopharynx was sterile at birth; but in the first 2 days it became colonized by the usual nonhæmolytic streptococci, Gram-negative cocci of the pharyngeal type, occasional diphtheroid bacilli, and a certain number of coliform bacilli. By the 2nd or 3rd day pneumococci and influenza bacilli were often present, and their frequency was found to depend on their distribution among the adults in immediate attendance on the child, and particularly on their presence or absence in the mother. By serological typing it was, in many cases, possible to demonstrate the identity of the strain of pneumococcus isolated from the mother and the child.

In recent years several groups of investigators have undertaken systematic studies of the nasopharyngeal flora of samples of the normal population in the course of which the same persons have been repeatedly swabbed during months or years (see, for instance, Noble, Fisher and Brainard 1928, Blackburn *et al.* 1930, Gundel and Linden 1931, Gundel and Schwarz 1932). These have revealed a striking tendency for any given individual to maintain a characteristic flora over considerable periods of time; and recent improvements in technique have made it possible to establish this point with added certainty and precision.

Thus Gundel and Schwarz (1932), using antisera corresponding to the 29 new types of pneumococci identified by Cooper (see Cooper, Edwards and Rosenstein 1929, Park 1930), were able to show that any one person tended to carry a single serological type of pneumococcus over a period of at least several months. Thus, one subject yielded a Type VI pneumococcus in May, June, July, August, September and October 1931, another a Type XVIII pneumococcus in May, June, July, September and October, and so on. Unpublished observations by Lovell and Straker have yielded results of the same kind. The nasopharyngeal flora of any given person changes in certain of its characters if observations are carried out over a sufficiently long period of time—influenza bacilli may be carried for months and then disappear, or be so reduced in frequency that their presence is not detected by the method employed; one type of pneumococcus may give place to another; meningococci may be present for months or years and then be lost, and so on—but over any short period there is a very definite tendency for each individual to cultivate a flora that, in certain particulars, is peculiar to himself.

Whatever the complex of determining factors may be, it is quite clear that there is a considerable local resistance to the colonization of any stray invaders from the outside world.

The Significance of the Bactericidal or Inhibitory Mechanisms Operating on Body Surfaces.

It is extremely difficult to assess the relative protective value of the factors that we have described above, as compared with the mechanisms that we shall discuss in subsequent chapters. It is obvious that they often fail; but how frequently they succeed we cannot tell.

That they are of real importance can easily be shown experimentally by noting the different doses of bacteria that are required to induce experimental infection by different routes.

To take a single example from many available, the dose of *Bact. aertrycke* that will, on the average, lead to a fatal infection when injected intraperitoneally into a mouse of 18 — 22 gms. is somewhere between 1,000 and 1,000,000 times smaller than the dose that will lead to the same result when administered by the mouth. A series of observed figures for groups of 85 mice per dose (intraperitoneal injection) and of 75 mice per dose (*per os* infection) are given in Table VIII.

TABLE VIII

SHOWING THE PERCENTAGE MORTALITIES OBSERVED IN GROUPS OF MICE AFTER THE ADMINISTRATION OF VARIOUS DOSES OF A VIRULENT STRAIN OF *Bact. aertrycke* INTRAPERITONEALLY OR *per os*.

Number of Bacilli administered.	Mortality per cent.	
	Intraperitoneal.	<i>Per os</i> .
1,000,000,000	Not tested	48.75
10,000,000	98.8	28.25
100,000	84.7	22.50
1,000	65.9	15.00
10	28.2	Not tested

SUMMARY

There are a series of mechanisms, concerning the exact nature of which we as yet know relatively little, that tend toward the mechanical removal or local destruction of most of those bacteria that reach the various body surfaces from the outside world. These mechanisms vary in nature from one locality to another, each body surface—the skin, the conjunctivæ, the mucous membranes of the nose, mouth, pharynx, intestinal tract, genital tract and so on—tends to develop and maintain its own characteristic bacterial flora, the flora of any one locality differing both qualitatively and quantitatively from that of another, and the flora of a particular locality (say the nasopharynx) showing minor but significant differences as we pass from one person to another, or study a single person over a prolonged period of time.

The resistance thus afforded to colonization of bacterial immigrants is certainly an important factor in the body's first line of defence, but its effectiveness in relation to any particular natural infection, or under any specified set

of conditions, cannot at present be estimated with any accuracy. We know only that this barrier is, in fact, often penetrated, and that the fate of the host then depends on the mechanisms of antibacterial or antitoxic immunity that we shall consider in subsequent chapters.

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CHAPTER V

ANTIGENS AND ANTIBODIES

WHEN a man or animal contracts an infectious disease, substances make their appearance in the blood serum that react in the test-tube with the organism that is causing the infection, or with its products. They may cause the clumping, or agglutination, of a uniformly dispersed suspension of bacteria; they may kill them, often with a partial disruption of the bacterial cells that we refer to as *lysis*; they may alter them so that they are more easily taken up by phagocytes; they may form a precipitate when added to an extract of the bacterial cells; they may neutralize a bacterial toxin; and so on.

Substances of this kind can be caused to appear in the serum by the injection into the tissues of materials of the most diverse kind: bacteria, living or dead; bacterial toxins or extracts; foreign cells, such as the red blood corpuscles of some other species; foreign proteins such as egg albumin, or the blood serum of some other animal, or a vegetable protein. The substances that appear in the serum are called *antibodies*; the substances that induce their formation, and react with them when formed, are called *antigens*.

Since these antigen-antibody reactions play a large part in the defence mechanism of the host we must consider them in some detail before describing what happens when bacteria gain access to the tissues.

For our immediate purpose it will suffice to sketch our present conceptions of the processes involved, stressing those points that seem significant from our restricted view-point, and ignoring in large part many subsidiary problems that have, from time to time, loomed large in immunological controversy.

We must start with a more precise definition of our terms.

Definitions and Terminology.

The two essential reagents may be briefly defined as follows:

An ANTIGEN is any substance that, when introduced parenterally into the animal tissues, stimulates the production of an antibody, and, when mixed with that antibody, reacts with it in some observable way.

An ANTIBODY is any substance that makes its appearance in the blood serum or body fluids of an animal in response to the stimulus provided by the parenteral introduction of an antigen into the tissues and, when mixed with that antigen, reacts with it in some observable way.

The term *parenteral* is introduced into this definition to emphasize the fact that the antigen must reach the tissues in an unaltered state, without pre-

liminary disintegration by those digestive fluids which it would meet in the alimentary canal.

It will be noted that an antigen has two essential attributes. It must react with the specific antibody—the antibody produced in response to its introduction into the tissues—and it must have the power of stimulating the production of that antibody. We shall note the existence of substances which react specifically with a given antibody without possessing the power of stimulating its formation. Such substances are not true and complete antigens, though they are derived from them and retain the chemical groupings on which their specificity depends.

An antibody, as defined, has also two attributes—that of specific reaction with the antigen and that of being produced in response to the stimulus provided by the access of that antigen to the tissues. In practice it is not customary to insist on the demonstration of the second attribute. There are numerous cases in which we find in the blood of normal men or animals substances that react with various foreign cells or bacteria in ways that are essentially similar to those observed when we mix an antigen with a specific antibody that has been produced by artificial immunization, or by an attack of an infective disease. It is customary to refer to these substances as “*natural*” or “*normal*” antibodies. The problem of their origin is discussed in Chapter X.

In addition to deciding upon a generic term for each of the two reagents involved in an antigen-antibody reaction, it soon became necessary to name in some way the particular substances that were assumed to be involved in the different reactions which were from time to time observed. Knowing little or nothing of their real nature, it was natural to adopt the plan of naming them according to what they did, instead of according to what they were. Names were therefore given to the antibodies describing the reaction that was observed when each was mixed with its corresponding antigen, the suffix *-in* being added to the descriptive term. An antibody that gave a precipitate when mixed with a soluble antigen was a *precipitin*; an antibody that caused the agglutination of a suspension of cells or bacteria was an *agglutinin*; an antibody that caused lysis was a *lysin*—a *hæmolysin* if it acted upon red blood corpuscles, a *bacteriolysin* if it acted upon bacteria; an antibody that rendered a bacterial or other cell more liable to phagocytosis was an *opsonin*; and so on.

The actual material, organized or unorganized, with which these antibodies reacted was in general provided with some well-recognized name; so that one could speak of bacteria, or of red blood corpuscles, or of crude protein solutions, such as horse serum or egg white, as antigens. But it was clearly recognized that such materials were highly complex, and that the antigenic property was almost certainly confined to some particular part of the material in question. It thus became necessary to use a term, derived from the name of the antibody, to denote the essential part of the crude material which acted as a stimulus to antibody formation and reacted with the antibody when formed. This was accomplished by adding the suffix *-ogen* to the name of the antibody.

Thus bacteria, when injected into the tissues, act as antigens and stimulate the production of agglutinins; but the particular part of the bacterial substance which provides the specific stimulus is spoken of as an *agglutino-gen*, and one bacterium may possess several different agglutinogens (see Chapter VI). This nomenclature still has its uses, but it may be misleading. We shall see that one and the same antibody may act as an agglutinin, a lysin or an opsonin; the corresponding antigen thus functioning as an agglutino-gen, a precipitino-gen or an opsonino-gen—though it happens that the last of these terms has never found its way into current use. It is sometimes convenient to give a thing different names according to the particular function it is fulfilling at the moment, and for this reason the terms agglutinin, precipitin, etc., will probably be retained; but the tendency is to use the term *antigen* indifferently for each active constituent of a crude antigenic material, and to let the context make clear the particular reaction that is being studied.

These general rules of nomenclature have certain exceptions. In all the examples we have considered above it is a possible view, though one-sided and misleading, to regard the antibody as the active agent, as doing something to the antigen. But there is a group of antigenic substances—the bacterial and vegetable toxins—which do something dramatic to the animals to whose tissues they gain access. If the main effect of the antibody is to neutralize this toxic effect, it is named by placing the prefix *anti-* before the name of the substance whose activity it neutralizes. Thus we speak of the antibodies that neutralize the bacterial toxins as *antitoxins*; those that neutralize certain vegetable poisons, such as abrin or ricin, as *antiabrin*, *antiricin*, etc.

The controversies with regard to the nature of the antigen-antibody reactions have centred largely round quantitative phenomena, and it will be more convenient to discuss the views of such pioneers as Ehrlich, Bordet, and Arrhenius and Madsen after we have considered each kind of reaction in turn, and noted the quantitative data that have to be allowed for in any general theory. There is, however, one aspect of the antigen-antibody reactions that is beyond question—their striking specificity—and, since the work of recent years has made it quite clear on what this specificity depends, it will aid discussion if we abandon any attempt at the historical method of approach and deal at once with our present knowledge of the qualitative chemical factors concerned. We can then consider the various antigen-antibody reactions with this cardinal point clear in our minds.

THE CHEMICAL NATURE OF ANTIGENS

We are concerned here with antigens in the purest state in which we can obtain them, and we shall, in general, be dealing with substances in solution. The *in vitro* reaction that enables us most easily to detect an antigen in this state is its capacity for forming a precipitate when mixed, under suitable conditions, with a serum containing the corresponding antibody. When it has been shown that a particular substance reacts specifically with its corre-

sponding antibody in the test-tube, we have still to determine whether it has the power of inducing the formation of that antibody when injected into the tissues of a suitable laboratory animal—whether it is an antigen in the full sense.

As the result of very extensive studies along these lines it would appear that, with a few possible exceptions to be noted later, all substances that are fully antigenic are proteins or have a protein component.

The study of native proteins, in the purest form available, has provided strong support for the view that immunological specificity is determined by chemical structure.

Thus, we can differentiate by immunological tests between serum albumin and serum globulin (Dale and Hartley 1916), between the egg albumin of the duck and of the hen (Dakin and Dale 1919), between the hæmoglobins of different animal species (Higashi 1922, Landsteiner and Heidelberger 1923, Hektoen and Schulhof 1923), and between different vegetable proteins (Wells and Osborne 1911, Wells 1915, Jones and Gersdorff 1923, Lewis and Wells 1925, Wells *et al.* 1927).

We know also that altering the complex protein molecule by heat, by racemization, or by hydrolysis, soon changes its antigenic behaviour; though the exact relations between chemical and immunological changes are, under these conditions, very difficult to define (see Hartley 1931, Wells 1929).

Far more significant information has been gained by altering the chemical structure of proteins along certain limited and well-defined lines, and noting the resulting changes in their immunological reactions.

Obermayer and Pick (1906) (see also Pick 1912) showed that the nitration or halogenation of proteins—that is the introduction of the nitro-group or of a halogen element such as iodine—profoundly altered the antigenic reactions of the treated protein. Serum proteins so treated lost their species specificity, but they gained a new specificity, shared by normally unrelated serum proteins that had been chemically altered by the same procedure. Thus an antiserum prepared against the nitrated serum of a particular animal species failed to react with the unaltered serum of that species, but reacted with a wide range of nitrated sera. Since it was known that the nitro-group and the halogen elements entered into the benzene ring of certain of the amino-acids which build up the complex protein molecule, Obermayer and Pick were led to attach particular importance to these chemical groupings as factors determining immunological specificity. Wormall (1930) has recently carried out further studies along these lines.

Landsteiner and his colleagues, however, have shown that the salt-forming groups of the amino-acids (the carboxyl-, hydroxyl- and amino-groupings) play an equally important part. By esterification, methylation and acetylation, they have succeeded in altering the immunological specificity of proteins (see Landsteiner and Prázek 1914, Landsteiner and Lampl 1917*a*, Landsteiner 1917).

All the above procedures have this in common, they start with a protein that has a native specificity and seek to alter it by making particular changes

in its chemical structure. More recently Landsteiner and his colleagues have approached the problem along different lines. Starting with substances of known and relatively simple composition, they have succeeded in building up synthetic antigens, the immunological specificity of which is in part determined by known chemical groupings.

The general method employed has been to prepare diazo-compounds of the simple substances under test, and to utilize the diazo-reaction as a means of linking them to some convenient protein molecule, usually to the protein of horse serum, chicken serum, etc. In any one experiment the diazo-compound under test is linked to two immunologically unrelated proteins. One synthetic antigen is injected into a suitable animal to prepare the antiserum. The other synthetic antigen is used to test the reactions of the serum *in vitro*. Since the protein components of the two antigens are immunologically distinct, the reactions which occur must be due to the attached grouping common to both. In this way it has been possible to prepare antisera that give specific precipitation with synthetic antigens in which the active grouping consists of such substances as metanilic acid, para-arsanilic acid, lævo-, dextro- and meso-tartaric acid, glucosides or galactosides (see Landsteiner and Lampl 1917b, Landsteiner 1918, 1920, 1930, Landsteiner and van der Scheer 1928, 1929, Landsteiner and Levine 1930, Avery and Goebel 1929). These results have afforded a clear-cut demonstration of an immunological specificity dependent on differences in chemical structure of the kind that determine the ordinary interactions between organic compounds; and it will be noted that the differences between the lævo- and dextro-isomers of an optically active compound such as tartaric acid are sufficient to confer specificity in the immunological sense.

To obtain precipitation Landsteiner found it necessary, in most cases, to use a complete synthetic antigen. The serum prepared against a metanilic-acid-horse-serum antigen, for instance, gave a precipitate with a metanilic-acid-chicken-serum antigen, but not with metanilic acid alone. This relation he found to be a general one, but he also found that the relatively simple substances prepared as a preliminary to linkage with protein would unite specifically with the antibody present in the antiserum, and so inhibit the precipitation of any complete synthetic antigen containing the same reactive grouping. Marrack and Smith (1932) have recently demonstrated, by an ingenious colorimetric technique, the direct union between certain azo-dyes and the antibodies produced by the inoculation of the corresponding synthetic antigens.

Landsteiner was thus led to formulate the conception of the partial antigen—the *haptén*, as he calls it—the part of the antigenic complex that carries the specific reacting group. These haptens, although they are not capable of giving rise to the formation of the corresponding antibodies, will combine specifically with the antibodies that are produced in response to the injection of the complete antigens.

We may note here that many substances—and particularly many of the polysaccharides that have been separated from bacterial cells—are hapten-like in failing to stimulate antibody formation *in vivo*, but differ from the simpler

haptens of Landsteiner in that they give specific precipitation in the test-tube when mixed with the corresponding anti-serum. It is probable that this precipitating capacity is a function of molecular size and complexity. Landsteiner and van der Scheer (1932) have recently shown that certain relatively simple substances, such as the azo-dyes prepared from *p*-aminosuccinanilic acid, *p*-aminoadipanic acid and *p*-aminosuberanic acid, will give specific precipitation with the corresponding antisera without previous linkage to protein, and that the strength of the reaction with these compounds appears to run parallel to the length of the aliphatic chain.

We might thus represent our present conception of a complete antigen and of a separated hapten as follows, endowing our antigen, for simplicity, with a single hapten grouping.

- | | | |
|------------------|---|---|
| Complete Antigen | { | Hapten—confers immunological specificity. |
| | | Protein Component—confers power of stimulating antibody formation; and, with simple haptens, the power of forming a precipitate with antibody in the test-tube. |
- Simple Hapten—has no power of stimulating antibody formation; but unites with antibody forming a compound that remains in solution.
- Complex Hapten—has no power of stimulating antibody formation; but forms a precipitate with antibody in the test-tube.

Following Landsteiner and others, we have regarded all haptens as devoid of the power of stimulating antibody formation; but observations have recently been recorded which suggest that certain of the more complex haptens may act as antigens under exceptional conditions. Thus, the polysaccharide fractions that have been separated from various types of pneumococci, when tested by the ordinary methods of inoculation into laboratory animals, have no antigenic action; but it has been reported that these haptens, when injected into the skin of human subjects in very small doses, may lead to the formation of specific antibodies (see Francis and Tillett 1930, Finland and Sutliff 1931, 1932, Zozaya and Clark 1932).

It is possible that polysaccharides owe such antigenic power as they possess to the same factor that apparently determines their capacity for precipitating the antibody *in vitro*, that is, to their structural complexity and high molecular weight.

That the state of dispersion of an antigenic material or compound may have some connection with the stimulation of antibody production in the tissues is suggested by reports (Zozaya 1932, Zozaya and Clark 1933) that the injection into animals of polysaccharides adsorbed on to collodion particles, charcoal, aluminium hydroxide or casein, may be followed by the appearance of specific antibodies in the blood.

It seems possible, then, that the protein which bears the specific hapten grouping may be acting as a physical rather than as a chemical agent, presenting the hapten to the tissues in a form which stimulates the antibody-producing mechanism, and that its place could be taken by any other component having similar physical properties; but our evidence is as yet much too scanty to justify any definite conclusion on this intriguing problem.

THE CHEMICAL NATURE OF ANTIBODIES

Of the chemical nature of the serum antibodies we know as yet very little beyond the fact that they are closely associated with the serum proteins, and particularly with the pseudoglobulin and euglobulin fractions as obtained

by any of the usual methods of fractional precipitation (see Hartley 1931, Wells 1929).

Certain observations, such as those of Landsteiner and Jägle (1903), Muir (1903), Bail and Tsuda (1909), Spät (1910), Kosaki (1918), Huntoon and his colleagues (1921*a, b*) and Locke and Hirsch (1925) have shown that antibodies may be dissociated from an antigen-antibody complex, giving an almost protein-free solution. (See also Frankel and Olitzki 1930, Olitzki and Frankel 1931, Frankel 1932, Olitzki 1932.) But, as Eagle (1930) has pointed out, the amount of antibody required for sensitization may well be too small to be detectable by the usual tests for proteins.

We may assume for our present purposes that antibodies either *are* globulins or are associated so closely with them that their behaviour is in large part determined by that of the globulin molecule. Marrack and Smith (1930), for instance, have recently shown that diphtheria antitoxin behaves as though it were identical with the pseudoglobulin of the serum, except for its specific affinity for diphtheria toxin.

We may regard any antigen-antibody reaction as involving a union between a hapten group borne by the antigen and a specific combining group borne by the antibody. The result of this union is to anchor to the antigen a molecule, or particle, of serum globulin, to which the reactive group of the antibody is attached. The antigen itself may be in solution—usually colloidal solution—or it may be attached to a cell or to cell debris. Any one kind of cell, such as a bacterium, may of course possess a variety of haptens or antigens; this indeed is almost always the case, as we shall see in the next chapter. For the moment, however, we are not concerned with complexities of antigenic structure but with the general nature of the reactions between antigens and antibodies and we may pass to the consideration of the various *in vitro* reactions that have been studied.

THE "IN VITRO" ANTIGEN-ANTIBODY REACTIONS

It will help to clear the issues if we ignore the sequence in which our knowledge has been gained and deal first with the precipitin reaction in which both antigen and antibody are in solution. We shall thus avoid the complications that are introduced when the antigen is attached to bacterial or other cells.

The Precipitin Reaction.

This reaction was first described by Kraus (1897) who used as his antigenic reagents filtrates of bacterial cultures. Since then it has been applied to a wide variety of antigens and antibodies.

It was, perhaps, a natural outcome of our terminology that we should think of the antigen as being flocculated by the precipitin of the antiserum, just as we think of bacteria as being flocculated by agglutinin; and, in a sense, this is a true picture. But, as we now know, by far the greater part of the precipitate comes from the antiserum, so that it would be a closer approximation

to the facts if we thought of the antigen as precipitating the antibody-containing globulin. Welsh and Chapman (1906), who emphasized the wide discrepancy between the amount of antigen and antibody in certain precipitating mixtures, put forward the view that the precipitate is composed almost entirely of antibody; but this is certainly not the case. Recent careful quantitative work indicates that the precipitate is composed of antigen and antibody in proportions that vary according to the proportions originally present in the reacting mixture.

Heidelberger and Kendall (1929) have studied this problem, using as antigen a purified sample of the polysaccharide hapten isolated from Type III pneumococci and as antibody a solution of antibody-containing globulin obtained from a Type III antipneumococcal serum. In their tests they kept constant the amount of antiserum, and varied the amount of antigen. By determining the total nitrogen in the antibody solution, and in the supernatant fluid after precipitation was complete, they were able to estimate by difference the weight of antibody-globulin contained in the precipitate. The supernatant fluids were tested for residual antigen and antibody by adding one or other reagent and noting whether any further precipitation occurred. The weight of antigen added to each tube was known, so that the proportions by weight could be calculated in those tubes in which no residual antigen could be detected in the supernatant fluid. The tube to which the smallest amount of antigen was added, and in which an excess of antibody remained in the supernatant fluid, gave a precipitate with an antigen-antibody ratio of 1 : 125. In a neighbouring tube the ratio was 1 : 106. In the tube that showed the first trace of residual antigen in the supernatant fluid it was 1 : 69. Marrack and Smith (1931b) record somewhat similar results. As antigens they employed azo-proteins and iodo-proteins prepared from purified horse-pseudoglobulin and crystalline egg albumin. Antisera were prepared by the immunization of rabbits. In the precipitin tests the amount of antiserum was kept constant and the amount of antigen was varied. The amount of azo-protein antigen in the precipitates was determined spectrometrically, the amount of iodo-protein by the estimation of iodine. The antigen : antibody ratio in the precipitates varied according to the ratio in the reacting system. When this contained 1 part of antigen to 6 parts of antiserum the antigen nitrogen in the precipitate was 24 per cent. When 9 parts of antigen were mixed with 1 part of antibody the antigen nitrogen in the precipitate rose to 68 per cent. (see also Marrack and Smith 1931a). The experiments of Dean and Webb (1926), which are discussed in detail later, also indicate quite clearly that the antigen contributes an appreciable fraction to the precipitate, and that the ratio of antigen and antibody in the precipitate varies with the ratio in the reacting mixture, at least in the region of antigen excess. Certain results obtained by the titration of the residual antibody in the supernatant fluid of those tubes in which antiserum was present in excess, suggested that, in this region, antigen and antibody were combining in constant proportions, a finding that differs from those of Heidelberger and Kendall and of Marrack and Smith.

We may picture a precipitin reaction as occurring in at least two stages. In the first stage the antigen unites with the antibody globulin, the hapten groupings of the former combining with the specific reacting groupings of the latter. The balance of evidence at present available suggests that union may occur in varying proportions, compounds of the general formula $A_m G_n$ being formed, where A represents antibody and G represents antigen (see Marrack and Smith 1931a, b). We should picture the antibody, and in most cases the antigen, as having very numerous reacting groupings attached to each molecule. In the second stage, the antigen-antibody compound is flocculated by the electrolytes of the saline in which it is suspended.

Since the antigen and antibody are each soluble in saline some change must be associated with the formation of the antigen-antibody compound that renders it salt sensitive.

It is usually held, and there seem good reasons for this view, that the essential change occurs in the antibody globulin—at least in those antigen-antibody compounds in which the antibody is in great excess.

In this connection some experiments carried out by Hartley (1925) are of considerable interest. Various sera were dried, and a portion of each dried serum was thoroughly extracted with ether. These dried sera—extracted and non-extracted—were redissolved in saline and injected into rabbits. It was found that extraction with ether had not appreciably lessened their antigenic value as judged by their power to stimulate the formation of antibodies. The antisera so prepared were dried, and a portion of each was thoroughly extracted with ether. All these dried reagents were dissolved in saline, and precipitin tests were put up in which each was tested against the other. The standard of comparison was supplied by unextracted antigen reacting with unextracted antiserum. No decrease in the strength of the reaction was noted when ether-extracted antigen was mixed with unextracted antiserum. When ether-extracted antiserum was mixed with unextracted antigen the reaction was diminished—the titre of the serum was lowered. When ether-extracted antiserum was mixed with ether-extracted antigen, no precipitation occurred. It would appear, then, that the precipitability of the antigen-antibody complex—its tendency to become predominantly lyophobic—depends on the presence of some ether-soluble constituent.

The actual flocculation has been shown to depend on the presence of electrolytes. The action of electrolytes was, however, first studied in connection with agglutination, and may be more conveniently discussed under that head. As would be expected, flocculation is hastened by those procedures that increase the frequency of impacts between the particles of antigen-antibody compound—by diminishing the total volume of fluid, and so reducing the space between the particles; by shaking; or by the agitation due to convection currents in a tube partially immersed in a water-bath. It is also hastened by increasing the concentration of electrolyte up to an optimal point, beyond which further increase may cause a retardation. An increase in temperature up to an optimum also increases the speed of flocculation; and this effect does not seem to be entirely due to an increase in the frequency of impacts (see Eagle 1932). With some reacting systems water-bath temperatures (37° – 55° C.) may be found unsuitable for precipitation tests, since the antigen-antibody complex may be partly soluble within this range.

The most obvious method of titrating the strength of a precipitating antiserum is to add progressive dilutions of the serum to a constant amount of antigen, and note the highest dilution that still gives a precipitate. This technique has not proved satisfactory, since the titres recorded have been relatively low. This is due to the fact that the bulk of precipitable material usually comes from the antiserum itself; so that, with increasing dilutions, the end-point of visible precipitation is soon reached. It was noticed by many of the earlier workers that the antigen might often be diluted to a far greater extent than the antiserum and still give a copious precipitate. With

some systems, for instance, an antigen diluted many thousand times will give good flocculation with a 1:20 – 1:50 dilution of a serum that ceases to give a visible precipitate, with any amount of antigen, when diluted to 1:100. This led to the practice of keeping the amount of serum constant, often at a dilution of about 1:20 – 1:40, and varying the dilution of the antigen. It was found that different sera did, indeed, give different end-points when titrated in this way; but the rationale of the procedure was never very clear, and there was no obvious way of estimating the relative potency of two sera, one of which, at a dilution of say 1:40 gave a precipitate with a specimen of antigen diluted to 1:500, while the other, at the same dilution, gave a precipitate with an antigen diluted to 1:5,000.

This unsatisfactory state of affairs was resolved by the studies of Dean and Webb (1926). The antigen they employed was horse serum, and their antisera were prepared in rabbits. They noted, not the end-point of flocculation, but the tube of a graded series in which flocculation first appeared. They kept the amount of antiserum constant, and varied the amount of antigen, the total amount of fluid in each tube being, of course, kept constant by adding the required amount of saline. Table IX illustrates their results. It shows the state of affairs 32 minutes from the start of an experiment in which a 1:20 dilution of a particular antiserum was titrated against increasing dilutions of antigen.

TABLE IX

SHOWING THE STATE OF REACTING MIXTURES 32 MINUTES AFTER MIXING A 1:20 DILUTION OF ANTISERUM WITH INCREASING DILUTIONS OF ANTIGEN (HORSE SERUM).

Dilution of Antigen.	Appearance of Mixture.
1:10	Clear.
1:20	Clear.
1:40	Trace of opalescence.
1:80	Opalescence.
1:160	Opalescence.
1:320	Turbid: small particles.
1:640	Turbid.
1:1,280	Opalescence.
1:2,560	Trace of opalescence.
1:5,120	Trace of opalescence.
1:10,240	Trace of opalescence.
1:20,480	Clear.

Here the process of flocculation is most advanced in the tube in which a 1:20 dilution of antiserum is reacting with an equal volume of a 1:320 dilution of antigen. The ratio of antigen to antibody in this tube is, in terms of the *amounts* of the two reagents, 1:16. Mixtures in which this ratio is departed from, in the direction either of antigen or of antibody excess, flocculate more slowly. Great excess of either reagent results in a very marked retardation. By titrations in which the dilutions of antigen were more closely spaced—the amounts of antigen in successive tubes forming an arithmetical instead of a geometrical series—Dean and Webb were able to determine the ratio giving

most rapid flocculation to within a very narrow margin of experimental error.

When titrations of this kind were carried out with different dilutions of the same antiserum, it was found that the ratio giving first flocculation was constant for all dilutions of antiserum. If a 1 : 20 dilution of antiserum flocculated most rapidly with a 1 : 320 dilution of antigen, then a 1 : 40 dilution of antiserum flocculated most rapidly with a 1 : 640 dilution of antigen, a 1 : 10 dilution with a 1 : 160 dilution of antigen, and so on.

Dean and Webb applied to this most-rapidly-flocculating antigen : antibody ratio the term *optimal proportions*. Tests for residual antigen and antibody in the supernatant fluids, after precipitation had occurred, led them to the conclusion that the *optimal proportions* were identical with the *equivalent proportions*; i.e., that most rapid flocculation occurred in the mixture in which antigen and antibody were present in such amounts that none of either reagent remained uncombined, or that a trace of each reagent remained detectable in the supernatant fluid.

This obviously afforded an admirable method for determining the antibody content of any specimen of antiserum, or the antigen content of any antigen-containing material. Dean and Webb titrated by this method 33 specimens of anti-horse precipitating sera prepared in rabbits and found that the optimal antigen : antibody ratio varied from 1 : 177 to 1 : 14. It will be noted that the serum with the lowest ratio has the highest antibody content. A ratio of 1 : 177 indicates that 1 part by volume of antigen (horse serum) combines with 177 parts by volume of antiserum. A ratio of 1 : 14 indicates that 1 part by volume of antigen combines with 14 parts by volume of antiserum. The second serum thus has an antibody concentration about 12·6 times that of the first. The correctness of this comparative titration does not, it will be noted, depend on the correspondence of the optimal ratio with chemical equivalence of the reacting substances. Provided that the optimal ratio bears a constant relation to the equivalent ratio, the optimal ratios of any two antisera will give a true measure of their relative antibody content.

That the method of Dean and Webb does in fact allow titrations of antigen or antibody content to be carried out with a high degree of accuracy is now quite clear. Taylor, Adair and Adair (1932) have, for instance, recorded studies with two purified antigens—crystalline egg albumin and horse-serum globulin—in which they have used antisera prepared against these antigens to estimate the percentage of egg albumin in egg white, and of globulin in horse serum. Their results are in close agreement with previous determinations by accepted methods of chemical analysis. Thus they record the following comparative figures.

Percentage of crystalline egg albumin in egg white :						Per cent.
Hopkins (1900)	.	.	.	.	.	6·0
Wu and Ling (1927)	.	.	.	.	.	8·5
Optimal-proportions method	.	.	.	.	.	7·29
Percentage of total globulin in horse serum :						Per cent.
Hammersten (1878)	.	.	.	.	.	4·57
Gibson and Banzhaf (1910)	.	.	.	.	.	4·07
Optimal-proportions method	.	.	.	.	.	4·46

In the optimal-proportions method as practised by Dean and Webb the amount of antiserum is held constant while the amount of antigen is varied. We might, then, refer to the antigen : antibody ratio determined by their technique as the *constant-antibody optimal ratio* or *constant-antibody optimum*.

Prior to these studies Ramon (1922) had shown that diphtheria antitoxin could be titrated by mixing falling amounts of antitoxin with a constant amount of toxin and noting which tube in such a series first showed flocculation. The fact that this test was introduced as a practical method of standardizing an important therapeutic reagent, and that controversy centred round its relation to the current *in vivo* methods of standardizing antitoxin, probably delayed the recognition of its wider significance. We might clearly refer to the antigen-antibody ratio in the first-flocculating tube of a Ramon titration as the *constant-antigen optimal ratio* or *constant-antigen optimum*.

What is the relation between the *constant-antibody optimum* and the *constant-antigen optimum*? The obvious expectation would be to find them identical. Dean and Webb concluded from their data that the *constant-antibody optimum* corresponded closely to the ratio of chemical equivalence. Ramon was able to show that the *constant-antigen optimum* corresponded closely to the neutralization of toxin by antitoxin. But in fact the two optimal ratios may be very different. Duncan (1932), working with a crude polysaccharide from a yeast-like fungus and the corresponding antiserum, records a constant-antibody ratio of 1 : 8 and a constant-antigen ratio of 1 : 64, the latter corresponding to a mixture containing a proportion of antiserum eight times as great as the former. Taylor (1933) notes, in the case of a horse-serum antihorse-serum system, that the proportion of serum at the constant-antigen optimum may be more than three times as great as at the constant-antibody optimum. In the case of crystalline egg albumin and its corresponding antiserum—a system that lends itself far more easily to exact quantitative study since the antigen has been purified—the divergence is smaller, but still appreciable. Ten antisera, tested by both methods, showed a mean value for the quotient $\frac{\text{serum proportion at constant-antigen optimum}}{\text{serum proportion at constant-antibody optimum}}$ of 1.65, and the individual values did not fluctuate widely about the mean, indicating that the relation of one ratio to the other is approximately constant for different antisera.

This rather curious relation may be expressed as follows. For any given quantity of antiserum there is an amount of antigen that gives most rapid flocculation; but this amount of antigen will flocculate still more rapidly in the presence of a larger amount of antiserum. If the amount of antiserum is still further increased a new optimum will be reached, beyond which the retarding effect of antibody excess will again come into play. It will be noted that the constant-antigen optimum always corresponds to a region of antibody excess as compared with the constant-antibody optimum.

Adopting a suggestion recently advanced by Miles (1933) and the views of Marrack and Smith in regard to the occurrence of antigen-antibody union in varying proportion, we may illustrate the position by a simple scheme representing the kind of thing that *might* happen, on the assumption that antigens and antibodies have each several combining groups. Let us, for simplicity, endow a hypothetical antigen and antibody with three combining groups

apiece. Suppose we take 6 molecules of antibody and add to them 6 molecules of antigen. We should have 6 molecules of the form AG , where A represents antibody and G represents antigen. Suppose we keep the amount of antibody constant at 6 molecules, but increase or decrease the amount of antigen. When we increase it, adding say 12 or 18 or more molecules we cannot increase the number of AG molecules—each compound molecule must have at least one A and there are only 6 A 's available—but we might alter the type of compound formed. Perhaps when we added 12 G molecules we should get 6 molecules of the form AG_2 , and with 18 G molecules 6 of the form AG_3 , a proportion of the combining groups on the G molecules remaining unsatisfied. Actually, if this or anything like it happened, we should probably have to add a considerable excess of G to get compound molecules of the form AG_n , so that there would be some G remaining in the supernatant fluid. When we decrease the antigen, say to 3, 2 and 1 molecules, we *must* decrease the number of AG molecules. If every A insists on having its combining groups satisfied we should get successively 3, 2 and 1 molecules of the form AG with 3, 4 and 5 A molecules free in the surrounding fluid. If this is not so, the constitution as well as the number of molecules may change. We may get 3 molecules of the form A_2G or 2 of the form A_3G . With 1 molecule of G we must have 3 A uncombined, since we have only given G three combining groups. Actually, again, we should probably always have some A in the supernatant fluid when the antigen-antibody compound had the form A_nG .

Now suppose that the condition $6AG$ represents the constant-antibody optimum. This must mean that molecules of the form AG_n are less flocculable than molecules of the form AG , since the number of compound molecules, and hence their frequency of impact, will be constant. It does not, however, follow that molecules of the form A_2G or A_3G , if these exist, are less flocculable than those of the form AG . There will be fewer A_2G or A_3G molecules per unit volume as we decrease the amount of antigen, and the number of impacts per unit of time will be fewer. Flocculation might be slower even if the flocculability of the molecules were greater. Now suppose we take the amount of antigen (6 molecules) that gave us optimal flocculation with 6 molecules of antibody, and increase the amount of antibody till we reach the constant-antigen optimum. We cannot increase the number of antigen-antibody molecules—each must have at least one G , and there are only 6 G 's available—but we might alter the constitution of the molecules. We might get first $6A_2G$ and then $6A_3G$. If A_2G were more flocculable than AG , but A_3G less flocculable than A_2G , then 12 molecules of A added to 6 molecules of G would give us our constant-antigen optimum. It may be that one optimal ratio is determined by the *number* of compound molecules in unit volume, the other by their *constitution*.

Such a schematic description as this has only an illustrative value. It rests on no adequate evidence. Heidelberger and Kendall (1929) and Marrack and Smith (1931b) have, as we have seen, demonstrated varying proportions of antigen and antibody *in the precipitate as a whole*. Heidelberger and Kendall have put forward a tentative explanation of their results on the basis of reversible reactions leading to the formation of three different compounds of different solubility, the varying proportion of antigen and antibody in the precipitate being accounted for by assuming varying proportions of two of these com-

pounds. Marrack and Smith (1931*b*) indicate objections to this view and favour the conception of the union of antigen and antibody in varying proportions, so that not two or three but n different compounds of the general formula A_mG_n will be formed. It is this conception that we have adopted here, though we have for simplicity allocated only three combining groups to each reagent. The conception that the different optima may depend on the concentration of the AG compound in the one case, and on its constitution and flocculability in the other, is clearly adumbrated by Miles (1933) in his recent studies on agglutination. The simple illustration we have given—much too simple to be true—must be taken as defining a problem to be solved, not as an attempt at its solution. We shall see how complex the problem really is when we have considered the other types of antigen-antibody reaction.

One further point we may refer to here. Which of the optimal ratios, if either, corresponds to chemical equivalence of antigen and antibody? Dean and Webb (1926) believed, as we have seen, that the reagents were present in equivalent proportions at the constant-antibody optimum, basing their view on the results obtained in testing for antigen and antibody in the supernatant fluids. Taylor (1931, 1933) reaches the same conclusion on the same grounds, and Smith (1932), working with purified polysaccharide from Type I pneumococcus and a Type I antipneumococcal serum, has obtained similar results. Duncan (1932), working with the system referred to above, in which the antibody proportion at the constant-antigen optimum was eight times that at the constant-antibody optimum, found that tests on the supernatants showed a close correspondence between equivalence, so judged, and the constant-antibody optimum, while the constant-antigen optimum was in a zone of gross antibody excess. So far as the reactions given by supernatants can be relied on, the balance of evidence is in favour of the view that chemical equivalence corresponds closely with the constant-antibody optimum.

The particular case of the Ramon test, in which the constant-antigen optimum appears to coincide with the point of neutralization of toxin by antitoxin, loses much of its significance in view of the fact (see Miles 1933) that in these mixtures the constant-antibody and constant-antigen ratios also approximate closely.

This problem will present itself again in connection with the agglutination reaction.

AGGLUTINATION

The agglutination of bacteria was first recorded by Gruber and Durham (1896); but any foreign cells—yeasts and other fungi, red blood corpuscles, etc.—will stimulate the formation of agglutinins when injected into a suitable animal, and will be specifically flocculated in the test-tube under the influence of the antiserum so produced.

The reacting system differs from that of a precipitation reaction in that the antigen is part of the structure of an organized cell; and it is the behaviour of the sensitized cell, not that of the isolated antigen-antibody compound, that we observe. We can, if we choose, watch the formation of bacterial aggregates under the microscope; but for quantitative purposes the macroscopic method in which the flocculation of a bacterial suspension is observed by the naked eye or with the aid of a hand lens is greatly to be preferred.

The reactions that determine agglutination are essentially similar to those that determine flocculation. The antibody-containing globulin of the antiserum unites, through its specific combining groups, with the hapten groups

of an antigen situated at the cell surface, or on a cell appendage (see Chapter VI). This renders the cells salt-sensitive; and, in the presence of electrolytes, they cohere in masses and form visible flocculi.

It was in connection with the agglutination reaction that the rôle of electrolytes in antigen-antibody reactions was first established (see Bordet 1899, Joos 1901, Bechhold 1904, Porges 1906, Porges and Prantschoff 1906). In distilled water bacteria remain dispersed in the presence of an antiserum that would cause rapid agglutination in normal saline; and the separation of the cells by centrifugation, followed by tests on the deposited cells and on the supernatant fluid, shows that the antibody has united with the antigen, and that the sensitized cells undergo prompt agglutination when resuspended in saline.

The main function of the electrolyte is quite clear; it decreases the potential difference between the bacterial cells and the surrounding medium to below a critical value, which lies in the range 10–15 millivolts, and so allows them to come together and cohere (see Northrop and De Kruif (1922)). The exact nature of the reaction between the electrolyte and the antigen-antibody compound is, however, not so clear. Bacteria are known to carry a negative charge, but it is by no means certain that the neutralization of this charge by the positive ion of the electrolyte is the sole reaction that occurs. Joos (1901), for example, believes that the electrolyte enters into chemical union with the antigen-antibody compound; and Northrop and De Kruif (1922) describe a decrease in the cohesive force of bacterial cells under the influence of electrolytes, this change being inhibited by the addition of serum.

The rate of agglutination, as of precipitation, is influenced by any of those physical factors that tend to increase the frequency of impacts between the sensitized cells. Thus, it is increased by shaking, or by the presence of active convection currents in tubes partially immersed in a water-bath. It occurs more rapidly at high than at low temperatures; so that it is an advantage to incubate the tubes at the highest temperature that will not affect the essential reagents—usually 55° C. or thereabouts. Whether the temperature effect is entirely attributable to increased frequency of impact is very doubtful.

The estimation of the agglutinin content of any given antiserum differs, as a technical problem, from the estimation of precipitin content, in that the visible flocculi in an agglutination test are formed of aggregates of sensitized bacterial cells. When, therefore, the amount of bacterial suspension is held constant, and the amount of antiserum is decreased from tube to tube by progressive dilution, the amount of flocculable material is not changed. In practice this method has worked excellently; and it is the universal custom to measure the agglutinin content of a serum in this way, and to express the result as a titre—the highest dilution of serum that will give visible, or “standard” agglutination, under suitable conditions as regards time, temperature, etc. Dreyer and his colleagues (see Dreyer and Inman 1917) have emphasized the importance of standardizing the bacterial suspensions employed, if uniform results are to be obtained, and have suggested the use of agglutinin “units”—the reciprocal of the titre divided by a factor depending on the sensitiveness of the suspension—for recording the results; though a titre reduced by the appropriate factor would, of course, ensure the uniformity desired.

In such a reaction we are, it will be noted, working to an end-point—to the limit of dilution at which visible flocculation is obtained, and it is necessary

to arrange time and temperature accordingly. With the rapidity of flocculation we are not concerned, except in so far as it is convenient to hasten the attainment of the true end-point. At any temperature different agglutinating mixtures require different times of incubation, and these must be determined empirically. With some bacteria and some antisera, agglutination is usually complete in an hour or less at 55° C.: with others 4 hours may be required. With slowly agglutinating mixtures it is always wise to take a final reading after leaving the tubes in the water-bath overnight.

The optimal-proportions method is not commonly employed in determining the agglutinin content of an antiserum. It has, indeed, no obvious advantages for this particular purpose since it would appear that the constant-antibody ratios given by any two sera bear the same relation to each other as the end-points as determined by the ordinary titration method (Duncan 1932; see also Miles 1933). There are, however, obvious possibilities in the application of this method to the problems of antigenic analysis that we shall discuss in the next chapter.

The occurrence of pro-zones in agglutination tests—the failure of agglutination in the presence of low dilutions of a serum that gives well-marked agglutination when diluted more highly—has been noted by many workers. Heuer (1922) and da Costa Cruz (1929) have noted that this phenomenon is a general one when very light bacterial suspensions are titrated, in constant amount, against increasing dilutions of a bacterial serum, and have pointed out that flocculation first occurs in a tube somewhere towards the middle of a suitably graduated series. This, of course, corresponds to the constant-antigen optimum. Duncan (1932) and Miles (1933) have studied this question in the light of our present knowledge of the significance of optimal proportions in antigen-antibody reactions in general. In the case of one of the systems studied by Duncan the constant-antigen ratio was 1:21.33, the constant-antibody ratio 1:3.55, the former corresponding to a mixture containing six times as much antibody as the latter. The results as regards equivalence were interesting. The limit of antigen excess was taken as being represented by the tube in which agglutination just failed to be complete, so that a detectable number of dispersed or imperfectly agglutinated bacilli remained in the supernatant fluid. The tube so selected corresponded closely with the constant-antibody optimum. The examination of the supernatant fluid for residual agglutinin gave results that were superficially very curious. The equivalent tube, as judged by this test, corresponded more nearly to the constant-antigen than to the constant-antibody optimum, but the relation of the tube that showed the last trace of residual agglutinin to the tube that gave most rapid flocculation in a constant-antigen series differed according to the serum dilution with which the series was prepared, whereas the optimal ratios themselves remained constant, as in the precipitation reaction. Duncan was able to show that this apparent discrepancy was due to the technical difficulty of detecting small amounts of residual agglutinin and inclines to the view that the constant-antibody optimum probably approximates to the equivalent antigen-antibody ratio.

The Lysis of Bacteria and Other Cells.

Nuttall (1888) noted that the defibrinated blood of certain animals had the power of killing certain bacteria. Buchner (1889) showed that this bactericidal power was possessed by the cell-free serum, but was lost when the serum was heated at 55° C. for an hour. Pfeiffer and his colleagues (see Pfeiffer 1893, 1894, 1895, Pfeiffer and Issaëff 1894, Issaëff 1894) found that cholera vibrios, when injected into the peritoneal cavity of immunized guinea-pigs, underwent

a granular degeneration followed by partial lysis. They also showed that this lysis was determined by specific antibodies, present in the blood and tissue fluids. These observations were followed by those of Bordet (1895) who showed that two substances are involved in the bacteriolytic action of immune sera. One of these, the specific bacteriolysin, or sensitizer, if present in a normal serum is seldom found in any considerable amount. It appears, or increases greatly in amount, in response to the injection of bacterial cells. It is not inactivated by heating at 55° C. for 30–60 minutes. The other substance is present in normal serum, and is not increased as the result of immunization. It is thermolabile, that is, it is inactivated by heating at 55° C. for one hour. To this substance Bordet gave the name *alexine*; it is better known as *complement*. A few years later Bordet (1898) described the phenomenon of serum hæmolysis—the specific lysis of red blood corpuscles by the serum of an animal “immunized” by repeated injections of the red cells of some other species. The hæmolysins behave as do the bacteriolysins—their lytic action depends on the presence of complement. They are far more easily studied, because the lysis of red blood corpuscles—the change from a turbid suspension to a clear red fluid—is easily observable to the naked eye, whereas the lysis of bacteria by an immune serum *in vitro* is not associated with so striking a change, and this reaction is usually studied by noting the degree of killing that occurs when a very light bacterial suspension is submitted to the action of the serum under test.

The action of the lytic antibody on red corpuscles or bacteria differs only in its secondary effects from that of any other antibody on any antigen. The antibody-containing globulin is anchored by its specific combining groups to hapten groups situated on the cell surface. The cell, so sensitized, takes up complement. This action is non-specific in the sense that almost any antigen sensitized by almost any antibody will absorb complement from serum, given the optimal conditions (see p. 74). The complement when taken up by the sensitized cell so alters the cell surface, or the limiting membrane, that substances retained by the normal cell diffuse out into the surrounding medium. In the case of the red cell the hæmoglobin thus diffuses, and the cell swells up, loses its typical bi-concave shape, and becomes almost transparent to light. It should be noted that there is no evidence that the cell or its contents undergo any profound chemical changes. Complement is thermolabile; but there is no evidence that it resembles an enzyme in any other way.

The immunological literature of the two decades following Bordet's observations abounds in controversial papers dealing with this phenomenon. Those who desire details will find them in the collected works of the two chief protagonists, Bordet and his colleagues on the one hand, and Ehrlich and his colleagues on the other, but the interest of these papers is now mainly historical. Controversy centred largely on the exact rôle of the lytic antibody. For Ehrlich it was an *amboceptor* (see p. 81), a special type of antibody with two combining groups; one with a specific affinity for the cell receptor with which it united, the other specially fitted to unite with complement and anchor it to the cell. Bordet insisted that there was no evidence of this

direct linking action. Complement was taken up by sensitized cells, not by normal cells. That was as far as the evidence went. There was no evidence that complement ever united with antibody that was not anchored to the cell; or that, when antigen-antibody union had occurred, the complement united with any special grouping of the antibody, or indeed with the antibody, as such, at all. In this controversy time has been on Bordet's side.

The problem of the titration of a hæmolytic serum differs from that of titrating precipitins or agglutinins, in that we are faced with three dependent variables instead of two. Our essential reagents are red cells, hæmolysin and complement. We heat our hæmolytic serum at 55°C . for one hour to inactivate its complement—clearly, we want to be able to vary our complement and hæmolysin independently—and we employ as complement the fresh unheated serum of a convenient animal such as the guinea-pig. Of our three reagents it is natural to keep our red cells constant, regarding our end-point as the lysis of a specified quantity of red cells, in a specified time, under specified conditions. It is a common practice to use a 5 per cent. suspension of red cells in saline, and to employ some convenient amount of this suspension such as 0.5 c.c. We have two reagents left, hæmolysin and complement. The natural, and usual, plan is to vary the amount of the reagent we want to measure—the hæmolytic serum—while keeping the complement constant. But here we meet a difficulty. We can only measure our reagents in terms of their activity, and they are dependent variables; the more complement we add, up to a point, the less hæmolysin we need to obtain complete lysis, and *vice versa*. We get out of the difficulty by making use of the kind of relation that exists between our variables. However much complement we add we need a certain minimal amount of hæmolysin. However much hæmolysin we add we need a certain minimal amount of complement. So we define our units of measurement as follows:

The Minimal Hæmolytic Dose (M.H.D) of hæmolysin is the smallest amount that will cause complete lysis of a particular amount of red cells, arbitrarily selected as standard, in the presence of excess of complement, in 1 hour at 37°C .

The Minimal Hæmolytic Dose (M.H.D) of complement is the smallest amount that will cause complete lysis of a particular amount of red cells, arbitrarily selected as standard, in the presence of excess of hæmolysin, in 1 hour at 37°C .

It will be noted that 37°C . is specified as the temperature of incubation. The effect of temperature on lysis is very striking. No lysis occurs at 0°C . At room temperature it is very slow and never attains completion within any time limit that could be employed in practice.

In our actual titration we proceed as follows. We first titrate our complement, which must be used fresh, adding varying dilutions to mixtures of red cells and a hæmolytic serum of known titre: a hæmolytic serum remains stable over a considerable period of time. In this titration we use excess of hæmolysin, say 4–6 M.H.D. We note the last tube that shows complete lysis. This gives us the M.H.D. of our complement. We now dilute our complement so that 3–5 M.H.D. are contained in the unit volume we intend to use, commonly 0.5 c.c., and with this we test our unknown hæmolytic serum. We add to each tube 0.5 c.c. of a 5 per cent. red-cell suspension, 0.5 c.c. of our diluted comple-

ment, and 0.5 c.c. of hæmolytic serum in dilutions that increase from tube to tube. After 1 hour at 37° C. we note the last tube that shows complete lysis, and the dilution in this tube gives us the titre (the M.H.D.) of our hæmolytic serum.

In titrating a bacteriolytic serum we do not, in our *in vitro* tests, observe lysis of bacteria as such. What we measure is the bactericidal effect. The titration, as usually carried out, does not differ in principle from the titration of a hæmolytic serum. A very light suspension of bacteria is employed, and the serum under test is mixed in increasing dilution with a constant amount of bacterial suspension and a constant amount of complement. The surviving bacteria are counted after varying intervals by some suitable cultural method, and the highest dilution of serum that produces a definite killing effect is noted. The method contains many possible sources of technical error. Gengou (1899), for instance, drew attention to the fact that bacteria are agglutinated as well as lysed by a specific antiserum, and that this will of itself reduce the bacterial count, since a clump of bacteria will produce a single colony. With long periods of incubation this is counterbalanced by the multiplication of the agglutinated bacteria, but it must be allowed for in estimating the significance of any reduction of the count over a short time interval. In carrying out titrations of this kind it was noted by Neisser and Wechsberg (1901) that there was often a marked pro-zone. A particular dilution of serum might exert no bactericidal action, while a much higher dilution might result in a complete killing of the bacteria. This phenomenon was seized on by the Ehrlich school as an example of the union of complement with free amboceptors, the hypothesis being that complement united indifferently with all the amboceptors present and that, when these were present in great excess, the chances would favour the union of all the complement present with amboceptors that were not united to bacterial cells. We know, however, from our experience with precipitation and agglutination tests, that an exactly similar inhibitory effect is produced by great excess of antibody in reactions in which complement plays no part; and it is clear that the custom of using in the bactericidal test a very thin bacterial suspension will favour the frequent occurrence of such zones of antibody excess. The phenomenon cannot, therefore, be accepted as affording support to the view that complement combines directly with antibody.

A point of considerable importance, so far as the immunological significance of the bactericidal reaction is concerned, is that its occurrence depends in large part on the nature of the bacterial cell. Certain bacteria, such as the cholera vibrio, the typhoid bacillus and most Gram-negative bacilli, undergo lysis readily when acted upon by complement after sensitization by the appropriate antibody. Other bacteria, such as the Gram-positive cocci, are insusceptible to the direct killing action of immune serum and complement.

A word may be added on the nature of complement. It is not a simple substance. The complementary action of a fresh, unheated serum is dependent on several different factors. Thus, such a serum can be divided into two fractions by methods which separate the globulin from the albumin. The fraction associated with the globulin is known as the mid-piece: the fraction associated with the albumin as the end-piece. These fractions are active together, inactive apart. Like the complementary serum itself they are both

thermolabile, being inactivated by heating at 58° C. for 1 hour (see Ferrata 1907, Brand 1907, Liefmann 1909, Skwirsky 1910, Amako 1911, Gengou 1911). But there are also two thermostable components. One of these can be selectively absorbed by yeast leaving the other components free (see Coca 1914, Whitehead, Gordon and Wormall 1925). The other is not absorbed by yeast but is selectively inactivated by ammonia (see Gordon, Whitehead and Wormall 1926a).

Complement Fixation.

This reaction has attained a considerable importance as an instrument in diagnostic technique, particularly in the case of the Wassermann reaction, which occupies a queer position of its own among the diagnostic serum reactions, in that the "antigen" employed is not in any way related to the causative organism of the disease (see p. 311).

Complement fixation was first described by Bordet and Gengou (1901). It depends on the fact that the adsorption of complement is a general property of antigen-antibody compounds, given the correct conditions. It is only in certain cases, as in hæmolysis or bacteriolysis, that this adsorption is followed by detectable changes in the antigen-antibody complex. We can, however, establish the fact of complement adsorption, or fixation, by adding to a mixture in which such fixation has occurred a suspension of sensitized red cells, and noting the absence of the hæmolysis that would have resulted had free complement been present.

The reaction itself may be represented as follows :

(a) Antigen + Antibody + Complement $\rightarrow$ Fixation.

The indicator test may be represented as follows :

Mixture (a) + Red Cells + Hæmolysin $\rightarrow$ No Hæmolysis.

We most commonly apply this reaction to test for the presence of an antibody, though it can equally well be applied to test for the presence of an antigen. If the appropriate antibody had not been present in our first mixture there would have been no fixation of complement, and we should have obtained hæmolysis in our indicator test.

It should be noted that the antigen concerned in fixation may be of the most varied kind—bacterial cells, bacterial filtrates or extracts, proteins such as horse serum, egg white, or any of the less crude antigenic substances that we have considered earlier in this chapter.

If we wish to carry out a complement fixation test quantitatively, as we usually do, we are again in the position of having three dependent variables—the antigen, the antiserum, and the complement. We can obtain a formally quantitative result by holding any two of these reagents constant and varying the third. Suppose, as is most commonly the case, that we want to determine how much of a particular antibody there is in a particular sample of serum. We can hold our antigen constant, say at a given volume of a given bacterial suspension; we can hold our complement constant, say at 3 M.H.D.; and we can vary the dilution of our serum, noting the highest dilution that will give complete fixation, and giving our answer in the form of a titre. Or we can hold our antigen and serum constant, and vary our com-

plement, noting how much complement is fixed by the particular amounts of antigen and serum that we have selected. Or we can hold our serum and complement constant, and vary our antigen, noting how much antigen is required to fix a constant amount of complement in the presence of a constant amount of serum. In any case we must include control tubes in which complement is allowed to react with antigen in the absence of serum, and with serum in the absence of antigen. Many antigens and some sera are able to fix, or inactivate, a certain amount of complement apart from the occurrence of any antigen-antibody reaction, and any such fixation must, of course, be allowed for in interpreting the results of the test.

Of the possible methods of titration, that in which the antigen and complement are held constant, while the amount of serum is varied, is the one most commonly employed. It has the advantage that we are varying the reagent the potency of which we want to measure.

The second method, in which the complement is varied, has been used and advocated by several workers, and provides an adequate diagnostic test. If accurate comparative results are required it has this difficulty. If a 1/5 dilution of Serum A fixes 6 M.H.D. in the presence of a certain amount of antigen, and a 1/5 dilution of Serum B in the presence of the same amount of antigen fixes 2 M.H.D. of complement, what are the relative amounts of antibody in the two sera? We have, at the moment, no knowledge on which to base an answer, though we shall usually be safe in assuming that Serum A has more antibody than Serum B.

The third method, that of varying the antigen, is not in current use for diagnostic purposes. It could only be used, with any approach to accuracy, by applying the method of optimal proportions.

The experimental study of the relation between complement fixation and precipitation has yielded results of great theoretical importance.

Gay (1905) showed that the precipitate formed by the interaction of an antiserum with the corresponding antigen frequently had the power of adsorbing complement. This suggested that precipitation and complement fixation might be two aspects of one and the same reaction. Muir and Martin (1906*b*) found that, although there was a close correlation between precipitation and complement fixation, the correlation was not absolute. They showed that complement fixation might occur in the absence of precipitation; and they noted that, in the presence of a constant amount of antiserum, there was a particular amount of antigen that gave maximal complement fixation, while amounts much greater or much less than this might fix little complement or none. Dean (1912) made clear the cause of earlier discrepancies. By a series of experiments in which varying amounts of antiserum were titrated against varying amounts of antigen he showed that, for any given amount of antiserum, two amounts of antigen could be determined, one that gave maximal precipitation and another that gave maximal complement fixation. In the particular system with which he was working maximal complement fixation occurred in a tube in which precipitation was retarded by an excess of antiserum. In a further series of experiments he showed that complement fixation

occurred during the early stages of precipitation, before any flocculi were visible to the naked eye. He concluded that the best conditions for fixation were supplied when the proportions of antiserum and antigen were such as to cause a slow aggregation of the antigen-antibody compound, so that the phase in which small particles were present, affording a large total adsorbing surface, was relatively prolonged. He suggested that the lack of complete parallelism between the two phenomena was not due to the presence of two different antibodies, but to the fact that the two secondary results were conditioned by different physical factors.

Goldsworthy (1928) has re-examined this problem in the light of the results obtained by Dean and Webb (1926). He has entirely confirmed Dean's conclusion that precipitation and complement fixation depend on a single antigen-antibody reaction, and that complement fixation occurs during the early stages of precipitation, when the adsorbing surface is at its maximum. His results indicate, however, that the maximal complement fixation observed by Dean in the presence of a slight excess of antibody, and the minimal fixation in the presence of excess of antigen, do not express a general relationship, but were determined by the particular antiserum with which Dean was working, and the conditions as regards time and temperature that he employed. The relation between the optimal antigen:antibody ratio for precipitation and the optimal ratio for complement fixation depends on several factors, including the temperature at which the reaction is carried out, the exact moment at which the complement is added to the other reagents, and the length of time for which the reaction is allowed to proceed. In all cases maximal complement fixation is obtained with the antigen:antibody ratio that will, under the particular conditions of the test, expose the complement to a maximal adsorbing surface.

Opsonins and Bacteriotropins.

It was shown quite early in the study of immunity that an antibacterial serum increased the phagocytosis of the corresponding bacterium by the leucocytes of normal or of immune animals (see Denys and Leclef 1895, Bordet 1897, Mennes 1897).

Wright and Douglas (1903, 1904), using a modification of a technique devised by Leishman (1902), showed that normal unheated serum contains active substances that act on bacteria, so altering them as to render them sensitive to phagocytosis by polymorphonuclear cells. These substances are inactivated by heating the serum at 60° C. for 15 minutes. To denote their action in preparing bacteria for ingestion Wright and Douglas called them *opsonins*.

Neufeld and Rimpau (1904, 1905) studied the effect of antipneumococcal and antistreptococcal sera on the phagocytosis of the corresponding bacteria. They showed that such sera contained specific antibodies, which united with the bacteria, and that these antibodies were not inactivated by heating at 60° C. for 30 minutes. They regarded the thermostability of these antibodies as differentiating them from Wright's opsonins; and they called them *bacteriotropins*.

The mode of action of the bacteriotropins is clearly analogous to that of the precipitins, agglutinins and lysins. Like these antibodies, they are relatively thermostable. Like them they unite specifically with an antigen carried by the bacterial cell. We can safely assume that the anchoring of the antibody

globulin to the antigen alters the condition at the cell surface in such a way as to make it easier for the leucocytes to engulf the bacteria, just as it makes the bacteria salt-sensitive, and, in certain cases, renders the cell membrane more permeable. An essential factor in this change at the cell surface is a lowering of the negative charge, and hence of the difference in electrical potential between the bacteria and the surrounding fluid.

Falk and Matsuda (1926) found that alterations in the charge carried by pneumococci induced by the addition of lanthanum nitrate or sodium oleate had a striking effect on the phagocytosis of these organisms, and Broom and Brown (1930) were able to decrease the phagocytosis of sensitized staphylococci from a high to a low level by preventing the usual reduction in surface charge by the addition of potassium ferrocyanide. Mudd and others (1929) studied the changes induced by specific antisera in four strains of acid-fast bacilli. They found that sensitization (*a*) increased the cohesiveness of the bacilli; (*b*) decreased the bacterial charge, as evidenced by a decrease in velocity of cataphoresis; (*c*) decreased the wettability of the bacilli by oil, as evidenced by their distribution at an interface between tricaprylin and normal saline, and (*d*) increased their susceptibility to phagocytosis.

There remains the problem of the relation of the normal opsonins of Wright and Douglas to the bacteriotropins of Neufeld and Rimpau. In their thermolability the opsonins resemble complement; and their identity with this serum constituent seemed at first to be rendered probable by the observation of Muir and Martin (1906*a*) that such complexes as red cells and hæmolysin, a protein antigen and its corresponding antibody, or bacteria and a specific antibacterial serum, all removed the opsonin from a normal serum, at the same time as they removed the complement. The observation of Neufeld and Hüne (1907) that absorbing a normal serum with yeast had a similar double effect, seemed to point in the same direction. But there were difficulties in this simple conception. The normal serum opsonins do not show the same strict specificity as the bacteriotropins—normal unheated serum promotes the phagocytosis of a wide variety of antigenically unrelated bacteria—but from the first there was evidence that suggested the presence of specific factors of one kind or another. Thus, it has been the general experience that the serum of any one person varies in its opsonic effect on different bacteria; that the sera of different individuals may show striking differences when tested against the same bacterium; and that any one person may show a variation in the opsonizing power of his serum for a particular bacterium, especially as the result of infection with that organism, or of artificial immunization. Variations of this kind have been studied extensively by Wright and his colleagues (see Wright 1909). Moreover, evidence pointing in the same direction was obtained by *in vitro* experiments. Bulloch and Western (1906) succeeded in removing the opsonic power of normal serum for particular bacteria by selective absorption. Other workers were unable to confirm these results, but the careful studies of Hektoen (1908) on the normal opsonins acting on the red blood corpuscles of different animal species afforded strong support for the correctness of Bulloch and Western's contention.

The explanation of these anomalous findings would appear to lie in the fact that the opsonic effect of normal serum resembles its hæmolytic and bacteriolytic effects in being dependent on both antibody and complement. Chapin

and Cowie (1907) showed that normal serum may have its opsonic power for a staphylococcus removed by absorption with that organism in the cold, but that such absorbed serum may still have the power of reactivating normal serum that has been inactivated by heat. The cocci that have been used for absorption, when washed and resuspended in saline, show little if any increased susceptibility to phagocytosis in the absence of unheated serum. Later (Cowie and Chapin 1907) they showed that normal serum loses almost all its opsonic power when diluted fifteen times with saline. If, however, such diluted serum is added to another sample of serum that has been inactivated by heating at 55° – 60° C. for 10 minutes the mixture is almost as active in promoting phagocytosis as was the original unheated, undiluted serum. It would appear that normal serum contains specific sensitizing antibodies, present in amounts so small that they are ineffective in a dilution of 1:15 or more. Even in undiluted serum these antibodies are unable, by themselves, to alter the bacterial surface sufficiently to promote phagocytosis; but when complement is adsorbed to the incompletely sensitized bacteria the necessary change in surface conditions is produced. It would seem, also, that complement is not without effect on the action of the bacteriotropins; for G. Dean (1907) has shown that the action of a heated antiserum is increased by the addition of a little unheated normal serum. Sleeswijk (1908) has confirmed Dean's results, and concludes that sensitization, as a preliminary to phagocytosis, is primarily dependent on a specific antibody. When this antibody is present in adequate concentration it can produce its effect in the absence of complement, though added complement may enhance it. When the antibody is present in very small amount complement is necessary to induce adequate sensitization. (See also Ward and Enders 1933).

It is of interest to note that the action of complement in opsonification appears to differ in some way from its action in hæmolysis; since, as Gordon, Whitehead and Wormald (1926b) have shown, the addition of ammonia renders complement inactive as a hæmolytic agent, but removes none of its opsonic activity.

The quantitative measurement of opsonic or bacteriotropic action is a technical problem of great difficulty. The system is a very complex one, including living phagocytic cells the uniform distribution of which is exceedingly difficult to ensure. It is impossible, even when the tubes are rotated by one of the various mechanisms that have been devised, to obtain results of the same order of accuracy as in the precipitin, agglutination or hæmolytic reactions.

In the method employed by Wright and his colleagues, serum, leucocytes and bacterial suspensions are mixed in capillary tubes and incubated at 37° C. for 15–30 minutes. Their contents are then expelled on to slides. Films are prepared and stained, and the bacteria contained in the first 50–100 leucocytes encountered are enumerated. The relative opsonic effect of two sera is expressed as the ratio between the numbers of bacteria taken up, under their influence, by the same number of leucocytes. As might be supposed, the experimental error is a high one (see Greenwood 1913). An alternative method, suggested by Klein (1907), is to fix on some particular degree of phagocytosis as an arbitrary end-point, and to dilute the serum under test until this standard

is reached and passed. But, at least with normal unheated sera, we shall be diluting two different reagents at the same time.

Within recent years a method has been applied to the study of certain immunological problems that, while making no pretence at the quantitative titration of any particular antibody, depends in part at least on an opsonic effect. Wright (1914) noted that the whole blood of an animal was often bactericidal, when the separated serum or plasma showed no such effect. Heist, Solis-Cohen and Solis-Cohen (1918), Robertson and Sia (1924), and many others have carried out similar studies using whole blood, to which a suitable anti-coagulant has been added, or serum-leucocyte mixtures. In any case the method employed is to subject varying numbers of the bacteria under test to this complex bactericidal system and to determine the number that are killed.

The Toxin-Antitoxin Reaction.

This reaction hardly falls in the same category as those we have been considering, since the titrations are carried out *in vivo*. We have, however, noted that toxin and antitoxin react in the test-tube giving specific precipitation, resembling in this respect other antigens and antibodies, and it is necessary to discuss some of the peculiarities recorded in the *in vivo* tests, since they have formed the basis of much of the controversy with regard to the mechanism of antigen-antibody reactions in general.

The starting-point of any *in vivo* titration is the definition of units of measurement. We have discussed in Chapter II the difficulties that are inherent in measurements of this kind, and the definitions given here, which apply to diphtheria toxin and antitoxin, must be read in the light of that discussion.

The Minimal Lethal Dose (M.L.D.) of diphtheria toxin is the least amount that will, on the average, kill a guinea-pig of 250 gms. weight within 4 days after subcutaneous inoculation.

The unit of antitoxin was originally defined by Ehrlich (1897) as the least amount that would completely neutralize 100 M.L.D. of the toxin with which he was working. This definition is now obsolete. Ehrlich's original unit of antitoxin has been accepted as a general standard of reference (see Chapter XXI); and the exact amount of any particular toxin which may be completely neutralized by that unit is of no importance. For the standardization of antitoxin two further doses of toxin were defined.

The Limes Nul (L_0) dose of toxin is the largest amount of toxin which, when mixed with one unit of antitoxin and injected subcutaneously into a guinea-pig of 250 gms. weight, will, on the average, give rise to no observable reaction, local or general.

The Limes Tod (L_+) dose of toxin is the smallest amount of toxin which, when mixed with one unit of antitoxin and injected subcutaneously into a guinea-pig of 250 gms. weight, will, on the average, kill that guinea-pig within 4 days.

THE EHRLICH PHENOMENON.—A naïve argument from the definitions would lead to the obvious conclusion that, for any given toxin, $L_+ - L_0 = 1$ M.L.D.

In fact it does not. When mixtures of various amounts of toxin with one unit of antitoxin are tested by subcutaneous injection in the guinea-pig the difference between the L_+ and L_0 dose may lie anywhere between about 10 and 100 M.L.D.

THE PARTIAL NEUTRALIZATION OF TOXIN BY ANTITOXIN.—If to the L_0 dose of toxin varying amounts of antitoxin are added and the amount of unneutralized toxin in each mixture is determined by *in vivo* tests, it is found that the amounts of toxin neutralized by different fractions of one unit of antitoxin are not proportional to the fractions employed. The results obtained by almost all workers, which differ only in one particular from those recorded by Ehrlich, show that a fraction of a unit of antitoxin neutralizes more than the equivalent amount of toxin. Thus, the amount of free toxin in a mixture of one L_0 dose of toxin with one-fourth of a unit of antitoxin is much less than three-quarters of the L_0 dose. As the amount of the antitoxin in the mixtures is increased, equal increments neutralize proportionately less and less toxin, until, when one unit has been added, complete neutralization is attained (see, for instance, Arrhenius and Madsen 1902, 1904, Arrhenius 1915, Glenny *et al.* 1925).

THE DANYSZ PHENOMENON.—Danysz (1902) showed that if a constant amount of toxin is added to a constant amount of antitoxin the toxicity of the mixture varies according to the way in which the addition is made. If, for instance, the amounts of toxin and antitoxin are equivalent and the toxin is added all at once to the antitoxin, the mixture is non-toxic to the guinea-pig; but, if the same amount of toxin is added to the same amount of antitoxin in two or more fractions, an interval of 15 minutes or more being allowed to elapse between each addition of toxin, the mixture may be highly toxic.

These departures from the relations that might be expected to hold if neutralization were due to firm chemical union in constant proportions are not peculiar to diphtheria toxin and antitoxin, but characterize toxin-antitoxin reactions in general (see, for instance, Burnet 1931).

THE ABSORPTION OF ANTIBODY BY ANTIGEN AS A GENERAL PHENOMENON.—We have noted in the case of each of the antigen-antibody reactions that the first, and essential, stage is the union of the specific combining groups of the antibody with the hapten groups of the antigen, and we have seen that this union can be demonstrated by showing that the antibody has been removed from the fluid after the reaction has occurred. This is often referred to as the "*absorption*" of antibody by antigen; and, when we study such absorption quantitatively, we find that the reaction proceeds in very much the same way as does the neutralization of toxin by antitoxin.

In the case of precipitation we have noted that most observers have recorded a varying ratio of antigen and antibody in the precipitate according as one or other reagent predominates in the reacting mixture. Absorption of agglutinins, or of other antibodies, tells the same story.

Thus, Eisenberg and Volk (1902) showed that, if a constant amount of serum is absorbed with varying amounts of bacteria, the amount of agglutinin absorbed is not proportional to the amount of bacteria employed. If a certain amount of bacteria removes a certain fraction of agglutinin, twice that amount will remove much less than twice as much agglutinin, and the amount required to remove all the agglutinin is always a high multiple of that required to remove say one-half. Craw (1905) showed that the way in which a

constant number of bacteria are added to an agglutinating serum has a marked effect on the degree of absorption. Much more agglutinin is removed if the bacteria are added in fractions, with intervals between the additions, than if they are added all at once. This is clearly analogous to the Danysz phenomenon.

THE CLASSICAL THEORIES OF ANTIGEN-ANTIBODY REACTIONS AND THEIR MODERN ASPECT

We are now in a position to examine very briefly—far too briefly for historical justice—the classical theories of the antigen-antibody reactions.

Ehrlich's Side-chain Theory. The theory propounded by Ehrlich (1898, 1900) may be summarized as follows. He regarded any living cell as being endowed with attached groupings, or side chains, the normal function of which is to anchor nutrient substances, and in some cases to act upon and modify them, as a preliminary to their incorporation into the essential cell substance. These side chains, or *receptors*, thus form the point of contact between the cell and any substance that gains access to the tissue fluids in which the cell is bathed. In Ehrlich's view the antigens, whatever their nature, attach themselves to these cell receptors. Since the antigens are in all cases foreign substances, which have no part in the normal economy of the cell, the receptors in question are diverted from their normal function. Stimulated by this derangement of its essential mechanism, the cell produces new receptors of the type thrown out of action and, acting in accordance with a frequent physiological habit, the process is carried to excess. The superfluous receptors are shed by the cell into the surrounding fluids, and it is these shed receptors that constitute the serum antibodies. The essential feature of these antibodies is that they unite specifically with the antigens. Ehrlich therefore endowed each of his receptors with a specific chemical grouping, the *haptophore group*, which enters into chemical union with a corresponding specific group borne by the antigen. If neutralization alone resulted the requirements were met at this stage. If, however, the antigen became altered in some way as the result of union with the antibody, as in precipitation or agglutination, Ehrlich postulated the existence of another grouping in the receptor—the *ergophore group*—which determines the particular change in the condition of the antigen that occurs after its union with antibody. In those cases in which the addition of complement to the antigen-antibody complex is needed to induce a given change in the antigen, as in hæmolysis or bacteriolysis, Ehrlich postulated the existence of special receptors with two haptophore groups, one of which attaches itself to the antigen and the other to the complement. These double receptors he called *amboceptors*. The haptophore that attaches itself to the antigen-containing cell is the *cytophilic group*. The haptophore that attaches itself to the complement is the *complementophilic group*. He referred these three different kinds of receptors to three different orders.

Fig. 3 gives a diagrammatic representation of Ehrlich's general conception.

If we regard Ehrlich's theory *qualitatively* in the light of our present know-

ledge we can see its fertility. With modifications that are necessitated by facts that we know now, but that were not known then, it forms a good working model of our current views. It suggests a theory of the formation of antibodies which must be rejected in its crude form (see Chapter XI); but this does not concern us here. The haptophore group of the antigen is Landsteiner's *haptén*, clearly recognizable. And the haptophore group of the receptor is the combining group of the antibody. The ergophore group we can discard. It is replaced for us by a complex including the antibody globulin

and the electrolytes that flocculate it. The amboceptor we have dealt with above. We must reject it, endowing any antibody with the power of forming a complement-fixing compound when united to the corresponding antigen.

The thing to note is that Ehrlich clung firmly to the concept of specificity determined by chemical structure; and in this he was demonstrably right.

On the quantitative side his conceptions were much less happy. To explain the anomalies to which we have referred, he expanded and elaborated his theory to include a wide variety of differences in chemical affinity, and endowed his hypothetical reagents with an increasing complexity of structure. Some of his conceptions were in accord with a wide range of experimental observations, for instance the existence of a non-toxic modification of toxin—

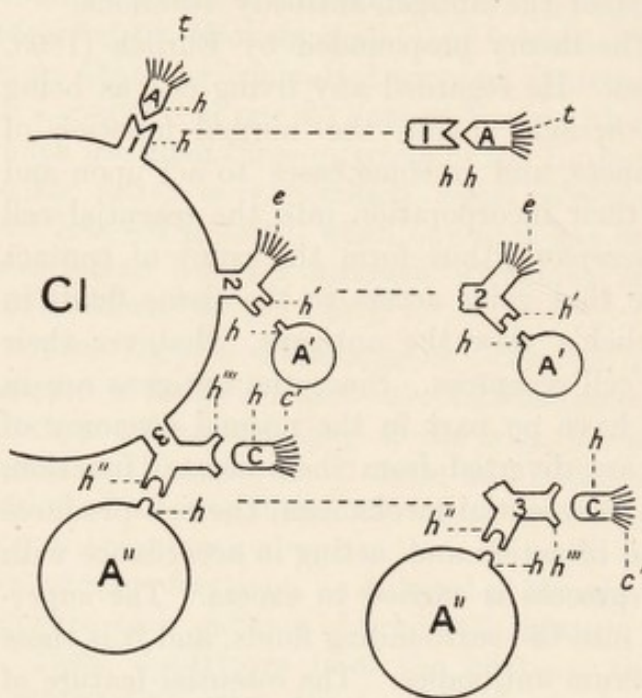


FIG. 3.

Cl. Cell.

1. Receptor of 1st order with its haptophore group *h*.
 2. Receptor of 2nd order with its haptophore group *h'* and its ergophore group *e*.
 3. Receptor of 3rd order with its two haptophore groups, *h''* the cytophilic group, and *h'''* the complementophilic group.
- A, A', A''. Various antigens, each with its haptophore group *h*; A has in addition an ergophore, or toxophore group *t*.
- C. Complement, with its haptophore group *h*, and its ergophore group *c'*.

toxoid—which retains its combining affinity after losing its toxicity. But others only fitted the facts they were created to explain, and had to be modified or remodelled with each succeeding observation.

The Theory of Arrhenius and Madsen.—Arrhenius and Madsen (1902, 1904, Arrhenius 1915) sought to explain the quantitative relations of the antigen-antibody reactions by an appeal to the law of mass-action.

If antigen-antibody reactions were reversible, so that an appreciable fraction of the antigen-antibody compound was present in the dissociated form, the equilibrium would be determined by the well-known formula:

$$(A)(G) = K(AG)$$

where (A) represents the molecular concentration of antibody, (G) the molecular concentration of antigen, (AG) the molecular concentration of the antigen-antibody compound, and K is the dissociation constant.

In the particular case of the neutralization of toxin by antitoxin Arrhenius and Madsen found good agreement between their calculated and observed values, over the greater part but not the whole of their range of observations, on the assumption that one molecule of toxin unites with one molecule of antitoxin to form two molecules of toxin-antitoxin compound, when the equilibrium equation becomes :

$$(T)(A) = K(TA)^2$$

They were, moreover, able to point to a close analogy in the progressive neutralization of the hæmolytic action of ammonia by the addition of increasing amounts of boric acid.

This theory would clearly account for many of the facts observed. In order to eliminate all free toxin it would be necessary to add more than the equivalent amount of antitoxin ; so that we could account for the discrepancy between the L_0 and L_+ dose. The equation based on the mass-action law gives a fair fit to the observed curve of partial neutralization. Moreover (see p. 384) there is no doubt that certain toxin-antitoxin compounds readily undergo dissociation ; and the same is true for certain viruses and virus-sensitizing antibodies (see pp. 266, 267). It is, indeed, quite probable that the phenomena of dissociation and mass-action will form a part of our picture of the antigen-antibody reactions, when that picture is completed ; but they are unlikely to form the whole of it.

The theory of Arrhenius and Madsen does not account very successfully for the Danysz phenomenon. It presents a chemical analogy involving an intermediate reaction which prevents the completion of the original reaction when the final fraction of one of the reagents is added ; but the analogy is a little forced.

The Theory of Bordet.—Bordet (1899, 1903) denies the applicability of the laws of ordinary chemical union to the reactions between antigens and antibodies. He regards them as belonging to that class of colloidal reaction in which the conditions that determine quantitative relationships are physical rather than chemical. On this view the laws that describe adsorption at surfaces and interfaces should be found to hold true.

The general formula that describes such quantitative relations is the Freundlich isotherm (Freundlich 1922, Freundlich and Neumann 1909). If x is the amount of an adsorbable substance adsorbed by surface m , C is the concentration of the substance left in the fluid at equilibrium, a is a constant depending on the units of measurement, and n is a constant whose value is less than unity : then :

$$\frac{x}{m} = aC^n$$

This may clearly be written :

$$\log.x - \log.m = \log.a + n\log.C$$

If we take x to represent adsorption at unit surface, we get :

$$\log x = \log a + n \log C.$$

Many observers have shown that the absorption curves obtained in antigen-antibody reactions do, in fact, correspond over a large part of the range with a Freundlich isotherm, but the agreement rarely if ever extends up to the point of complete neutralization or absorption ; and it would, in truth, be very difficult in many cases to say whether a curve based on the mass-action law, or a curve based on the adsorption formula, gave the better fit. Many of the curves recorded suffer from a sad paucity of points established by direct experiment.

Bordet's conception is an obvious simplification. It accounts for the $L_+ - L_0$ discrepancy, since the amount of toxin adsorbed, or neutralized, by one unit of antitoxin will vary with the concentration of toxin in the reacting mixture. It accounts, over a wide part of the range, for the results obtained in the partial neutralization of toxins, or in the absorption of antibodies. It accounts for the Danysz phenomenon—an exactly similar phenomenon may, for instance, be demonstrated in the absorption of dyes by blotting-paper. But it ignores specificity.

The controversy between the adsorption school and the chemical-union school has, in truth, become more than a little artificial in view of recent developments in colloid and organic chemistry.

So far as we are concerned, the present position is fairly clear.

The antigen-antibody reactions depend on specific affinities determined by chemical structure of the kind familiar to the organic chemist—using “structure” in the modern sense, to include not only the nature of particular active groupings but the spatial arrangement of these, or of other groupings, in relation to one another. The quantitative behaviour of these reactions depends on a variety of factors, one of which is almost certainly the complexity and large molecular size of the reacting substances. These probably carry many combining groups. It is at least possible that they combine in varying, not in constant proportions. The compounds formed may be dissociable, to a varying degree, though in many instances the dissociation would seem to be minimal. When combination has occurred, in whatever proportion other than exact equivalence, it is possible that some secondary change in the compound produced may alter its capacity for further reaction. It has, for instance, been noted that the union of antigen with antibody may become firmer with lapse of time. In certain of the secondary reactions, as for instance in the fixation of complement, we are probably dealing with a non-specific adsorption effect. But, in truth, the systems with which we are concerned are altogether too complex to allow us to determine their behaviour in detail from the kinetic point of view. And this does not matter very much, though it will be an intellectual satisfaction when we can describe our phenomena in more exact terms. We know the kind of quantitative relations that hold, and we can at least obtain comparative measurements of our reagents

with a fair degree of accuracy. In particular, we know that the line of advance—the line that will allow us to interfere more successfully in the reactions between host and parasite—lies across the field that is being mapped out by the structural organic chemist.

THE UNITY OR DIVERSITY OF ANTIBODIES

One more question falls for discussion in this chapter, though it has in truth been answered as we have considered each antigen-antibody reaction in turn. Are precipitins, agglutinins, lysins, complement-fixing antibodies, opsonins and antitoxins, different *kinds* of antibody, or are they the same kind of antibody doing different things?

The former view held the field for many years; but the latter—the unitarian hypothesis—has now gained almost universal acceptance. This has been due mainly to the work of Dean (1917) in this country, to that of Zinsser (1921) in America, and to Nicolle and Césari (1922) in France; though a similar view had been adumbrated by Bail and Hoke (1908). We have seen how Dean established the essential identity of precipitation and complement fixation. We have noted that precipitation is a general form of reaction between all antigens and all antibodies, including toxin and antitoxin. And we have seen that the union of antigen and antibody at the surface of a bacterial cell sensitizes it alike to the flocculating action of electrolytes, to the lytic action of complement, and to the phagocytic action of leucocytes.

The available evidence lends strong support to the view expressed by Dean:

“In all the serum reactions . . . the immediate consequence is an aggregation of globulin particles round the antigen. The degree to which the aggregation or precipitation proceeds depends on the experimental conditions, on the relative proportions of antigen and antibody in the mixture, on the nature of the antigen-containing substance, red corpuscle, bacillus, or normal serum, on the presence of some third factor. . . . In other words the various serum reactions are various methods of observing and measuring one single reaction.”

This conception of the serum reactions does not, of course, in any way modify our belief in a multiplicity of antibodies corresponding to a multiplicity of antigens. A red cell, a bacillus, a crude protein solution such as horse serum, contain many antigens and give rise to many antibodies. The unitarian hypothesis, as Zinsser (1921) has emphasized, implies simply that the injection into the tissues of a chemically pure antigen will lead to the formation of one antibody, and one only.

When we discuss, in the next chapter, the antigenic structure of bacteria, we shall see that there is a sense in which it would be correct to differentiate one antibody from another in terms of function—to say, for instance, that a particular antibody is an agglutinin but not a lysin. This difference in functional activity, however, is determined not by a difference in the nature of

the antibody but by a difference in the structural position of the antigen to which it is attached.

SUMMARY

(1) Complete antigens have the power of stimulating the production of antibodies when introduced parenterally into the tissues, and of reacting specifically in the test-tube with the antibodies so formed.

(2) The specificity of the antigen-antibody reactions depends on the chemical structure of particular groupings borne by the antigen—the hapten groups—and corresponding combining groups borne by the antibody.

(3) Separated haptens combine with the antibody. If they are relatively simple the union is followed by no detectable change in the antigen-antibody compound. If they are relatively complex the compound formed may be precipitated by electrolytes. Typical haptens have no power of inducing antibody formation when injected into the tissues.

(4) Complete antigens are protein in nature, or have a protein component.

(5) Antibodies are closely associated with the globulin of the serum in which they are contained.

(6) The various antigen-antibody reactions—precipitation, agglutination, lysis, complement fixation, opsonification, neutralization of toxin—are all conditioned by union of antigen with antibody, leading to the formation of a compound that includes the antibody globulin.

(7) The secondary reactions that occur are determined by the nature of the antigenic material and by the presence of certain secondary factors.

(8) If the antigen is in solution, and if electrolytes are present, the antigen-antibody compound will be precipitated as a result of a lowering of the surface charge, and perhaps of other changes in the physical state of the particles.

(9) If the antigen is attached to bacterial or other cells, the cells will be agglutinated under the same conditions.

(10) If the antigen is attached to cells, if complement is present, and if the cells are susceptible to its action, lysis will occur.

(11) Antigen-antibody compounds in general have the power of fixing complement under suitable conditions, although its fixation may result in no detectable secondary change.

(12) Bacteria and other cells that have been sensitized by an antigen-antibody union occurring at their surface are rendered susceptible to phagocytosis.

(13) The quantitative relations that are observed in antigen-antibody reactions appear to be determined by highly complex factors. Among these the relative amounts of antigen and antibody in the reacting system appear to play a predominating part, not only in affecting the speed of the reaction but in determining the constitution of the compound formed. Following the primary chemical union between antigen and antibody it is possible that dissociation and mass-action effects, and in some instances a secondary non-specific adsorption, play a part in the reaction as a whole.

(14) The available evidence supports the view that such names as *precipitin*, *agglutinin*, *lysin*, *opsonin*, *bacteriotropin*, etc., do not designate different antibodies but the same antibody doing different things. Corresponding to any single antigen there is probably a single antibody, the functional activity of that antibody depending on the nature of the antigen, on its situation—whether free in solution or attached to a bacterial or other cell—and on the secondary factors referred to under previous headings.

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CHAPTER VI

THE ANTIGENIC STRUCTURE OF BACTERIA

A COMPLETE description of the antigenic structure of a bacterial cell would include (a) the number and kind of different antigens present, (b) their relative proportions, and (c) their position in the cell or cell appendages.

Such a description is not yet possible in any instance. But our advance has been very rapid during the past decade, and we can now construct rough working models of the antigenic structure of certain bacterial species. With the aid of these we have already been able to solve many immunological puzzles; and it seems certain that further advance along the same lines will enable us to solve many more.

We may start our discussion by considering the methods that are available for studies of this kind.

Before we do so we may enter one caveat. In later parts of this chapter we shall refer frequently to antigens, or antigenic components, or haptens, that have been isolated by chemical methods from bacterial cells. It would be highly inconvenient—sometimes our ignorance would make it impossible—to indicate in each case the position in the complete-antigen → simple-hapten hierarchy that each of these components occupies. It may be taken as a general rule that each component acts as an antigen in the full sense while attached to the bacterial cell, but that many of them, and particularly the bacterial polysaccharides, are converted into relatively complex haptens when separated from the cell and submitted to chemical purification.

ANTIGENIC ANALYSIS BY SELECTIVE QUALITATIVE ABSORPTION OF ANTIBODIES

The fact that a single bacterial cell contains many antigens was established during the earliest studies on the agglutination reaction. The original observations by Gruber and Durham (1896) showed that the specificity of this reaction was not absolute in the biological sense. For instance, while the colon bacillus was sharply differentiated from the typhoid bacillus and both from certain vibrios, these vibrios were not so clearly differentiated from one another. Durham (1901) as the result of a more detailed study of these group agglutinations enunciated quite clearly the hypothesis of a multiplicity—as he called it, a *mosaic*—of antigens within a single bacterial cell. Employing small letters to denote the antigenic bacterial components, and capitals to denote the corre-

sponding agglutinins in the antisera, he suggested that the actual agglutinins and agglutinogens involved might be represented as follows.

Agglutinogens.		Agglutinins.	
Bacterium 1.	<i>a, b, c, d, e.</i>	Serum 1.	A, B, C, D, E.
Bacterium 2.	<i>c, d, e, f, g, h.</i>	Serum 2.	C, D, E, F, G, H.
Bacterium 3.	<i>e, f, g, h, j, k.</i>	Serum 3.	E, F, G, H, J, K.

The serum prepared against Bacterium 1 would contain the agglutinins A, B, C, D, E, and in virtue of the presence of each and all of these it would agglutinate the corresponding bacterium. It would also agglutinate Bacterium 2 in virtue of agglutinins C, D, and E, and Bacterium 3 in virtue of agglutinin E.

The method that has been employed in analysing the antigenic structure of a particular bacterial strain—the method of agglutinin absorption—was introduced by Castellani in 1902; though a precisely similar method had been previously employed by Ehrlich and his co-workers in demonstrating the multiplicity of hæmolytic antibodies. When an agglutinating serum is exposed to an excess of bacterial cells that contain some or all of the corresponding agglutinogens the agglutinins that find their counterparts in the antigenic structure of the bacteria are bound, or absorbed, and thus removed from the fluid in which the bacteria are suspended. If the bacteria are separated by centrifugation, the supernatant fluid can be tested as regards its remaining agglutinins. For instance, taking the illustrative example given above, if Serum 1 were absorbed with an excess of Bacterium 1 all the agglutinins A, B, C, D, and E would be removed and the supernatant fluid would have no agglutinating action. If it were absorbed with Bacterium 2, C, D and E would be removed, but A and B would remain. The supernatant fluid would not agglutinate Bacterium 2—the absorbing strain—nor Bacterium 3 (since that bacterium does not contain agglutinogens *a* or *b*); but it would still agglutinate Bacterium 1 in virtue of the remaining agglutinins A and B. If it were absorbed with Bacterium 3, the single common agglutinin E would be removed. The supernatant fluid would (as always) fail to agglutinate the absorbing strain; but it would still agglutinate Bacterium 1 in virtue of agglutinins A, B, C, and D, and Bacterium 2 in virtue of agglutinins C and D.

This simple method has enabled us to study in considerable detail the antigenic make-up of the bacterial species with which we have been concerned, and to establish the existence within a single species of types or races which cannot be differentiated in any other way.

A note may be interpolated here with regard to two terms that are sometimes loosely applied. The antiserum that is produced by the inoculation into a suitable animal of a particular bacterium is frequently referred to as a *homologous* serum. A serum that agglutinates the same bacterium but has been produced by the inoculation of some other bacterium, differing in one or more of its antigenic components, is termed a *heterologous* serum. Used in this sense the terms are useful and logical, and may be applied either to an antiserum in relation to a bacterium, or to two antisera or two bacteria in relation to one another, implying in either case complete correspondence between active combining

groups. Thus, a serum that contains the active antibody groupings A, B, C, D is homologous with a bacterium containing the active antigenic groupings *a, b, c, d*; two bacteria each containing the active antigenic groupings *d, e, f, g* are homologous with one another, and so on. But an antiserum containing the active groupings A, B, C, D is not homologous, either with a bacterium containing the active groupings *a, b, c, e*, or *a, b, c, d, e*, or *a, b, c*. The terms cannot, logically, be applied to single antigens or single antibodies. They describe the relation between a group of different antibodies in a serum and a group of antigens attached to a bacterial cell, or between the group of antigens attached to one bacterial cell and the group attached to another. We are also, it should be noted, using the terms qualitatively, not quantitatively.

We have not yet considered the relations of an antiserum that contains a high concentration of antibody A and a low concentration of antibody B to two bacteria, one of which contains a large amount of antigen *a* and a small amount of antigen *b*, while the other contains a small amount of *a* and a large amount of *b*. Before doing so we may describe the test required to establish complete qualitative homology.

Suppose that we have an unknown bacterium *x*, which we suspect to be antigenically identical with a known bacterium *y*, and suppose that we have available an anti-*y* serum. We absorb the anti-*y* serum with *x* and then test it against *y*. If it now fails to agglutinate *y* we may assume that all the active antigenic groupings in *y* are also present in *x*. But we have not excluded the possibility that *x* has additional active antigenic groupings, not present in *y*. So we prepare an anti-*x* serum, and then absorb it with *y*. If, after absorption, it fails to agglutinate *x*, we assume that all the active antigenic groupings present in *y* are also present in *x*. That is, *x* and *y* are antigenically homologous. This double cross-absorption method is often spoken of as the "mirror test."

Antigenic Analysis by Selective Quantitative Absorption of Antibodies. This type of analysis is in its earliest infancy. It seeks to differentiate between a bacterium with the active antigenic structure $xa + yb$ and another with the active antigenic structure $ma + nb$, where *x, y, m* and *n* represent significantly different numbers. If the corresponding antisera are absorbed to exhaustion with massive doses of either bacterium, all agglutinins will be removed, for the bacteria and their antisera are qualitatively identical. But, with a proper adjustment of the absorbing dose, it is possible to show that the absorbing effect of the two bacteria differs significantly.

A good example of this kind of antigenic difference has recently been recorded by Wilson and Miles (1932) in the case of two nearly related organisms, *Brucella abortus* and *Brucella melitensis*, both of which may give rise to undulant fever in man, though the most frequent rôle of the former is as the causative organism of contagious abortion in cows. When very heavy absorbing suspensions are used ($150 \times 10^9 - 300 \times 10^9$ bacteria per c.c.) the abortus strains remove all agglutinins from an anti-melitensis serum, and *vice versa*. But with a smaller absorbing dose (3×10^9 bacteria per c.c.) a melitensis strain removes all agglutinins from an anti-melitensis serum but removes only some 50-75 per cent. of the total agglutinins from an anti-abortus serum, while an abortus strain removes all agglutinins from an anti-abortus serum but only about 75 per cent. of the agglutinins from an anti-melitensis serum. This is clearly the kind of result that would be expected if *Brucella abortus* and *Brucella melitensis* contained the same two antigens in different proportions. Let us assume for the moment that the antibodies in the sera correspond quantitatively as well as qualitatively to the antigens in the organism against which the serum was prepared, or at least that an antigen excess in the organism will be represented by an antibody excess in the serum. Under these conditions a given dose of bacteria containing the antigens A and B in the proportions $3a + 2b$ might remove

all agglutinins from a serum containing the corresponding antibodies in the proportions $3A + 2B$; but the same dose of bacteria containing the same antigens in the proportions $2a + 3b$, while more than sufficient to remove all the B agglutinins, would leave some of the A agglutinins unabsorbed.

The Optimal Proportions Technique in the Quantitative Antigenic Analysis of Bacteria.—It will be obvious that the selection of the dose of bacteria for use in such differential absorptions as those described in the preceding section will be greatly facilitated by any technique that enables us to determine the equivalent proportions of a given bacterial antigen and the corresponding antibody in an agglutinating serum. We have seen that this can be done, on certain assumptions, by determining the constant-antibody or constant-antigen optima; though it is not yet entirely clear which optimum, if either, corresponds to exact equivalence in an agglutinating system. When groups of antigens and groups of antibodies are concerned, it may well be that no ratio between the antiserum and the bacterial suspension corresponds to exact equivalence between each antibody and each antigen. Duncan (1932*a, b*) and Miles (1933) have, however, demonstrated the utility of the method for studies of this kind.

It seems possible, indeed, that an elaboration of this method may enable us to explore the active antigenic groupings of bacteria without recourse to the rather laborious absorption method. Miles (1933) records detailed curves describing the flocculation rate of *Brucella* suspensions of varying density when acted on by varying dilutions of an anti-*Brucella* serum. In the constant-antigen curves, particularly in those describing the behaviour of very thin bacterial suspensions, there are two quite definite optima, and a probable third. It seems likely that these correspond to two or three different antigen-antibody combinations. When this method has been further developed, it may be possible to determine with some accuracy the proportions in which any particular series of antigens are distributed among different bacterial strains, types or species.

THE LOCALIZATION IN A BACTERIAL CELL OF DIFFERENT ANTIGENIC COMPONENTS

So far we have considered bacteria as a whole. It is, however, possible to identify particular antigenic constituents as being situated in particular parts of the bacterial cell. Smith and Reagh (1903) isolated a non-motile strain of the hog cholera bacillus, and compared its agglutination reactions with those of the normal, motile, flagellated type of this organism. They found that a serum prepared against the motile strain agglutinated both the motile and the non-motile strains; but the titre was much higher for the motile than for the non-motile, and the clumps formed by the motile strain were fluffy and formed rapidly, while the clumps formed by the non-motile strain were tight, small and granular, and formed far more slowly. A serum prepared against the non-motile strain agglutinated the motile and non-motile strains to the same degree, giving in each case the slow, granular type of agglutination. The serum prepared against the motile strain, after absorption with the non-motile strain, had lost its power of agglutinating the non-motile bacilli, but

retained its power of agglutinating the motile strain. The serum prepared against the non-motile strain lost its agglutination for that strain when absorbed with the motile strain. Smith and Reagh concluded that the normal, motile hog cholera bacillus has two kinds of antigens, one contained in the flagella, the other in the cell body. The non-motile type has lost the flagellar antigens and retains the body antigens only. Beyer and Reagh (1904) extended these observations and showed that the flagellar antigens were so altered by heating to 70° C. for 15 minutes that the heated bacilli no longer gave the flagellar type of agglutination, but reacted like the non-motile strain. The somatic, or body antigens were not affected by this treatment and still gave the characteristic slow, granular agglutination (see also Orcutt 1924, Craigie 1931).

These observations of Smith and Reagh and of Beyer and Reagh demonstrated all the essential points of difference between the flagellar and somatic types of agglutination, but they passed almost unnoticed until they were recorded again by Weil and Felix (1917) in connection with their work on the diagnostic significance of the agglutination of a particular strain of proteus bacillus by the blood of a patient suffering from typhus fever. The proteus bacillus, in its normal, flagellated form, gives rise to a thin, widely spreading growth covering the surface of the agar medium on which it is grown. This was called by Weil and Felix the *Hauch* form—the “exhalation” form. A non-flagellated variant grew in isolated colonies with no thin, spreading growth between them; this was the *Ohne Hauch* form—the form without an exhalation. As is the way in laboratory shorthand these soon became the “H” and “O” forms; and so the thermolabile, flagellar antigens are now the H antigens, and the thermostable, somatic antigens are the O antigens. This is in the best traditions of scientific terminology, so long as we define exactly what we mean by H and O. But we have been a little careless about this, at least as regards the O type of antigen, and this carelessness has led to some confusion.

The Study of Antigenic Variation as a Guide to Antigenic Structure.

Flagellar and somatic antigens were first recognized as separate entities by studying non-flagellated variants of normally flagellated bacilli. Bacterial variation may involve a far more deep-seated change in antigenic structure, and the detailed study of such changes has played a very important part in the development of our present views on antigenic anatomy.

The most significant variation, from our present point of view, was first described by Arkwright (1920, 1921, 1924) in members of the coli-typhoid-dysentery group. He noted that a particular type of variant was of relatively common occurrence among these bacilli; and this variant differed from the parent form in several characteristic ways. Thus, the normal parent form gave smooth colonies on a solid medium and a diffuse growth in broth, and was not auto-agglutinable in normal saline (0.85 per cent.). The variant form gave rough or granular colonies on solid media and a granular growth in broth, and was auto-agglutinable in normal saline, though a stable suspension could

usually be prepared in distilled water, or in a saline solution with a greatly decreased salt-content (0.2–0.4 per cent.). These differences in colony form, growth in broth, and salt-sensitiveness were associated with a profound change in antigenic structure. The normal parent form reacted specifically as regards its agglutinability by immune sera; the variant was agglutinated not only by its own antiserum but by antisera prepared against many other bacteria in the rough state, some of which were only distantly related to the parent form according to the ordinarily accepted bacteriological criteria. By the usual transition—through shorthand to symbols—the normal form, giving smooth colonies, became the *Smooth* (or S) form; the variant, giving rough colonies, became the *Rough* (or R) form. And here again we have become entangled in the web of our words, for we have never defined exactly the criteria of roughness and smoothness. The implicit meaning is certainly not the meaning we want; since, as we shall see, the correlation between colonial form and antigenic structure is by no means constant. If we give *Smooth* and *Rough* their obvious or commonsense meaning, then we shall want new terms for the underlying changes that really interest us. If we employ the legitimate licence of scientific terminology, and say that by *Smooth* we mean one sort of antigenic structure and by *Rough* another, then we must at least define quite clearly what we mean. Up to the present we have evaded our difficulties, and so we find that the rough type of one species is recorded as having the more important characters of the smooth type of another species, as in the case of the anthrax bacillus (see Preisz 1904, Eisenberg 1912, Bordet and Renaux 1930, Tomcsik and Szongott 1932, 1933); or it becomes necessary to invent new descriptive terms, such as *matt* and *glossy*, as in the case of the hæmolytic streptococcus (see Todd 1928*a, b*, Todd and Lancefield 1928).

In most of the cases with which we are familiar the change from smoothness to roughness (the S→R variation) is associated with a loss of virulence—an association of obvious importance to the immunologist—as well as with a particular kind of change in antigenic structure. We have very good reasons for believing that the change in antigenic structure determines the loss of virulence, and it certainly determines the change in antigenic value of the variant strain regarded as an immunizing agent. We may, then, for our immediate purpose and to avoid confusion, explicitly limit ourselves to this antigenic variation and its consequences, noting that if we define the S→R variation in this way we are acting in defiance of general usage.

In terms of antigenic structure we may say that this variation consists in the *loss of the heat-stable somatic antigen that characterizes the surface of the normal virulent bacterial cell*. This loss may be associated with an uncovering of some other somatic antigen, which then dominates the antigenic behaviour of the strain.

Variation of this type has now been described in a wide range of different bacterial species—in the *Pasteurella* Group (de Kruif 1921, Webster 1925), in pneumococci (Griffith 1923, Reimann 1925, 1927), in staphylococci (Bigger, Boland and O'Meara 1927), in streptococci (Todd 1928) and in a host of other organisms. We are probably justified in regarding it as a type of variation to which pathogenic bacteria are inherently liable.

Sometimes (see White 1932, 1933) this variation may proceed still further, and another antigenic constituent may be lost. The antigenic behaviour of

the organism may then be dominated by a component that, in the normal state, was altogether latent. It may be noted (*a*) that the $S \rightarrow R$ variation is quite independent of the $H \rightarrow O$ variation (rough variants are often flagellated), and (*b*) that loss variations of the $S \rightarrow R$ or more deeply seated types are often irreversible.

There is another kind of antigenic variation to which reference must be made before we pass to the next section of our discussion.

Andrewes (1922, 1925) described a curious phasic variation in the H antigens of certain flagellated species. Taking two bacteria, *x* and *y*, that showed the flagellar type of agglutination when tested either against an anti-*x* or against an anti-*y* serum, and therefore possessed at least one H antigen in common, he absorbed the anti-*x* serum with bacillus *y* and thus obtained a serum that agglutinated *x* but not *y*. He then took a broth culture of *x* and plated it on a solid medium, thus obtaining separate colonies. Subculturing from several of these, he obtained different cultures, each representing a single bacillus in the original broth culture. When he tested these different cultures against the anti-*x* serum, rendered specific by absorption, and against the anti-*y* serum, he found that his subcultures fell sharply into two classes. Those of one class were agglutinated to titre by the specific anti-*x* serum, but not at all by the anti-*y* serum. Those of the other were agglutinated to titre by the anti-*y* serum, but not at all by the specific anti-*x* serum. The only possible conclusion would seem to be that the original culture of *x* showed cross-agglutination with the anti-*y* serum not because each bacillus in the culture possessed two antigenic components, say *a* and *b*, in their flagella, *a* being specific for *x* and *b* being shared by *y*, but because some of the bacilli possessed *a* alone, and others *b* alone. We need not, for the moment, worry as to whether the *a* bacilli do or do not possess a trace of *b* and *vice versa*. Similarly Andrewes was able to show that the *y* culture, that agglutinated with anti-*x* and anti-*y* sera, contained some bacilli possessing the common flagellar antigen *b* and others containing an antigen *c* specific for *y*. The bacilli that possessed the group antigen only were referred to as being in the *group phase*; those that possessed the specific antigen only as being in the *specific phase*. If plate cultures were prepared from a strain that was momentarily in the specific phase, bacilli in the group phase turned up sooner or later; similarly a strain in the group phase gave rise to substrains in the specific phase. The variations might occur in either direction. Species or types of bacteria that show this curious variation in their flagellar antigens are termed *diphasic*. We do not know of any example of diphasic variation affecting the somatic antigens.

THE APPLICATION OF CHEMICAL METHODS IN THE ANALYSIS OF ANTIGENIC STRUCTURE

This extension of a purely serological technique, obvious though it seems, has in fact been explored only during the last decade; but it has already yielded information of the greatest interest and importance.

Zinsser and Parker (1923) isolated substances from pneumococci, staphylococci, the influenza bacillus, the typhoid bacillus and the tubercle bacillus, which gave none of the ordinary protein reactions, except a very weak xanthoprotein reaction, but gave specific precipitation and complement fixation with the corresponding antisera. These non-protein antigens failed to stimulate antibody production on injection.

In the same year Heidelberger and Avery published the first of a series of papers dealing with a chemical study of the antigenic constituents of the

pneumococcus (Heidelberger and Avery 1923, 1924, Avery and Heidelberger 1923, 1925, Avery, Heidelberger and Goebel 1925, Avery and Morgan 1925, Avery and Neill 1925). From the three classical types of pneumococci partial antigens were separated, which were found to have the chemical structure of complex polysaccharides, failed to stimulate antibody production *in vivo*, but gave specific precipitation at extraordinarily high dilutions when mixed with antisera prepared by the inoculation of rabbits with the corresponding strains of pneumococci. They also studied the antigenic properties of the protein fraction precipitated with acetic acid during the separation of the type-specific polysaccharide from the bacterial cells.

This method—the extraction of pure, or relatively pure chemical substances from the bacterial cells, and the examination of their antigenic properties by precipitin tests *in vitro* and by inoculation into animals—is now being developed over a wide field.

There is another way in which chemical methods may be applied in the investigation of antigenic structure. Bacteria may be treated with various chemical solvents, or other reagents, with or without heat, and the effect of this treatment on their immunological behaviour may be studied (see White 1927, 1928, 1929, 1932, 1933).

Illustrative examples of the information that has been obtained by these various methods will be considered in relation to certain of those bacterial groups that have been intensively studied from this point of view. But it will be convenient to state our general conclusions before we set out the particular data on which they are based, and to sketch our present picture of antigenic structure so that we may have it clear in our minds when considering each different example in turn.

THE ANTIGENIC STRUCTURE OF THE BACTERIAL CELL

Our present knowledge suggests that the spatial arrangement of the antigenic material contained in a bacterial cell is a factor of primary importance in determining that cell's immunological behaviour. Our conception with regard to the way in which such components are actually arranged can be set out most easily in diagrammatic form. But it must be emphasized that our diagram is not a picture. By placing an antigen at the cell surface we are implying that it behaves as though it were there. By placing it beneath the surface we mean that it seems, in the normal form of the organism, to be overshadowed by some other bacterial component. How the components are really arranged we do not know, except that we can certainly allocate some to flagella or capsules, and are almost certainly right in supposing that changes in antigenic behaviour are associated with changes at the cell surface. It is quite likely that antigenic variation is associated with a change in the amount of a particular antigenic component, as well as a change in its situation. A component, for instance, that has been unmasked by the loss of another component may be produced in greater amount when it assumes a dominant

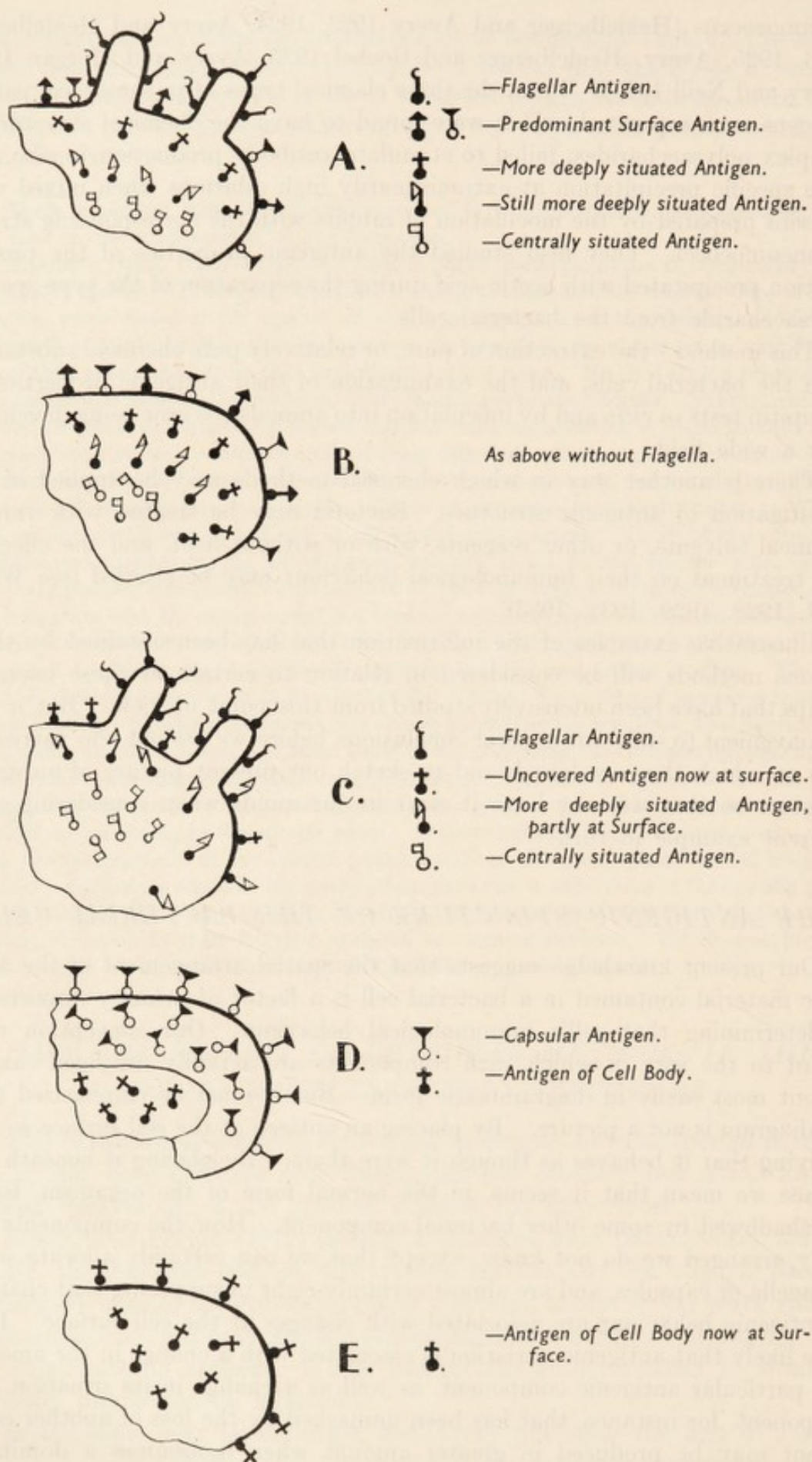


FIG. 4.

position on the active surface of the cell. Perhaps in some cases it is only represented in the normal cell in a rudimentary form.

Bearing these caveats in mind we may consider the diagrammatic arrangements of antigens set out in Fig. 4.

At A is a portion of a flagellated bacillus—with grotesquely but conveniently stumpy flagella—in its normal, virulent, smooth form, with one antigen at the surface of its flagella, a second and a third antigen at the cell surface, a fourth antigen situated more deeply and masked by antigens two and three, a fifth antigen situated more deeply still, and a sixth antigen situated centrally in the cell body.

At B is a portion of a bacillus having the same antigens, but without flagella. We might regard it as an O variant derived from A. Alternatively we might alter the nature of the antigens, maintaining their arrangement, and regard it as the normal, smooth form of any non-flagellated organism.

At C is a rough variant from A. It has kept its flagella and its normal flagellar antigen; but it has lost the antigenic components that determined the nature of the cell surface in the normal, smooth form. A deeper antigen has been unmasked. It will be noted that a few of the antigenic components that originally lay more deeply still are represented as having now found a place at the cell surface.

In some cases (see White 1933) antigenic variation by loss may proceed still further. The characteristic rough somatic antigen may disappear, and the bacterial surface may be dominated by components that lay very deeply in the normal, smooth form.

At D is an organism, such as the pneumococcus, having a capsule that consists mainly of one kind of antigen (or hapten). Beneath it, in the cell body, is another antigen which is entirely covered by the capsule.

At E is a rough variant from D. The capsule has gone, and with it the characteristic capsular antigen. The character of the cell surface is now determined by the antigen that before was hidden.

All these diagrams, and particularly D and E, are much too simple to be true. There are probably a very large number of antigens, or antigenic groupings, in any bacterial cell. But it is true that the character of the cell surface appears in most cases to be dominated by a few characteristic antigenic components, and that this surface may be profoundly altered by the replacement of these components by a few others. It is probable that the S → R variation is not a sudden, all-or-none mutation, but a more gradual or step-like process, with intermediate forms in which the normal surface antigens are still present, though in diminished amount (see, for instance, Wilson 1930).

Antigenic Structure in Relation to the Antigen-Antibody Reactions.—When antibodies unite with antigens that form part of a bacterial cell, the effect on the cell will vary according to the position that the antigen occupies.

If the antigen is situated on the surface of the flagella the antigen-antibody compound will be formed in this situation, the usual change in the direction of salt-sensitiveness will occur, and, in the presence of electrolytes, the

bacilli will flocculate in the large, loose clumps characteristic of flagellar agglutination.

Antigen-antibody union in this situation does not, however, render the bacterium sensitive to lysis by complement, or to ingestion by phagocytic cells (see Felix 1924, Braun and Nodake 1924, Felix and Robertson 1928). For sensitization of this type to occur antigen-antibody union must take place at the bacterial surface.

It would seem, also, that the aggregates formed in flagellar agglutination are not of the kind that readily fix complement; though there is some uncertainty on this point (see Hofmeier 1927, Springut 1927). It seems possible that the absence, or paucity, of complement fixation may be due simply to the physical character of the aggregates and the rapidity with which they are formed.

The more deeply seated antigens will play no part in the serological reactions of the intact cell in its normal smooth form. They are inaccessible to the antibody. In suspensions that have been allowed to autolyse, or that have been broken up in some other way, these antigens may be liberated. They will then unite with the corresponding antibody to form a precipitate, and if complement is present and the conditions favourable this complement will be fixed.

To revert for a moment to the problem of the unity or diversity of antibodies, it is logical to say that an antibody acting on a flagellar antigen is an agglutinin, but not a lysin or a complement-fixing antibody or an opsonin; that an antibody acting on a surface antigen is an agglutinin and a lysin, and a complement-fixing antibody, and an opsonin; and that an antibody acting on an antigen that is normally situated below the bacterial surface is, when tested against bacterial extracts or autolysates, a precipitin and a complement-fixing antibody, but is not, so far as the normal cell is concerned, an agglutinin, or a lysin or a complement-fixing antibody or an opsonin. But none of these differences in reaction are determined by differences in the nature of the antibody, apart from its combining group; they depend on differences in the situation of the antigen with which it unites.

THE IMMUNOLOGICAL IMPORTANCE OF THE BACTERIAL SURFACE

It is at its surface that a bacterial cell reacts with the antibodies in the fluid that surrounds it. We have noted that the immunological behaviour of a normal cell, or of any of its variants, is determined by the character of the surface antigens. It would appear (see White 1933) that this dominance of the surface antigens is not confined to reactions in the test-tube. The antibodies produced as the result of inoculating bacteria into laboratory animals seem to correspond to the bacterial surface rather than to the bacteria as a whole. We have noted also that rough variants are usually avirulent, and avirulent bacteria are *ipso facto* of minor interest to the student of infective

disease. The antigens that primarily concern us are those that characterize the surface of the normal, smooth, virulent forms of pathogenic bacteria. Direct evidence that this is so will be found in Chapter VIII.

ILLUSTRATIVE EXAMPLES

The Antigenic Structure of Pneumococci.—The fact that pneumococci are divisible into sharply demarcated serological types has long been known (see Neufeld and Händel 1909, Dochez and Gillespie 1913, Dochez and Avery 1915, Avery *et al.* 1917); and we have already noted that Heidelberger and Avery succeeded in isolating from the three classical types of pneumococci complex polysaccharides that had the characteristics of haptens, in that they gave specific precipitation in the test-tube with the corresponding type-specific antisera, but failed to stimulate antibody production *in vivo*.

The more important chemical characters of these three haptens are summarized in Table X (Avery and Heidelberger 1925).

TABLE X
SHOWING THE CHARACTERS OF THE TYPE-SPECIFIC HAPTENS OF PNEUMOCOCCI.
(After Avery and Heidelberger.)

Type.	Optical Rotation.	Per cent. Nitrogen.	Substances obtained on Hydrolysis.	Dilution giving Specific Precipitation.
I	300°	5	Galacturonic acid and amino-sugar derivative	1 : 6,000,000
II	74°	0	Glucose	1 : 5,000,000
III	33°	0	Glucose and glucuronic acid . .	1 : 6,000,000

These haptens are certainly contained in the well-developed capsule that is so prominent a feature of a virulent strain of pneumococcus. Probably the capsule is composed mainly or entirely of this polysaccharide material.

When pneumococci undergo the S → R variation they lose their capsule, and with it the type-specific polysaccharide. They no longer agglutinate specifically with type-specific antisera, but react to antisera prepared against autolysed cultures of any pneumococcal type, or against certain streptococci.

In addition to the type-specific polysaccharide haptens, Avery and Heidelberger (1923) isolated from pneumococci a nucleo-protein fraction, precipitable by acetic acid. This fraction gave a precipitate with antisera corresponding to each of the three types.

We may, then, regard the capsular surface of a virulent pneumococcus as dominated antigenically by a type-specific polysaccharide; and we may note that some 32 different pneumococcal types have now been described (see Cooper *et al.* 1932). Beneath this capsule lies a nucleo-protein antigen, which perhaps dominates the surface of all pneumococci in the rough form.

This is certainly not a complete picture of the antigenic structure of the pneumococcus. There is a considerable body of evidence indicating the presence of other antigens.

Thus Enders (1930, 1932) has described a type-specific antigen in Type I pneumococcus that differs from the type-specific polysaccharide of Heidelberger and Avery; and Felton (1932) has described type-specific antigens in Type I and Type II pneumococci that appear to differ both from Heidelberger and Avery's haptens and from Enders' antigen.

Tillett, Goebel and Avery (1930) have isolated a carbohydrate substance from smooth and rough strains of pneumococci that gives precipitation with Type I, Type II and

Type III antisera. It would appear (Bailey and Shorb 1931, Eisler and Howard 1932) that the heterogenetic antigen of Forssman (see p. 156) or some antigenic substance with a similar active grouping is also present in the pneumococcus as a species.

The Antigenic Structure of Haemolytic Streptococci.—Haemolytic streptococci, like pneumococci, have long been known to be antigenically diverse. They are awkward organisms to handle serologically, since they show a marked tendency to auto-agglutination; but recent studies, and especially those of Griffith (1926, 1927), have established the existence of numerous serological types, that can be differentiated from one another by agglutinin absorption.

Like other pathogenic bacteria, haemolytic streptococci undergo a $S \rightarrow R$ variation. At least, they undergo a variation which is characterized by a loss of the type-specific surface antigen associated with the loss of virulence. Todd (1928*a, b*), who first clearly described this change, rejected the terms "smooth" and "rough," because, in fact, the virulent colony with the type-specific antigen is rougher than the avirulent colony in which the type-specific antigen is lost. He called the virulent form *matt*, the avirulent *glossy*, but we may retain S and R, if we define these shorthand terms as we did on p. 95.

Lancefield (1928) has separated antigenic components from haemolytic streptococci by chemical methods. She finds that the component that confers type-specificity is acid-soluble and contains some 14 per cent. of protein nitrogen. In its purified state it is hapten-like, in that it gives specific precipitation in the test-tube but fails to stimulate antibody-production *in vivo*. In addition to this type-specific antigen there is a polysaccharide component that is shared by many types of haemolytic streptococci, but differentiates the species into large sub-groups (Lancefield 1933). And there is a nucleoprotein antigen that is shared by a wide variety of streptococci, haemolytic and non-haemolytic. Studies by Todd and Lancefield (1928, see also Lancefield and Todd 1928) have shown that the change from the virulent *matt* to the avirulent *glossy* form is associated with the loss of the type-specific component, the polysaccharide component being retained. We may, then, regard the surface of virulent haemolytic streptococci as being dominated by the type-specific acid-soluble hapten.

The Antigenic Structure of the Typhoid-Paratyphoid Group.—In this group the analysis of antigenic structure by agglutination and agglutinin absorption has been developed in considerable detail. Chemical separation and purification of the very numerous antigens that the serologist has identified have inevitably lagged far behind; but we know enough to correlate chemical with serological findings in a few instances, and to feel confident that the same general type of structure applies over the whole field.

The great majority of the species or types of bacilli that fall within this group are flagellated. We have therefore to deal with both flagellar and somatic antigens. Until recent years ignorance of this structural differentiation and of the diphasic variation that affects many of the flagellar antigens prevented any escape from the confusion that enveloped this particular problem. It is to the studies of White (1926, 1929, 1930) and of Kauffmann (1929, 1930, see also Kauffmann and Mitsui 1930) that we owe the greater part of our present knowledge of the antigenic structure of the organisms in question.

Taking first the normal smooth forms of bacilli, some illustrative examples are set out in Table XI (see Lovell 1932), which gives the antigenic components of twelve of the types or species that fall within this group—some 36 have now been identified.

At the outset we are met with the difficulty that English workers following White, and German workers following Kauffmann, have chosen a different system of numbering and lettering to indicate the same series of antigenic components. White uses Roman numerals for his surface somatic antigens, so does Kauffmann; but White's factor III is Kauffmann's factor IX, White's II is Kauffmann's IV, and so on. White uses capital letters for his flagellar antigens. Kauffmann uses small italics for the flagellar antigens in the type or specific phase, and Arabic numerals for the flagellar antigens in the group phase. Noting this possible source of confusion for the benefit of those who wish to

TABLE XI

Bacterial Species or Type.	White.			Kauffmann.		
	O Antigens.	H Antigens.		O Antigens.	H Antigens.	
		Specific.	Group.		Specific.	Group.
Typhoid	III	S	—	IX	<i>d</i>	—
Enteritidis (Gaertner)	III	R, P ₁ , P ₂	—	IX	<i>g, o, m</i>	—
Dublin	III	R, P ₁ , P ₃	—	IX	<i>g, p</i>	—
Dar-es-Salaam	III	D ₁ , N ₁ , M ₁ , Y	—	IX	<i>e, n, l, w</i>	—
Paratyphoid A	VI	T	—	I, II	<i>a</i>	—
Paratyphoid B	I, II	Specific	G, A	IV, V	<i>b</i>	1, 2
Aertrycke (Breslau) . .	I, II	Specific	G, A, B, C	IV, V	<i>i</i>	1, 2, 3
Reading	II	D ₁ , D ₂	G, B, E ₁ , E ₂	IV	<i>e, h</i>	1, 4, 5
Eastbourne	III	D ₁ , D ₂	G, E ₁ , E ₂			
Hirschfeld (Eastern Paratyphoid C, East- ern Suipestifer)	V	W	G, E ₁	VI, VII	<i>c</i>	1, 4, 5
Western Paratyphoid C (Western Suipesti- fer Kunzendorf)	V	—	G, E ₁ , E ₂	VI, VII	—	1, 3, 4, 5
Bareilly	V	Specific	G, A, B, C, E ₁ , E ₂			

consult the original papers and ignoring the minor differences in White's and Kauffmann's findings, we may consider what the tabulated data actually imply.

Taking a few organisms as examples, and following White's terminology, we note that the typhoid bacillus has a single antigen III at the cell surface and another antigen S at the surface of its flagella. It is not diphasic; so the absence of group antigens is indicated by a minus sign. It should perhaps be added that White adds a (? +) sign to his factor III, indicating that absorption experiments suggest the presence of some other antigen in small amount. In abstracting these data from the more extensive tables given by Lovell (1932) signs of this kind have been omitted for simplicity. Turning to the aertrycke bacillus we find that it has at its surface two antigens I and II. When in the specific phase it has at its flagellar surface an antigen that White labels "*specific*." When in the group phase it has at its flagellar surface four antigenic components G, A, B and C, of which G is shared by all other diphasic members of this group, while the distribution of A, B and C is much more restricted. The Western type of the paratyphoid C bacillus is a curious exception to the general rule. It exists in one phase only; but that phase is group, not specific, in character. Its flagellar antigens are G, E₁ and E₂.

We are, then, dealing with an antigenically complex group, even if we confine our attention to the surface components of the normal, smooth forms.

The flagellar antigens we may dismiss very summarily. They are relatively heat-labile, being rendered inactive so far as test-tube reactions are concerned by exposure to a temperature of 60°–70° C. for 30–60 minutes. In this they contrast sharply with the surface somatic antigens, which will withstand boiling. Bacilli also become inagglutinable by anti-flagellar sera after extraction with absolute alcohol. Neither heat inactivation nor alcohol inactivation removes completely the capacity of stimulating the production of flagellar agglutinins *in vivo*, though this capacity may be greatly reduced. The flagellar antigens, and the corresponding agglutinins, are of considerable importance in diagnostic reactions (see Chapter XVIII), but they appear to be of little if any importance in the production of effective immunity.

Coming to the antigenic structure of the body of the bacterial cell we may inquire what lies beneath the surface antigens that we have tabulated above. We may note, in passing, that O (non-flagellated) variants of many of these bacteria occur in nature;

that these variants, so long as they retain their normal surface antigens, are fully virulent when tested in laboratory animals; and that they have occasionally been recovered from natural infections in man (see p. 299).

Our knowledge of the deeper antigenic structure of the bacilli of this group is mainly derived from the studies of White (1926, 1927, 1928, 1929, 1931*a*, *b*, 1932, 1933). The $S \rightarrow R$ variation is common and typical. The R variants give rough colonies and a granular growth in broth. They have lost the surface antigens that characterize the smooth form, and they have lost their virulence. These rough variants are very difficult to study serologically, since they tend to agglutinate spontaneously in saline; but this salt-sensitiveness can be removed by extraction with alcohol, ether or chloroform (White 1927, cf. Hartley's observations, p. 63). The salt-sensitiveness of the rough form is of interest as suggesting that the salt-insensitiveness of the virulent, smooth form may be due to the presence of the normal surface antigen (White 1928, 1932). The antigen that dominates the bacterial surface when the normal surface antigens have been lost—White calls it the " R " antigen—is shared by all the members of this group and by other more distinctly related species. Thus, so far as the somatic antigens are concerned, a rough variant of the typhoid bacillus behaves in the same way as a rough variant of a paratyphoid B bacillus, or of any other member of the group. In addition to the R antigen, or hapten, the surface of the rough variant appears to contain a minor amount of another antigen, or hapten (White 1933). This, the ρ antigen, is only partially unmasked in the usual rough variant; but a further loss variation may occur, in which the R antigen disappears. The surface of the cell is then dominated by the ρ antigen, or more probably by a pair of antigens ρ_1 and ρ_2 (White 1932, 1933). Situated still more deeply in the bacterial cell is another antigen " T " (White 1933). It would seem that this antigen never reaches the bacterial surface in young and actively growing cells; but it is exposed by extracting R or ρ strains with alcohol, or by allowing such strains to undergo partial autolysis.

If, therefore, we take a normal smooth virulent form—neglecting the flagellar antigens—and pass inwards from the surface we shall meet a series of antigenic components, only the most superficial of which will be characteristic of the particular type or species with which we are dealing. In the typhoid bacillus we shall meet in succession antigens $III : R : \rho_1 \rho_2 : T$, in the paratyphoid B bacillus $I, II : R : \rho_1 \rho_2 : T$, and so on.

Some day, perhaps, we shall employ antigenic formulæ to describe any given bacterial species. If we do, it is likely that we shall often give the surface formula only; for this will describe any variant quite adequately, and will tell us what we most want to know—the surface structure of the virulent smooth form.

When we come to chemical methods of analysis, our data are a little sketchy; but they are highly significant.

Furth and Landsteiner (1928, 1929) isolated several different components from bacilli of the typhoid-paratyphoid group, working with normal, smooth strains and with rough variants. Some of these components were polysaccharides, some were not. These studies taken in conjunction with those of White (1929, 1931*b*) make it clear that the surface antigens of the normal, smooth forms (I, II, III, IV and so on in White's terminology) are polysaccharides. There seems indeed to be a general rule, to which there are as usual a few exceptions, that the surface antigen of a virulent bacillus is a polysaccharide of one sort or another. The R, ρ_1 and ρ_2 antigens are also polysaccharides, or at least they behave as such in the ordinary tests (see White 1931*b*, 1932, 1933).

The T antigen is a protein substance, soluble in acid-alcohol (White 1933). There is another acid-soluble protein, which White (1932) has labelled " Q "; but this appears to be distributed more widely throughout the bacterial cell.

The Antigenic Constituents of Other Bacteria.—We may perhaps add a note on the antigenic components that have been isolated by chemical means from other species of bacteria.

The capsulated bacillus of Friedländer has given results entirely analogous to those obtained with the pneumococcus (Heidelberger, Goebel and Avery 1925, Julianelle 1926). The species may be divided into a number of sharply demarcated serological types. This type-specificity is conferred by a polysaccharide hapten present in the capsule. The body of the bacillus contains a nucleo-protein antigen that is shared by all types.

Polysaccharide haptens have also been isolated from the tubercle bacillus (Laidlaw and Dudley 1925, Enders 1929), from *Bact. lactis aerogenes* (Tomcsik and Kurotchkin 1928), from cholera vibrios (Landsteiner and Levine 1927, Jermoljewa and Bujanowskaja 1930), from Shiga's dysentery bacillus (Meyer 1930, Morgan 1931), from the anthrax bacillus (Tomcsik 1930, Schockaert 1928, Tomcsik and Szongott 1932) and from yeasts (Mueller and Tomcsik 1924, Stone and Garrod 1931, Duncan 1932a).

The case of the anthrax bacillus presents points of interest. The normal virulent form of this bacillus is capsulated and gives a rough colony on agar; the avirulent variant is non-capsulated and gives a smooth colony on agar (Preisiz 1904, Eisenberg 1912). This, then, is one of the cases in which the normal relation between smoothness and virulence is reversed. It was natural to suppose that the polysaccharide component isolated from *B. anthracis* was the capsular material; but Tomcsik and Szongott (1932, 1933) report that this is not the case. They state that the polysaccharide component is common to the virulent, capsulated, rough-colony-forming type and to the avirulent, non-capsulated, smooth-colony-forming type, and that the capsule is formed of a protein substance that is precipitated by copper sulphate, and has a nitrogen content of 10 per cent. It is of interest to compare these findings with those of Todd and Lancefield in the case of the hæmolytic streptococcus.

We have already mentioned the presence of Forssman's heterogenetic antigen in pneumococci. It would appear that this antigen, and perhaps other antigenic components with active groupings related to the haptens of the red cells, are present in a wide variety of bacteria—dysentery bacilli, bacilli of the typhoid-paratyphoid group and the anthrax bacillus among others (see Rothacker 1913, Jijima 1923, Fujita 1924, Schmidt 1925, Meyer 1926, 1930, 1931, Powell 1926, Yasui 1929, Combiesco *et al.* 1930, Eisler 1931, Eisler and Howard 1931).

The Sharing of Antigenic Components between Different Bacterial Types and Species.

In conclusion we may note certain instances in which a particular antigenic component is shared by bacterial types or species that are nearly or distantly related, or even by bacteria and micro-organisms that belong to a different class. We have discussed the group reactions that occur among members of the typhoid-paratyphoid group. We should perhaps note that this sharing of antigenic components is not confined to the flagellar antigens or to those situated deeply in the cell body, but extends to the surface somatic antigens of the virulent, smooth forms that are our particular concern. Thus the typhoid bacillus, Gaertner's bacillus and the Dublin, Dar-es-Salaam and Eastbourne types all share factor III. The paratyphoid B bacillus and the aertrycke type share factors I and II, and so on.

But there are other instances in which organisms far more widely separated in their other relationships share a common antigenic component. Thus (Avery *et al.* 1925, Julianelle 1926), the capsular polysaccharide of the Type II pneu-

mococcus is closely similar to the capsular polysaccharide of Type B in Julian-elle's classification of Friedländer's bacilli, and also to a polysaccharide isolated from a species of yeast (Sugg, Richardson and Neill 1929); and a polysaccharide obtained by the partial hydrolysis of gum arabic reacts specifically with antisera prepared against Type II and Type III pneumococci (Heidelberg, Avery and Goebel 1929). We have, indeed, already noted a still more curious sharing of antigens in our reference to the Forssman antigen, which occurs in the red cells of some species and in the tissues of others, but would appear to possess some active grouping that is represented either in some of the bacterial polysaccharides or in substances closely associated with them.

There is, indeed, nothing very bizarre in this occurrence of the same, or closely similar, antigenic groupings in living cells that have no close systematic relationship. The specificity that antigen-antibody reactions detect is, as we have seen, a chemical one. The fact that it is also biological depends on the way in which the chemical substances concerned are distributed in nature. It is quite probable that, as our search extends, we shall come on one instance after another in which antigenically similar substances are found in entirely dissimilar biological situations.

SUMMARY

(1) We can study the antigenic structure of bacterial cells by several different methods: (*a*) by the selective qualitative absorption of agglutinins, (*b*) by selective quantitative absorption, (*c*) by quantitative estimations of antigens based on the optimal proportions method, (*d*) by noting the changes in antigenic behaviour associated with particular types of bacterial variation, (*e*) by separating antigenic components from bacterial cells by chemical treatment and studying the immunological reactions of the components so separated, and (*f*) by treating bacteria with various chemical reagents and observing how their antigenic behaviour is altered by such treatment.

Of these methods (*a*), (*d*) and (*e*) have been most extensively explored, and our present knowledge of the antigenic structure of bacteria is derived mainly from data that have been obtained along these lines.

(2) The general picture that is suggested to us by these data is that of a bacterial cell with antigenic components distributed in an orderly fashion: some on flagella, some in capsules, where these cell excrescences are present, others on the cell surface. Below this surface there are others still: some just below it, others situated more deeply. In certain types of bacterial variation the normal surface antigens are lost, and some of the deeper antigens are exposed.

(3) This ordered antigenic anatomy determines differences in the functional activity of the different antigens when the bacterial cell, or its constituents, are exposed to the action of an antibody-containing serum. Union of flagellar antigens with their corresponding antibodies leads to agglutination, but not to lysis or opsonification. Union of surface antigens with their corresponding

antibodies leads to all three results, assuming that the cell is of a type that is susceptible to lysis by complement. The more deeply seated antigens cannot unite with their corresponding antibodies so long as they remain in the interior of the cell. When they are liberated they react and give rise to a precipitate.

(4) The antigens situated at the surface of the bacterial cell are of particular importance from the immunological point of view, since it is by reactions in this situation that the cell is rendered susceptible to ingestion by phagocytes or to lysis by complement.

There are indications, also, that antigens at the cell surface play a prominent part in stimulating the production of antibodies when intact bacteria gain access to the tissues.

(5) Illustrative examples are found in the recorded studies of the antigenic structure of pneumococci, hæmolytic streptococci, bacilli of the typhoid-paratyphoid group and certain other organisms.

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CHAPTER VII

THE MECHANISM OF ANTITOXIC IMMUNITY

For our immediate purpose this type of immunity may be dealt with very briefly.

Apart from certain cases of natural insusceptibility, such as the insusceptibility of birds and of cold-blooded animals to tetanus toxin (Vaillard 1892), immunity to bacterial exotoxins depends on the presence in the circulating blood of antitoxins which exert a specific neutralizing action. These antibodies may have been naturally acquired, as the result of past infections, overt or latent; or they may have been produced in response to purposeful immunization with modified or unmodified toxin. They can be passively transferred from one animal to another—naturally from mother to offspring *via* the placental vessels or in the colostrum; artificially by injecting an antitoxic serum into a susceptible host.

The original observations on this type of immunity were made by von Behring and Kitasato (1890), who showed that the sera of animals that had received repeated injections of non-lethal doses of tetanus toxin or of diphtheria toxin had acquired the property of specifically neutralizing these toxins and thus preventing their poisonous effects.

Since that time a host of antitoxic sera have been prepared and exploited for prophylactic or curative purposes. We are here concerned only with anti-exotoxins—in the sense in which these were defined in Chapter III. Among these we should include—in addition to diphtheria and tetanus antitoxins—the antitoxins that neutralize the erythrogenic toxin produced by the hæmolytic streptococcus and the toxins of the *Staphylococcus aureus*, of Shiga's dysentery bacillus, and of a variety of pathogenic anaerobes, such as *Cl. welchii*, *Cl. septique*, *Cl. œdematiens* and the lamb-dysentery bacillus. The antitoxin which neutralizes the toxin of *Cl. botulinum* is, of course, a typical anti-exotoxin; but, as we have explained above, it is doubtful whether *Cl. botulinum* should be included among the pathogenic bacteria in the ordinary sense, since there is little evidence that it is capable of giving rise to infection *in vivo*.

A characteristic of antitoxic immunity is the relatively low concentration of antitoxin in the circulating blood which suffices to afford protection against natural infection, provided that it has been produced as the result of active immunization. In the particular case of diphtheria in man, we have good reasons for believing that less than 0.01 unit of antitoxin per c.c. of blood serum will render a person immune to the natural disease (see Glenny 1925). Under

such conditions the initial toxin produced is neutralized as it passes from the site of infection before it reaches the susceptible cells. But it is clear that this small amount of circulating antitoxin will not suffice for long, and that more will be needed to neutralize additional toxin as and when it is produced. An actively immune person or animal differs from a non-immune not only in possessing circulating antitoxin but in the readiness and rapidity with which more antitoxin is produced in response to the stimulus provided by the access of toxin to the tissues (see Chapter XI). The equilibrium between the rate of toxin-production by the bacterium and the rate of antitoxin-production by the host is, in the immune animal, readjusted to the host's advantage. A degree of antitoxic immunity that afforded ample protection against infection with the toxigenic organism could of course be broken down by the inoculation, in a single dose, of an overwhelming amount of toxin.

The position is very different once the toxin has become anchored to the cell.

Glenny and Hopkins (1925) have studied the effect of the administration of various doses of antitoxin at different times after the intradermal injection of a Schick dose of diphtheria toxin into a guinea-pig. This amount of toxin produces a characteristic local reaction at the site of inoculation; 0.001 unit of antitoxin is sufficient to prevent this reaction if the toxin and antitoxin are mixed together before inoculation. If, however, the toxin is injected before the antitoxin, neither 10 units of antitoxin injected intravenously 15 minutes later, nor 1,000 units injected intravenously 30 minutes later, are sufficient to prevent the appearance of a small reaction at the site of the injection of the toxin. The size of the reaction is, however, very greatly reduced as compared with that which occurs when no antitoxin is given. If 10 units of antitoxin are administered intravenously 30 minutes after the intradermal injection of the toxin, or 1,000 units intravenously after $1\frac{1}{2}$ hours, or intramuscularly after 45 minutes, the size of the lesion is reduced to about half its usual diameter. A slight reduction in the size of the reaction is obtained if 1,000 units are injected intravenously after $2\frac{1}{2}$ hours, or intramuscularly after $1\frac{3}{4}$ hours; but the subcutaneous injection of this amount of antitoxin 15 minutes after the intradermal inoculation of toxin leads to no reduction in the size of the reaction. We may note the relatively enormous dose of antitoxin that is required to neutralize the effect of the toxin under the conditions of this experiment; 1,000 units of antitoxin are equivalent to 1,000,000 times the dose of toxin injected into the skin, yet this amount administered intravenously 30 minutes after the intradermal injection fails to suppress completely the local toxic effect.

The increased resistance afforded by specific antitoxic immunity is determined, almost entirely, by the interception and neutralization of the toxin before it reaches the susceptible cells.

A purely antitoxic immunity affords effective protection against such an infection as diphtheria, in which the tissue invasion is minimal and the ill effects are due almost entirely to the toxæmia. Does it afford effective protection against those bacteria that are invasive as well as toxigenic? We should

expect that it would protect only against that part of the total pathological effect that is due to the action of the toxin itself. If the toxin plays any important part in the invasive process, the antitoxin might to that extent lessen the extent of infection. But we should expect modification of the infection rather than complete immunity. The available evidence is in accord with our expectations.

Thus, the presence of circulating antitoxin, active against the rash-producing toxin of the hæmolytic streptococcus, affords protection against clinical scarlet fever, that is, against the obvious effects of the toxin itself; but it does not appear to protect against the local lesion in the throat—the acute tonsillitis (see Okell 1932). Again, it would seem (Burt-White, Colebrook and others 1930, Baird and Cruickshank 1930, Stent 1930) that the presence of circulating antitoxin, as revealed by a negative Dick test (see p. 147) in pregnant women during the later stages of gestation, affords little if any protection against a severe or fatal infection with hæmolytic streptococci during the puerperium. These clinical findings are borne out by the results of experiment. Thus, Parish and Okell (1927) found that a potent antitoxic serum, which would protect rabbits against acute toxæmic death following the intravenous injection of large doses of living cultures of hæmolytic streptococci, did not prevent a fatal infection leading to death after a week or more, associated with multiple foci of infection.

Closely analogous results have been obtained in experimental staphylococcal infections in the rabbit (Burnet 1929, Burnet and Kellaway 1930, Kellaway, Burnet and Williams 1930). The potent staphylococcal toxin is neutralized, in multiple proportions, by the specific antitoxin; and a rabbit can be actively or passively immunized against the poisonous effect of a toxin-containing filtrate. But such immunized animals are still susceptible to the injection of living staphylococci and succumb to a pyæmic infection, though they survive rather longer than non-immunized controls.

Thus, we may say that antitoxic immunity is highly effective *so far as it goes*—much more effective, within its own sphere, than the specific antibacterial immunity discussed in the next chapter; for it is direct in its action, does not depend on the efficient functioning of a complex system of phagocytic cells, and is complete and final in its effect on the noxious agent. If the invasive power of an infecting toxigenic organism is so low that the normal antibacterial defence mechanism can be relied on to suppress it, or to hold it in check, an added antitoxic immunity will afford almost complete protection against the corresponding disease.

This chapter is itself a summary.

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CHAPTER VIII

THE MECHANISMS CONCERNED IN SPECIFIC ANTIBACTERIAL IMMUNITY

HAVING described in outline what happens when antigens react with antibodies in the test-tube we are in a position to study the events that occur when a bacterial parasite gains access to the tissues.

The only sound basis for a discussion of this subject would be to treat it as a problem in comparative pathology, describing in turn the reaction to infection of unicellular organisms, of simple multicellular animals, of the invertebrates, and of the vertebrates, culminating in the mammals and man. It happens that, owing to the classical studies of Metchnikoff, our earlier knowledge of the cellular reactions involved in immunity was developed largely along these lines ; and any serious student of the subject should make himself familiar with the work of Metchnikoff and his school (see Metchnikoff 1901). For our present purpose, however, it will suffice to note that the mechanisms that are described in this chapter are clearly traceable, in their gradually increasing complexity and differentiation, from the simplest to the most highly organized of animal hosts. In the pages immediately following we shall be concerned almost entirely with happenings in the small mammals of the laboratory ; and it will be convenient to deal first with the reactions of normal animals, and then to consider how these reactions are modified by active or passive immunization.

The Reactions which follow the Intravenous Injection of Dyes or Suspensions of Inert Particles into Normal Animals.

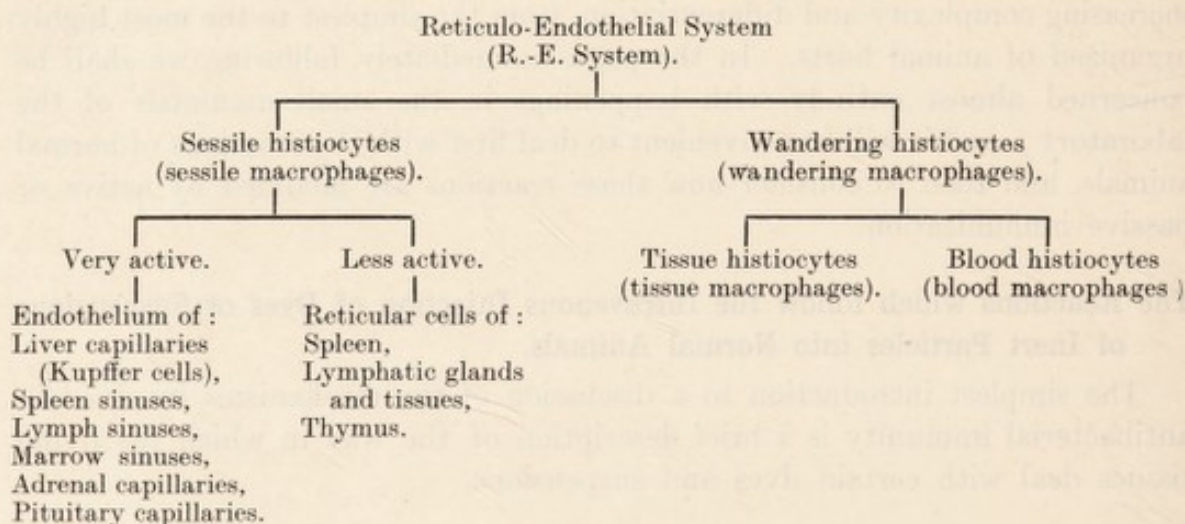
The simplest introduction to a discussion of the mechanisms involved in antibacterial immunity is a brief description of the way in which the living tissues deal with certain dyes and suspensions.

In pre-bacteriological days Hoffmann and von Recklinghausen (1867) and Ponfick (1869) noted that particles of carmine and vermilion, when injected intravenously into animals, were deposited in various organs such as the spleen, liver, lymph nodes and bone-marrow, where they remained recognizable for weeks, and that they were not eliminated in the urine or the bile. More recently, and particularly within the last decade, extensive and detailed studies have been carried out on the effects of intravital staining with a variety of dyes such as trypan blue, vital red, pyrrhol blue (isamine blue), diaminefast scarlet and benzopurpurin, or with suspensions of finely divided carbon (Indian

ink) or colloidal ferric oxide (saccharated oxide of iron). An account of a recent investigation along these lines and a good review of the literature will be found in a series of papers by Cappell (1929, 1930).

Briefly, these studies have shown that the various dyes are stored, in the form of granules or in intracellular vacuoles, by certain cells that have a characteristic distribution throughout the body. The histologist calls these cells *histiocytes*, the immunologist knows them best by Metchnikoff's name, *macrophages*. The most active may be divided into two main types, the sessile and the wandering. The most active of the sessile histiocytes are those found in certain specialized areas of the vascular or lymphatic endothelium—the endothelium of the liver capillaries (Kupffer cells), of the spleen sinuses, of the venous sinusoids of the bone-marrow, of the capillaries and medullary sinusoids of the adrenals, of the capillaries of the pituitary gland, and of the sinuses of the lymph glands throughout the body. Somewhat less active in this respect are the reticulocytes, which do not form a lining to blood or lymphatic channels, but are disposed about the reticulum fibres in the interstices of the tissues. The wandering histiocytes are found throughout the tissue spaces, and some of them find their way into the circulating blood, particularly into the vessels of the internal organs.

The common character of dye-storage possessed by cells of this type has led many observers, and particularly Aschoff (1924), to regard them as an integrated system of cells fulfilling a particular bodily function. Aschoff has coined for them the generic term of *reticulo-endothelial system* (R.-E. system, for short). This system, as described by him, is summarized in schematic form as follows.



We are here concerned mainly with the more active phagocytic cells, and particularly with the sessile histiocytes of the lymph glands, liver, spleen and bone-marrow, and with the wandering histiocytes in general.

As Cappell emphasizes, there is no gradual transition from the less specialized endothelium to that in which the sessile histiocytes are found. The endothelium of the lymph sinuses abruptly assumes a pronounced vital-staining capacity, in marked contrast to that of the endothelium of the afferent and efferent lymphatics ; while the same contrast exists between the endothel-

ium of the spleen sinus, liver capillaries, etc., and the vascular endothelium in general.

There is probably some degree of functional differentiation between different parts of the reticulo-endothelial system. Some histiocyte depôts may be more active in removing effete blood-corpuscles or leucocytes, others in removing bacteria or smaller particles. There is also a difference between one animal species and another in the relative activity of the various depôts of sessile histiocytes. Thus the bone-marrow of the mouse is far less active than the bone-marrow of the rabbit (Cappell 1930). In birds, the mass of the reticulo-endothelial system is concentrated in the liver.

The histiocytes are not, however, the only phagocytic cells with which we are concerned. The polymorphonuclear leucocytes of the blood, although they do not take up vital stains in the same way as the cells of the reticulo-endothelial system, play an active part in removing particulate material from the circulation under certain specialized conditions.

The intervention of these cells is, for instance, brought about by the injection, directly into the blood stream, of an Indian ink suspension or of some similar material. Following such an inoculation, the polymorphonuclear cells accumulate in certain areas—most abundantly in the pulmonary capillaries, to a less extent in the veins and sinusoids of the liver, spleen and other viscera. The abundance of polymorphonuclear cells in the lungs, which may be associated with a definite leucopænia in the peripheral circulation, is so characteristic a feature of the reaction as to make it quite clear that some mechanism is at work which temporarily retains the circulating leucocytes in this particular situation. While in the lungs the polymorphonuclear cells take up a certain number of the injected carbon particles; but the degree of phagocytosis is relatively slight, and in marked contrast to the active engorgement of particles by the histiocytes of the reticulo-endothelial system (Dudgeon and Goadby 1931). In the course of a few hours the majority of the polymorphonuclear cells leave the lung capillaries. Some of them pass into the alveolar spaces, some along the lymphatics to the regional lymph glands; but the great majority appear to make their way back into the general circulation and so pass to the spleen sinuses, where they appear to be taken up by macrophages.

There is still another mechanism at work in freeing the general circulation from injected foreign particles, in which no cellular elements are primarily concerned. Many suspensions, including carmine and Indian ink, are unstable when introduced into the blood stream. The dispersed particles are rapidly agglutinated into larger masses, and these appear to be filtered off from the circulating blood, during its passage through the finer capillaries. These aggregates are themselves phagocytosed at later stages. It has been noted that the aggregates of carbon particles contain large numbers of blood-platelets (Delrez and Govaerts 1918), and their formation is associated with a definite peripheral thrombopænia (Dudgeon and Goadby 1931). Whether the platelets play any active part in the process of flocculation is, however, very doubtful. The phenomenon of aggregation—certainly in the case of bacteria and presumably in that of any particulate material—appears to be unaffected after

the elimination of platelets by the injection of an antiplatelet serum (Govaerts 1921*a*, *b*, Bull and McKee 1922).

Confining ourselves for the moment to the particular case of the direct introduction of particulate matter into the blood stream, we may inquire in what ways our description needs modification or extension when our injected particles are living bacterial cells.

The Reactions which follow the Intravenous Injection of Bacteria into Normal Animals.

There is no doubt at all that the mechanism brought into play in clearing the blood stream from bacterial cells is in the main identical with that which frees it from inert particles of the same order of size (Wyssokowitch 1886, Werigo 1894, Opitz 1898, Bardach 1889, Métin 1900, Bail 1905, Bull 1914-16, Kyes 1916, Bartlett and Ozaki 1917, 1918, Wright 1927 and many others). The reticulo-endothelial cells, particularly those of the liver, spleen and bone-marrow, actively phagocytose the injected bacteria. The mechanism of aggregation, followed by the removal of the bacterial aggregates from the general circulation by their retention in the lung capillaries, is sometimes very prominent (Bull 1914-16, Bartlett and Ozaki 1917, 1918, Wright 1927, Dudgeon and Goadby 1931). This is accompanied, as in the case of the intravenous injection of carbon particles, by an accumulation of polymorphonuclear cells in the lung capillaries, associated with a temporary peripheral leucopænia (see Levaditi 1901, Andrewes 1910). But the polymorphonuclear cells appear to play a more active part in phagocytosing bacteria than in phagocytosing carbon particles (Dudgeon and Goadby 1931). It is probable that these cells with their ingested bacteria are subsequently carried to the reticulo-endothelial depôts in the spleen, liver and elsewhere, and are there phagocytosed by the sessile histiocytes. Such wandering histiocytes as are present in the lung capillaries ingest the bacterial cells, or cell-aggregates, directly.

So far then as we can regard bacteria merely as foreign particles, our description of the reaction of the tissues to the intravenous injection of vital stains or carbon particles needs little modification. But, in fact, the bacteria we inject are alive and capable of multiplication; and it is the balance between their capacity to multiply and the capacity of the tissues to remove them that determines the fate of the animal host.

Bull (1914-16) counted the viable bacteria in the systemic blood stream of dogs and rabbits at various intervals after the intravenous inoculation of a bacterial suspension. Using pneumococci and streptococci he found that there was at first a sharp and progressive fall in the number of organisms in the circulating blood, lasting until about the 5th hour. After this there was in most cases a secondary rise, varying in degree and persistence with the virulence of the organism and the resistance of the host. With strains of low virulence the numbers then declined until the blood became sterile. Typhoid bacilli, injected directly into the blood stream, showed a similar rapid fall in numbers within a few minutes of inoculation. In one experiment they fell

from 10,000,000 per c.c. 1 minute after injection to 40 per c.c. at the end of 15 minutes.

Wright (1927) has carried out a very detailed series of experiments on experimental pneumococcal septicæmia in the rabbit, and the following illustrative examples are taken from his paper.

TABLE XII

LIVING PNEUMOCOCCI PER C.C. OF CIRCULATING BLOOD AT STATED TIMES AFTER INOCULATION OF AVIRULENT, SLIGHTLY VIRULENT, AND HIGHLY VIRULENT PNEUMOCOCCI INTO NORMAL RABBITS.

Time.	Avirulent.	Slightly Virulent.	Highly Virulent.
Immediately	8,900,000	1,030,000	1,070,000
2 hours	206	20,800	137,000
5 „	2	340	25,000
24 „	0	1,300	1,510,000
48 „	—	134	Dead
96 „	—	0	—

In Table XII are set out the numbers of living pneumococci per c.c. of circulating blood at varying intervals after the intravenous injection of (a) an avirulent, (b) a slightly virulent, and (c) a highly virulent strain. In Fig. 5 the same series of observations are set out in chart form, the logarithms of the numbers of pneumococci per c.c. of circulating blood being plotted as ordinates against time as abscissæ. The figures need no comment. With an avirulent strain the clearance of the organisms from the blood stream is rapid and permanent. With a slightly virulent strain there is the same initial rapid clearance, so that 99·97 per cent. of the injected organisms have been removed from the blood stream by the end of the 5th hour. There is then a period during which the capacity of this strain of pneumococcus to multiply in the tissues—limited though it is—makes itself felt, and the number of viable organisms rises from 340 per c.c. at the 5th hour to 1,300 per c.c. at the 24th hour. But the clearing mechanism is now removing the newly generated bacterial cells at a greater rate than this particular strain can produce them. The number of organisms per c.c. sinks to 134 at the 48th hour, and the blood is sterile by the 96th. The highly virulent strain is not proof against the initial action of the clearing mechanism. By the 5th hour 97·7 per cent. of the injected pneumococci have been removed from the blood stream. But when the parasite's capacity for multiplication is brought fully into play it overwhelms the defence mechanism of the host. By the 24th hour the number of pneumococci per c.c. of circulating blood has risen to 1,510,000; and between the 24th and 48th hours the rabbit dies from acute pneumococcal septicæmia.

It is probable (Wright 1927) that the initial rapid clearing which follows the intravenous injection of a large dose of virulent pneumococci is the result of the lag phase in bacterial growth. When a subculture is made in the laboratory, by transferring a few loopfuls of a 24-hours' broth culture to a fresh tube of nutrient broth and incubating at 37° C., there is an interval of about 2 hours before any detectable multiplication occurs. After this the organisms pass into the phase of logarithmic growth, during which the number of viable organisms increases in geometrical progression. This phase lasts for some 6–8 hours. It is followed by the stationary phase, during which the death rate

and the birth rate—if we may use the latter term in reference to multiplication by simple binary fission—balance one another; so that the number of viable bacteria remains constant. This phase, in its turn, is followed by a prolonged phase of decline, during which the number of viable bacteria steadily decreases. When a 24-hours' culture of virulent pneumococci is injected into the ear-vein of a rabbit the clearing mechanism immediately comes into play, and its effects are not counterbalanced during the first few hours by any bacterial multiplication; so that the initial drop in the number of organisms in the circulating blood occurs just as it does with avirulent bacteria. Wright has shown that this initial clearance of virulent pneumococci can be almost eliminated by injecting a culture that is still in the logarithmic stage of growth.

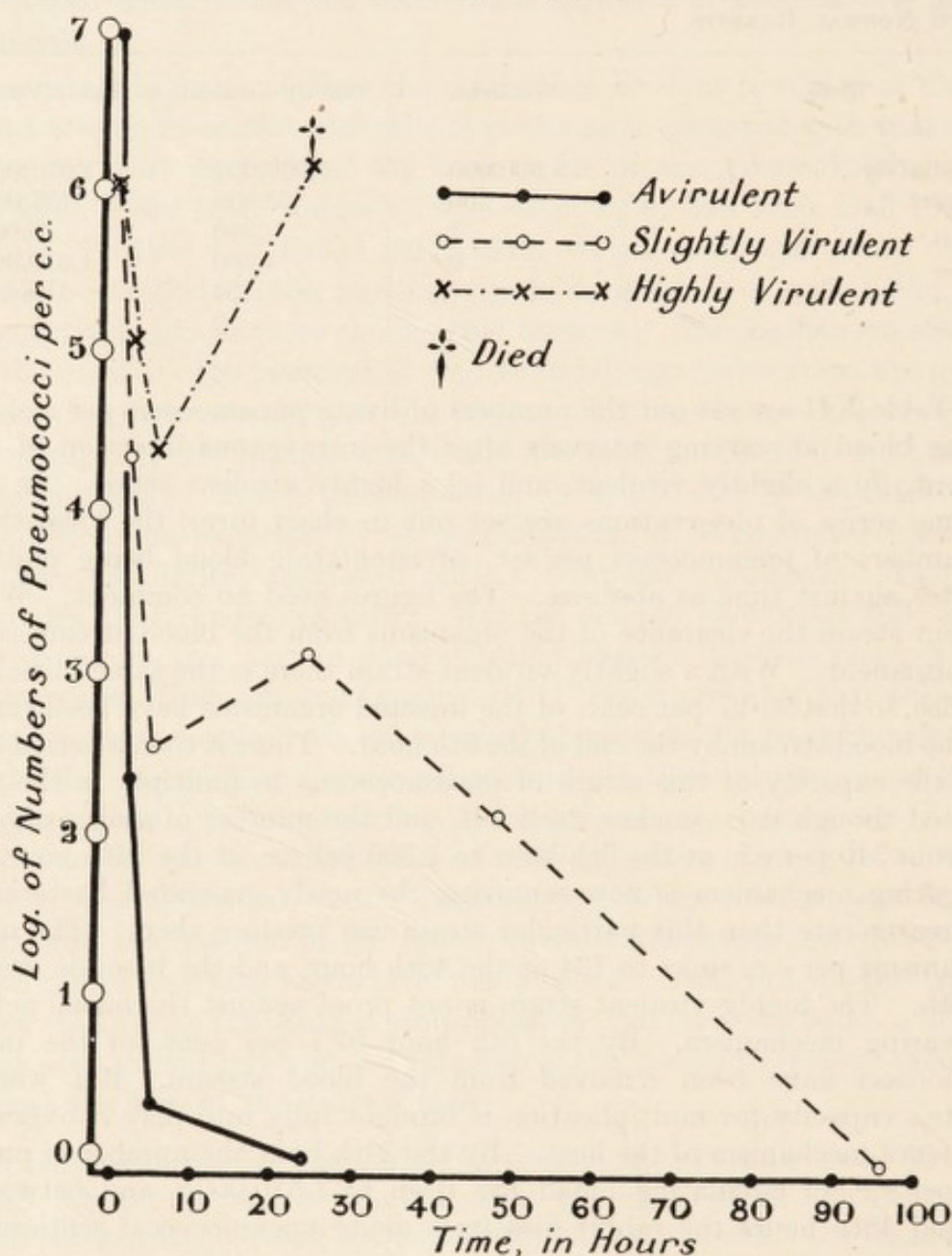


FIG. 5.

In the argument from analogy, which plays so large a part in the construction of our concepts of infection and resistance, we must be very careful to allow for the effects of our experimental technique. The reactions which we have described above are those that follow the sudden introduction into the

blood stream of a relatively small animal of a relatively enormous number of bacteria—a number of the order of 1,000,000,000. Such an occurrence must be of extreme rarity in natural infection, and it is possible that some part of the mechanism we have described—for instance, the aggregation of bacterial cells and the retention of the aggregates in the lung capillaries—may have been over-emphasized by the particular experimental procedure that we have chosen to employ. In any case there seems little doubt that the most significant happenings are those that occur between the 5th and 24th hours after an intravenous inoculation; when the fate of the host is being determined by the balance between bacterial multiplication, on the one hand, and the removal of the newly produced bacterial cells on the other.

Even when the tide has for the moment turned decisively in favour of the host, it does not follow that there will be a complete sterilization of the tissues. Bacteria will have been caught up in the liver, spleen and elsewhere, and there some of them may remain alive but relatively inactive for considerable periods.

Our knowledge of the exact method by which bacteria are *killed* in the tissues is woefully incomplete. There is no doubt at all that the majority of the bacteria taken up by phagocytic cells are destroyed by intracellular digestion; but the tacit assumption that a bacterium phagocytosed is, of necessity, a bacterium finally disposed of is certainly unwarranted.

It is possible, as Rous and Jones (1916) have suggested, that ingestion by a phagocytic cell may sometimes protect a bacterium against bactericidal substances in the body fluids, and that wandering phagocytes may sometimes play a part in the spread of infection within the tissues. But it is probable that any such happenings represent a chance and local breakdown in a mechanism that under most conditions is highly efficient.

The Reactions that follow the Intraperitoneal Injection of Bacteria or of Suspensions of Inert Particles into Normal Animals.

The small quantity of fluid present in the normal peritoneal cavity, or other serous sac, contains very few cells, and these consist almost entirely of mononuclears, mainly of the lymphocyte type (Dudgeon and Ross 1906, Cappell 1930, and many others).

Durham (1897), in his classical paper on the reaction to intraperitoneal infection, described the course of events that follows the injection of bacterial and other suspensions into the peritoneum of the guinea-pig. As regards the changes in the cellular contents of the exudate, there is a preliminary period lasting for about 1 hour or less during which no increase in cells can be detected. The cellular reaction, when it occurs, differs widely in degree and in character according to the kind of inoculum employed. With injections of dead bacteria, or of living strains of low virulence, there is a rapid emigration into the peritoneal cavity of polymorphonuclear leucocytes (or *microphages*, if we employ the terminology of Metchnikoff) which ingest the bacterial cells. These cells increase in number up to the 8th hour or later, and then slowly decline. As they decline, their place is taken by large mononuclear phagocytes (*macrophages*), which ingest those bacteria that still remain free in the fluid, together

with many of the polymorphonuclear cells and the bacteria that they have already phagocytosed. Durham puts the duration of the whole period of peritoneal reaction, from the time of the first appearance of the microphages to the final disappearance of the macrophages and the return to normal, as 4 to 7 days.

Although this microphage-macrophage succession may be regarded as the modal reaction in the peritoneal exudate following the injection of a bacterial suspension, it requires considerable modification in the light of subsequent studies (Dudgeon and Ross 1906, Buxton and Torrey 1906*a, b, c, d*, Cappell 1930). The cellular content of the exudate during the earlier hours of the reaction is by no means confined to polymorphonuclear cells, and other cells present during this period may be actively phagocytic. Cappell (1930) believes that the appearance of typical macrophages during the later stages of the reaction is due not to the emigration of fully formed cells of this type from some neighbouring depôt of histiocytes, such as the omentum, but to the gradual maturation of the small mononuclears that are present in the exudate from the earliest stages. These he regards as immature macrophages, and he believes them to be derived mainly from the small mononuclear cells that are so plentiful in the *tâches laiteuses* and adventitial sheaths of the omentum and mesenteries.

These events in the fluid exudate form only a part, perhaps only a minor part, of the general peritoneal reaction. Certain specialized regions of the peritoneal membrane, and of the tissues which are subjacent to it, play a very important rôle.

Among these specialized tissues the omentum is pre-eminent (Durham 1897, Buxton and Torrey 1906*d*, Dudgeon and Ross 1906). Just as particulate material injected into the circulation tends to be aggregated into masses that are caught up in the lung capillaries, so the particles or cells in a suspension injected into the peritoneum are aggregated into masses that are deposited on the omental surface; and, just as the polymorphonuclear cells of the circulating blood tend to collect in the lung capillaries together with the aggregated particles, so such cells as are present in the peritoneal exudate tend to collect on the surface of the omentum. On this surface, and in the interstices of the underlying tissue, macrophages are normally present, especially in the milk-spots, or *tâches laiteuses*; and these cells play from the first an active part in the phagocytosis of the deposited particles or cells. Ingestion completed, these cells migrate into the deeper omental tissues, changing in shape as they do so from the spheroidal or slightly irregular form of the macrophage of the body fluids or surfaces to enormously elongated trailing cells, which make their way through the interstices of the tissues. When such cells have phagocytosed large amounts of some indigestible material, such as carbon particles, they may be detected in the tissues for periods of weeks or months. The part played by polymorphonuclear cells in the omental reaction appears to vary with different inocula. According to the observations of Buxton and Torrey, they are absent or very scanty during the first few hours or so, but they then begin to collect in increasing numbers and to play their part in the phagocytic reaction.

The reaction that follows an intraperitoneal injection is not, however, limited to the peritoneal cavity. Indeed, it spreads beyond it with surprising rapidity.

Muscatello (1895) injected carmine suspensions intraperitoneally, killed the inoculated animals after various intervals, and studied the distribution of the dye. Within 1-2 hours he found particles of carmine in the liver and spleen. Similar particles, in smaller numbers, were found in the lungs, pancreas and testis. Durham (1897) noted the rapid passage of bacteria from the peritoneal cavity to the blood stream, and concluded that the most important route of transit was *via* the diaphragmatic lymphatics, the anterior mediastinal glands and the right lymphatic duct. The importance of the diaphragmatic route was also noted by Dudgeon and Ross, and the correctness of Durham's conclusions has been confirmed by many subsequent observers, notably in the careful studies of Buxton and his co-workers, and in the more recent studies of Bolton (1921).

Buxton and Torrey (1906*b*) found that, almost immediately after the intraperitoneal injection of an Indian-ink suspension, there was a rush of carbon particles into and through the lymphatics of the diaphragm. Thence they passed rapidly through the anterior mediastinal lymphatics and the corresponding lymph glands, reaching the blood stream within a very few minutes. During the earliest stages of this transit there was very little evidence of phagocytosis in the mediastinal glands or elsewhere; but, as time passed, an increasing number of carbon particles were found within the histiocytes of the lymph nodes.

In other experiments (Buxton 1906, Buxton and Torrey 1906*a*) the fate of living typhoid bacilli, injected intraperitoneally into rabbits, was followed by quantitative plating methods. The living bacilli passed into the general circulation almost immediately; and their numbers per c.c. of circulating blood reached a maximum after about an hour. After this there was a rapid decrease, and few bacilli could be recovered from the blood after about the 6th hour. When suspensions of liver and spleen tissue were plated it was found that there was a rapid accumulation of bacilli in these organs, and particularly in the liver, within a few minutes after an intraperitoneal injection. There was then a transitory decrease in the numbers of viable organisms, followed by a secondary increase lasting from the 2nd to the 6th hour, *i.e.*, the bacilli were increasing in the liver and the spleen while they were decreasing in the blood. After about the 6th hour there was a general decrease, lasting for several days, and more rapid in the liver than in the spleen. When animals were killed 4 days after injection viable bacilli were still recovered from the liver, though in small numbers; larger numbers were recovered from the spleen, and very large numbers from the mediastinal glands. Many observations on laboratory animals have shown that living bacilli may be recovered from the spleen weeks or months after experimental infection (Topley and Wilson 1923, Price-Jones 1927).

It may be noted that the rapid transit of particles or bacteria from the peritoneum to the blood stream is almost certainly the result of the mechanical pumping action of the rise and fall of the diaphragm in breathing. This is probably assisted by a special arrangement of the endothelial cells of the peritoneal membrane covering the diaphragm, which provides for a rapid passage of particles to the underlying lymphatics (Florey 1927). In this sense a massive peritoneal infection may be regarded as a special case, which approximates, in its effects on the body as a whole, to the direct introduction of bacteria into the blood stream.

The Reactions that follow the Injection of Bacteria or Inert Particles into Normal Animals by Other Routes.

It is not necessary to describe in any detail the course of events which follow the injection of particulate material by other routes, such as the intramuscular or subcutaneous. Cappell (1929) gives a full description of the spread of soluble and colloidal dyes, or of carbon particles, from the local site of injection, and reference may be made to his paper for fuller details on this point. It suffices for our present purpose to note that spread is slower and more restricted than after intraperitoneal inoculation, and that the injected particles or bacterial cells may pass so slowly on their centripetal journey that the great majority of them are caught up in the histiocyte depôts of the regional lymphatic glands before they can reach the blood stream. The centripetal transit is more rapid after intramuscular than after subcutaneous inoculation, owing to the increased rate of lymph flow resulting from muscular contractions.

It should be noted that it does not follow that the injection of bacteria directly into the blood stream is necessarily more fatal to the host than injection by other routes. Indeed, it would appear that the reverse is often the case, when small inocula are employed.

Lange and Gutdeutsch (1928) found that pneumococci, streptococci and *Pasteurella* were more fatal to mice when injected intraperitoneally than when injected subcutaneously or intravenously, and that the subcutaneous was rather more fatal than the intravenous route. In the case of *Erysipelothrix rhusiopathiae*, the bacillus causing swine erysipelas and mouse septicæmia, the subcutaneous route of injection was found to be more fatal than the intraperitoneal or intravenous. According to Sobernheim and Murata (1924), the dose of *B. anthracis* required to kill a guinea-pig after intravenous or intraperitoneal inoculation is about ten times the dose that will produce a fatal infection when injected by the subcutaneous or intracutaneous routes.

The probable reason for this apparent anomaly is that bacteria injected intravenously are at once exposed to the full force of the highly efficient clearing mechanism that protects the blood stream. If the number of bacilli injected is small, they may all be removed without a nidus of infection being established in the tissues. As we have seen, it is the progressive secondary infection of the blood stream from a centre of bacterial multiplication established somewhere in the tissues that is the usual precursor of a fatal septicæmic infection.

The Reactions that follow the Administration of Pathogenic Bacteria to Normal Animals by the Mouth.

Ørskov and his colleagues (Ørskov, Jensen and Kobayashi 1928, Ørskov and Moltke 1928, Ørskov and Lassen 1930) have recorded a series of experiments in which they fed mice with *Bact. aertrycke* and allied strains of the salmonella group by dropping measured amounts of a broth culture, or of a saline suspension, into the open mouth. The animals so treated were killed after intervals varying from a few hours to a month or more, and cultures were prepared from the blood, liver, spleen, mesenteric glands, the small intestine at different levels, and certain other situations. In this way it was possible to follow the spread of infection throughout the body. These observations are of particular interest because they are concerned with a natural disease of mice, and because the portal of entry is that by which the parasite gains

access to its host in the natural spread of the disease. The results obtained may be summarized as follows.

It would seem that the bacilli, when they gain access to the body by the mouth, fail to establish any immediate foothold in the intestine, and for the most part rapidly succumb. A certain number, however, enter the tissues from the alimentary tract and are carried to the mesenteric lymph glands. Later they enter the blood stream, probably *via* the thoracic duct, but are rapidly removed from the circulation by the reticulo-endothelial cells, particularly those of the liver and the spleen. During this phase the blood taken from the heart is sterile, and *Bact. aertrycke* cannot usually be recovered from the intestine; while the liver, spleen and mesenteric glands show its presence in increasing numbers. In many animals this stage is followed by a secondary bacteraemia, which increases in intensity until the animal succumbs. The intestine, which as stated above is rapidly freed from the invading bacteria during the primary stage of the infection, becomes secondarily infected during its later stages, probably by way of the bile-duct.

A similar series of experiments were carried out using *Bact. paratyphosum B* as the infecting agent—an organism closely related to *Bact. aertrycke*, but far less pathogenic for the mouse. The same rapid disappearance from the intestine was noted, and the same localization in the lymphatic glands; but in this case there appeared to be no tendency for the infection to spread beyond this primary focus, though the bacilli might persist in the mesenteric glands for weeks or months. A relatively avirulent variant of *Bact. aertrycke* behaved in the same way as *Bact. paratyphosum B*.

Bactericidal and Bacteriolytic Reactions.

It is very difficult to assess with any accuracy the part played by the lytic action of complement on sensitized bacterial cells. There is little doubt that this purely humoral effect is much less important than was at one time supposed, but it is certainly operative to some degree with certain organisms and under certain conditions.

Thus, apart from Pfeiffer's classical experiments (see pp. 70, 129), Buxton and Torrey (1906*d*) note that many of the typhoid bacilli that collect on the surface of the omentum after an intraperitoneal inoculation are destroyed by extracellular lysis instead of being ingested by macrophages, and many workers have recorded analogous observations. In immunity to such Gram-positive organisms as pneumococci or streptococci it would seem, however, that this purely humoral mechanism plays no part.

THE REACTION OF ACTIVELY IMMUNIZED ANIMALS AS COMPARED WITH THAT OF NORMAL ANIMALS

We have so far been concerned only with the reaction of normal animals to bacteria of varying virulence; and we have seen that the normal clearing mechanism, while highly effective against a strain that has little capacity for multiplication within the tissues, may be overwhelmed by a more virulent

strain that is capable of establishing multiple or extensive foci of active infection from which the circulation is continuously flooded.

So far as this particular mechanism is concerned, we can summarize the difference between an actively immunized and a normal animal very briefly, by saying that an immunized animal behaves towards a virulent strain of a

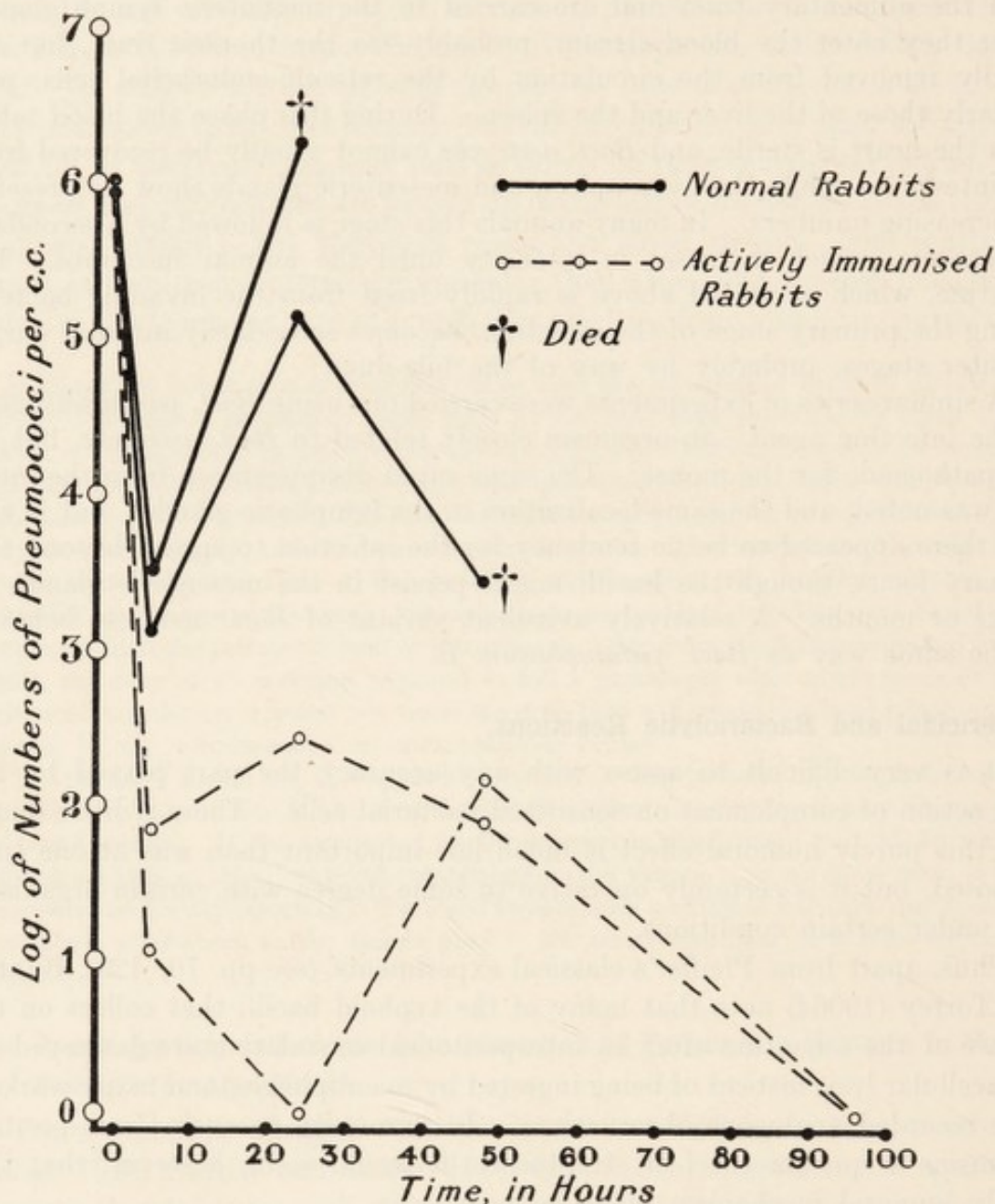


FIG. 6.

particular pathogenic bacterium in the same way as a normal animal behaves towards an avirulent, or slightly virulent, strain of the same bacterial species. Without any attempt to recapitulate in detail, we may quote a few illustrative examples.

Wright (1927) studied the response, to the intravenous injection of virulent pneumococci, of rabbits that had been immunized by the injection, at various

intervals before the test inoculation, of a killed culture of the same strain. Table XIII and Fig. 6 show the results obtained in two rabbits that had been immunized 3 months previously, and in two normal controls injected with the same dose of the same living culture. Comparison with Table XII and Fig. 5 will show that the immunized rabbits dealt with the highly virulent culture in the same way as the normal rabbit of Table XII dealt with the slightly virulent strain.

TABLE XIII

SHOWING THE NUMBER OF PNEUMOCOCCI PER C.C. OF CIRCULATING BLOOD AT VARIOUS TIMES AFTER INOCULATION OF A VIRULENT STRAIN INTO NORMAL AND INTO ACTIVELY IMMUNIZED RABBITS.

Time after Injection.	Normal.		Immunized.	
	Rabbit 247.	Rabbit 248.	Rabbit 299.	Rabbit 300.
Immediately	870,000	1,100,000	1,000,000	1,000,000
5 hours	1,300	3,300	12	68
24 „	142,000	1,953,000	0	289
48 „	2,800	Innumerable	149	79
96 „	Dead	Dead	0	0

The phagocytic reactions that we have described are illustrated in Figs. 7, 8, 9, and 10, for which I am indebted to Professor H. D. Wright.

The reaction in the lung capillaries in these immunized animals is illustrated by the following figures (Wright 1927) obtained from a film preparation.

Total number of pneumococci seen	893
Phagocytosed by polymorphonuclear cells	297
Phagocytosed by mononuclear cells	340
Total phagocytosed	637
Total outside phagocytes	256
In unphagocytosed aggregates associated with platelets.	206
In free aggregates	53

The results that have been obtained when immunized animals have been injected with virulent bacteria by other routes—by intraperitoneal or subcutaneous injection or by the mouth—are in entire conformity with those described above. An immunized animal reacts to a highly virulent bacterium as does a normal animal to one of lower virulence. The exact degree of difference in behaviour depends on the grade of immunity that has been established.

The immunity with which we are here concerned is, it should be noted, strictly specific. The influence of non-specific factors on immunity is considered in Chapter XIII.

The Passive Transference of Antibacterial Immunity.—A fact of the greatest significance is that the increased resistance that an actively immunized animal enjoys in virtue of the increased efficiency of the defence mechanism



FIG. 7.

Showing phagocytosis of pneumococci by macrophages in the liver of an immunized rabbit.



FIG. 10.

Lung of an immunized rabbit, showing phagocytosis of pneumococci in capillaries of alveolar walls.

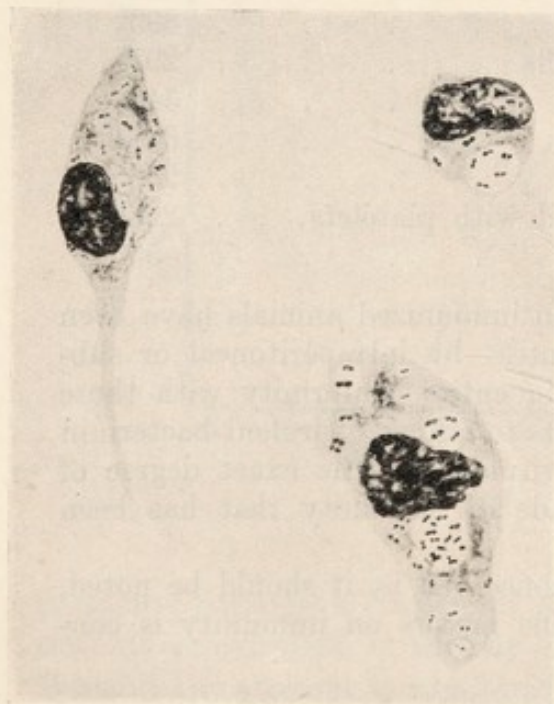


FIG. 8.

Showing phagocytosis of pneumococci by the Kupffer cells, from the liver of an immunized rabbit.

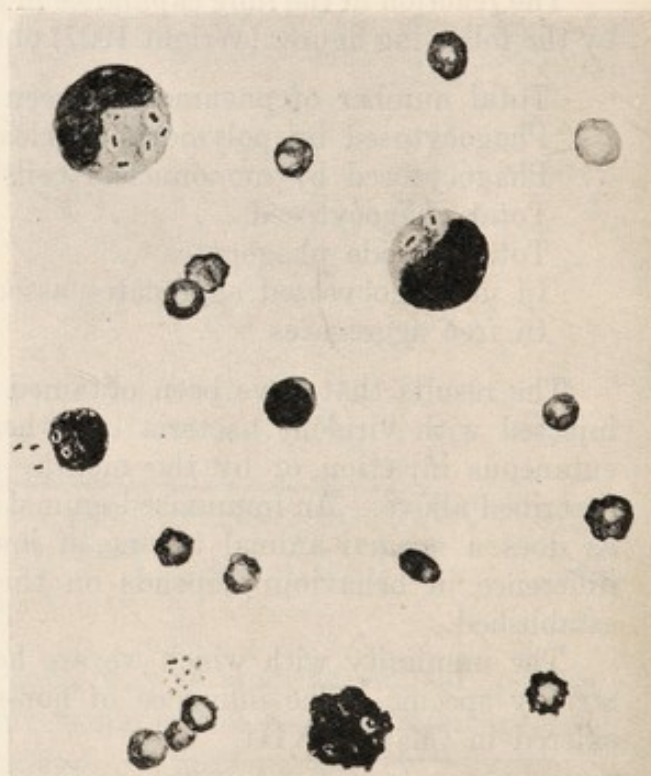


FIG. 9.

Blood from the liver of an immunized rabbit, showing phagocytosis of pneumococci.

considered above can be passively transferred from an immunized to a normal animal by injecting into the second the blood serum of the first.

It follows that we must regard the mechanism by which virulent bacteria are disposed of when once they have gained access to the tissues of an immunized

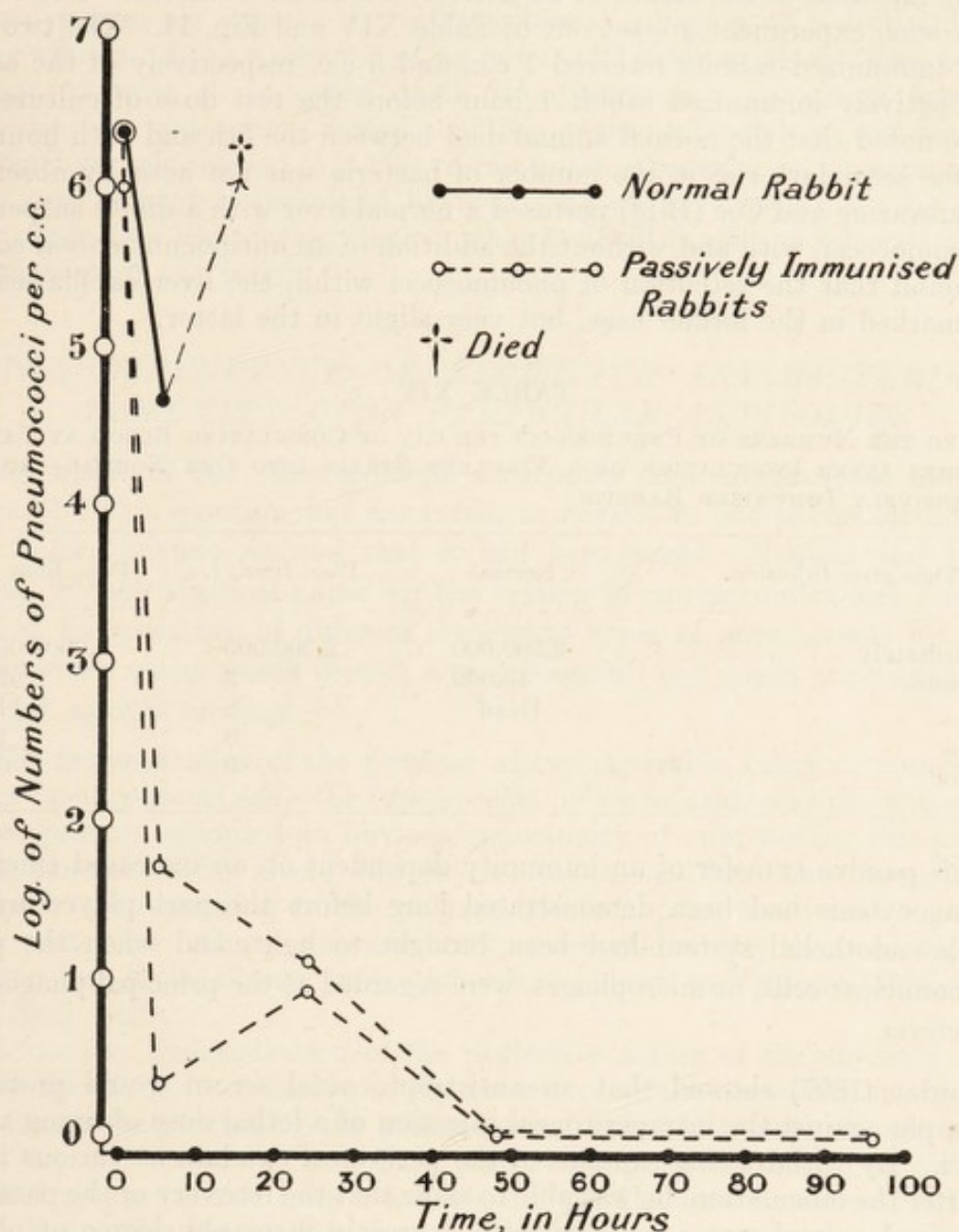


FIG. 11.

animal as consisting in the main of an integrated system of cellular reactions conditioned by the presence of specific antibodies in the blood or tissue fluids.

Again, a few illustrative examples will suffice.

Bull (1915a, b) found that, in a rabbit in which a bacteraemia had been established by the intravenous injection of pneumococci, the bacteria were removed from the circulation within about 15 minutes after the intravenous

injection of 0.2-0.5 c.c. of antipneumococcus serum per kilo-body-weight of the rabbit.

Wright (1927) showed that the clearing mechanism of a normal rabbit could be rendered highly effective against virulent pneumococci by the intravenous injection of the serum of an actively immunized animal. The results of one such experiment are set out in Table XIV and Fig. 11. The two passively immunized rabbits received 1 c.c. and 5 c.c. respectively of the serum of an actively immunized rabbit 1 hour before the test dose of culture. It will be noted that the normal animal died between the 5th and 24th hours, so that the secondary rise in the number of bacteria was not actually observed.

Manwaring and Coe (1914) perfused a normal liver with a dilute suspension of pneumococci, with and without the addition of an antipneumococcal serum, and found that the retention of pneumococci within the liver capillaries was very marked in the former case, but very slight in the latter.

TABLE XIV

SHOWING THE NUMBERS OF PNEUMOCOCCI PER C.C. OF CIRCULATING BLOOD AT VARIOUS TIMES AFTER INOCULATION OF A VIRULENT STRAIN INTO ONE NORMAL AND TWO PASSIVELY IMMUNIZED RABBITS.

Time after Injection.	Normal.	Pass. Imm. 1.	Pass. Imm. 2.
Immediately	2,300,000	2,300,000	2,000,000
5 hours	43,000	2	52
24 „	Dead	8	14
48 „	—	0	1
96 „	—	0	0

This passive transfer of an immunity dependent on an increased efficiency of phagocytosis had been demonstrated long before the part played by the reticulo-endothelial system had been brought to light, and when the polymorphonuclear cells, or microphages, were regarded as the principal phagocytes of bacteria.

Bordet (1897) showed that an antistreptococcal serum would protect a guinea-pig against the intraperitoneal injection of a lethal dose of living streptococci. By withdrawing samples of the peritoneal exudate at various intervals after the inoculation, he was able to show that the recovery of the passively immunized animal was associated with a greatly increased degree of phagocytosis, mainly by the polymorphonuclear cells. By a very ingenious experiment he demonstrated that the relatively slight degree of phagocytosis in the peritoneal exudate of the normal animal could not be ascribed to an absence of activity on the part of the leucocytes. If a suspension of *Proteus* bacilli were injected into the peritoneum of a normal guinea-pig at a time when virulent streptococci were multiplying actively therein without undergoing any considerable degree of phagocytosis, the leucocytes at once ingested the *Proteus* bacilli in enormous numbers.

It happened that the first experimental demonstration of passive anti-

bacterial, as opposed to antitoxic, immunity was made in a case in which the direct bacteriolytic action of the antiserum was particularly prominent.

Pfeiffer and his colleagues (see p. 70) found that the serum of a guinea-pig that had been immunized against *Vibrio cholerae* would protect a normal guinea-pig against the intraperitoneal injection of a lethal dose of living vibrios. Such a serum had no power to protect against a dose of dead vibrios so large as to cause death from toxæmia; its action was antibacterial, not antitoxic. Samples of the peritoneal exudate withdrawn at various intervals showed that the vibrios became granular in appearance, and they were ultimately destroyed by lysis. As we have seen, a similar change may occur, to a less marked degree, when typhoid bacilli are injected into the peritoneum of the rabbit.

THE DEPENDENCE OF EFFECTIVE ANTIBACTERIAL IMMUNITY UPON PARTICULAR ANTIBODIES

On which of the various serum antibodies does antibacterial immunity depend? This question was answered, in respect to one particular infection long before anyone realized that it had been asked. Neufeld and Händel (1909), in their classical paper on the testing of antipneumococcal sera, first showed the existence of different serological types of pneumococci by noting that a given serum would protect a mouse against one strain of pneumococcus but not against another.

The demonstration of the presence of two separable antigenic components in the pneumococcal cell—the type-specific polysaccharide and the non-specific nucleo-protein—afforded an obvious opportunity of approaching this problem along more satisfying lines; and the results obtained were unequivocal. The antibody corresponding to the polysaccharide hapten was found to have a high protective value: the antibody corresponding to the nucleo-protein antigen had little, if any, protective effect (Avery and Morgan 1925, Avery and Neill 1925).

A decisive demonstration of the protective action of the antibody acting on the specific polysaccharide, in the absence of antibodies acting on any other constituent or product of the pneumococcal cell, has recently been recorded by Avery and Goebel (1931). They prepared a synthetic antigen by obtaining purified polysaccharide from a culture of Type III pneumococcus and linking it to horse globulin, and with this antigen they immunized rabbits. The antisera so obtained protected mice against Type III pneumococci, but not against Type I or Type II pneumococci. We must not, of course, infer that no other antibody plays any part in antipneumococcal immunity; but we may certainly infer that this particular antibody is effective when acting by itself.

In this connection an observation of considerable interest was made by Sugg, Richardson and Neill (1929). It was found that a particular variety of yeast contained the specific polysaccharide component of the Type II pneumococcus. Vaccination of mice with a heated suspension of this yeast significantly increased their resistance to Type II pneumococcus, but not to Type I or Type III.

Julianelle (1926) has reported entirely analogous findings in the case of Friedländer's bacillus. A serum containing antibodies for one of the type-specific polysaccharides protects mice against the corresponding serological type. A serum containing only antibodies corresponding to the common nucleo-protein antigen affords no protection.

It was, however, in the case of the motile bacilli, possessing flagellar and somatic antigens, that the problem of the different immunizing value of different bacterial antigens was first stated in its clearest and most explicit form by Felix and his colleagues (see Felix 1924). The results of experimental studies carried out during the past few years have afforded strong support to his contention that the flagellar antigens, and the corresponding antibodies, play little if any part in the mechanism of specific antibacterial immunity, while the heat-stable somatic antigens and the antibodies that react with them are all important.

Arkwright (1927) immunized guinea-pigs by the injection of vaccines prepared from different strains of *Bact. typhosum* and *Bact. paratyphosum A*, and subsequently tested their resistance by the injection of living virulent bacilli.

In the case of *Bact. typhosum*, a vaccine prepared with a smooth, motile, virulent strain (containing the flagellar antigen and the surface antigen III) produced a significant increase in resistance. A vaccine prepared from a rough, motile variant (containing the flagellar antigen but not the surface antigen III) did not.

In the case of *Bact. paratyphosum A* four vaccines were used: (1) a smooth, motile strain, with the flagellar antigen and the surface antigen VI, (2) a non-motile, smooth variant, with no flagellar antigen but retaining the surface antigen VI, (3) a rough, motile variant, with the flagellar antigen but without the surface antigen VI, and (4) a rough, non-motile variant containing neither the flagellar antigen nor the surface antigen VI. Vaccines (1) and (2), containing the antigen VI, produced a marked increase in resistance. Vaccines (3) and (4), from which this antigen was absent, did not.

Ibrahim and Schütze (1928) obtained analogous results in the immunization of mice against *Bact. aertrycke*. They used seven different vaccines derived from smooth and rough strains and killed by heating for different times at different temperatures. They found that the rough variants, in which the specific polysaccharide antigen was absent, were ineffective as immunizing agents. The smooth variants, containing the specific polysaccharide antigen, produced a significant increase in resistance; but prolonged heating (2 hours) at 100° C. reduced their immunizing value.

Robertson and Felix (1930) have recently reported that an antiserum to *Cl. septicum*, containing antibodies against the heat-stable, somatic antigen, but none acting on the heat-labile, flagellar antigen, has a high protective value as tested in mice. Henderson (1932) has recently recorded analogous results with *Cl. chauvoei*.

Experiments, in which untreated mice and mice vaccinated with different bacterial suspensions were submitted to the risk of natural infection during a long-continued experimental epidemic of mouse typhoid, have yielded entirely concordant results (Greenwood, Topley and Wilson 1931). The total numbers in each of the vaccinated groups were large—over 300 mice—so that the difference noted may be regarded as certainly significant. The results are summarized in Table XV.

The column giving the antigenic structure shows the components on the surface of the bacterial cells and those carried by the flagella. All vaccines except E were saline suspensions, killed by heat and formalin. Vaccine E—a killed broth culture—was included because of the possibility that some bacterial product of immunizing value might be liberated into the culture fluid. The measure of resistance—the mean survival time in the epidemic cage limited to 60 days—was selected for reasons that will be referred to later. The significance of the standard deviation, which is attached to each survival time, has been considered in Chapter II.

TABLE XV

Group.	Vaccine.	Antigens.	Mean Survival Time in Epidemic Cage. Limited to 60 Days.
C	<i>Bact. aertrycke</i> (Smooth—Type and Group)	Surface I, II. Flag. Aertrycke Sp. Flag. G, A, B, C.	35.31 $\pm$ 0.884
E	<i>Bact. aertrycke</i> (Smooth—Type and Group— Broth culture)	Surface, I, II. Flag. Aertrycke Sp. Flag. G, A, B, C.	34.97 $\pm$ 0.845
F	<i>Bact. paratyphosum</i> B (Smooth—Group)	Surface, I, II. Flag. G, A.	35.06 $\pm$ 0.845
G	<i>Bact. paratyphosum</i> B (Smooth—Type)	Surface, I, II. Flag. Para. B. Sp.	33.71 $\pm$ 0.853
D	<i>Bact. aertrycke</i> (Rough—Type and Group)	Surface R. Flag. Aertrycke Sp. Flag. G, A, B, C.	29.44 $\pm$ 0.771
B	<i>Bact. typhosum</i> (Rough)	Surface R. Flag. S.	29.28 $\pm$ 0.732
A	<i>Staph. albus</i>	—	29.86 $\pm$ 0.714
H	Unvaccinated Controls	—	26.26 $\pm$ 0.641

It will be seen that the control and vaccinated groups can be divided into three classes on the basis of their survival time. The unvaccinated mice lived, on the average, for 26.26 days, and were thus significantly less resistant than any other group. The groups D, B and A were slightly more resistant. This increased resistance was clearly not specific. The rough polysaccharide antigen was not concerned, for the *Staph. albus* vaccine did not contain it. The flagellar antigens of *Bact. aertrycke* were not concerned, for neither the *Staph. albus* nor the rough *Bact. typhosum* contained them. The groups C, E, F and G were definitely more resistant; their mean survival time varied from 33.71 to 35.31 days. They all differed from the other groups in that they contained the specific polysaccharide surface antigens of *Bact. aertrycke* (I and II). They differed among themselves with regard to their flagellar antigens. The results indicate quite clearly that the specific polysaccharide surface antigen is the most important immunizing agent.

We may, then, take it as a general rule—a rule that we could have foretold in view of the facts considered in Chapter V—that the antibodies acting on the surface antigens of virulent, smooth bacilli are essential agents in effective antibacterial immunity; and that the efficiency of any bacterial vaccine depends in large part on the presence of these antigens. There is, as we have seen above, good reason for believing that the S→R variation, and the associated antigenic loss, may be a gradual or step-like process rather than a sudden

discontinuous variation. Strains which show few of the more obvious characters of roughness may be very deficient in the specific polysaccharide antigen.

THE EFFICACY OF ANTIBACTERIAL IMMUNITY

As we emphasized in the earliest pages of this book, immunity may be of very varying grade. The first effect of the mechanisms that we have discussed in this chapter is to free the circulating blood from bacteria, and so save the host from a fatal bacteræmia. The effect on the local foci of infection is less pronounced, and much less rapid. Bacteria may remain latent in such foci over considerable periods.

Thus (Topley 1929) of 64 vaccinated mice that had survived in apparent health for 28 days after an intraperitoneal injection of 1,000 virulent *Bact. aertrycke*, 31 were found to be harbouring that organism in the spleen. We need not doubt that a complete sterilization of the tissues often occurs; but we must not identify the absence of overt illness with a complete absence of infection, or illness followed by recovery with infection followed by a return to the uninfected state.

There is another factor which may limit the efficacy of immunity of this type. There are regions of the body in which bacterial infections are particularly dangerous, and when foci are established at these sites an immunity that is effective against lesions elsewhere seems to be of little avail. Thus Bull (1915*b*) notes that immunized animals, after rapidly freeing their blood stream of pneumococci and remaining in apparent health for several days, may later succumb to a pneumococcal meningitis.

ACTIVE ANTIBACTERIAL IMMUNIZATION AS THE RESULT OF INFECTION

We may close this chapter by considering what happens when a bacterium of moderate virulence obtains access to the tissues of a host that makes a successful immunizing response, and so recovers. We will take as an example a disease, such as typhoid fever, in which the bacterial parasite gains access to the blood stream and so is spread throughout the tissues during the early stages of the attack.

Fig. 12 shows diagrammatically the probable progress of an infection of this type. At A is represented the initial phase, during which the bacteria gain access through a mucous surface and pass to a regional lymphatic gland. At B the bacteria are proliferating in the gland, and there is an intermittent escape of some of them into the blood stream. At C these bacteria are shown as collecting in the liver and spleen, these organs being taken as examples of tissues rich in reticulo-endothelial cells, and hence concerned in the normal clearing mechanism of the blood. At D the bacteria are proliferating in those situations in which they have collected during the initial phases of infection, which correspond to the incubation period of a clinical attack of disease. At E there is a re-invasion of the blood stream from these foci of bacterial pro-

liferation, a well-marked bacteræmia, and a transport of bacteria to other tissues, not previously infected. This corresponds to the phase of clinical illness, and, in a fatal case, persists till death. Up to this stage no antibodies have appeared in the circulating blood; but at this point they usually make their appearance in cases that recover. At F is represented the phase of clinical recovery. The improved clearing mechanism, consequent on an

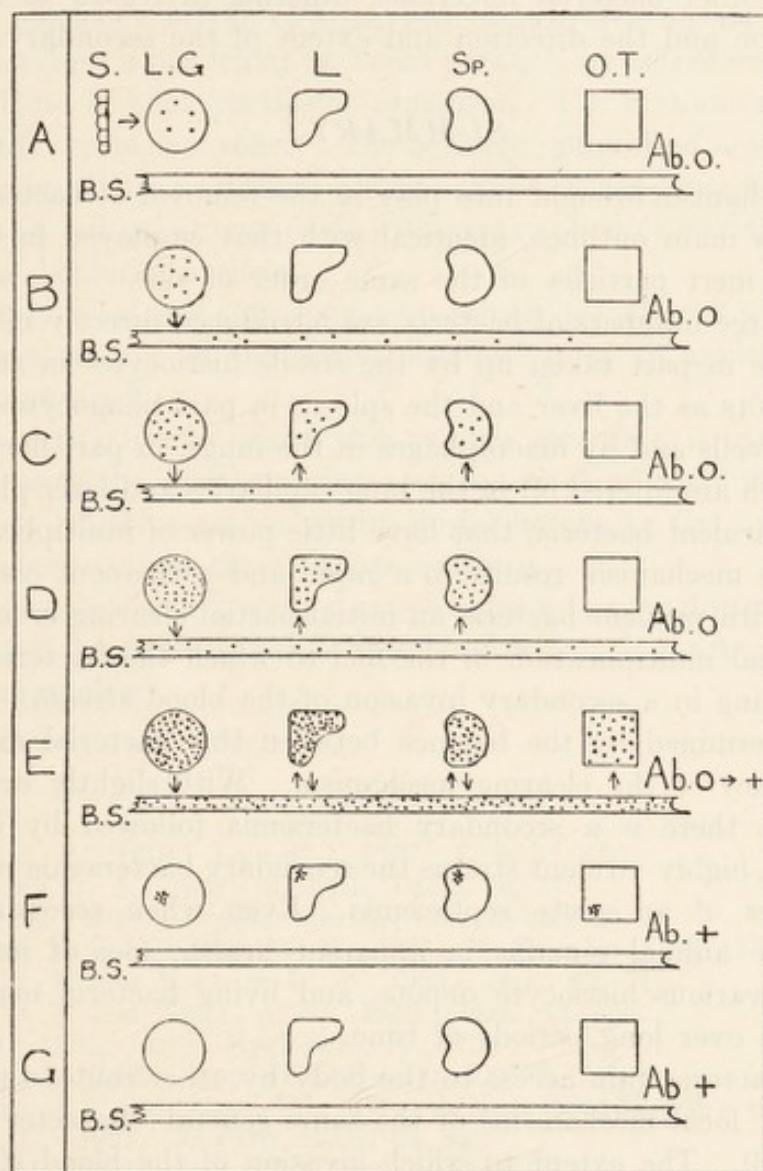


FIG. 12.

S = Mucous Surface. L.G. = Regional Lymphatic Glands. L = Liver. Sp. = Spleen. O.T. = Other Tissues. B.S. = Blood Stream. Ab.o. = Circulating Antibodies Absent. Ab. + = Circulating Antibodies present.

efficient antibody-producing response, has terminated the bacteræmic stage; and we are left with a few scattered foci of infection, in the spleen, liver and lymph nodes, and perhaps in other tissues. As we have seen, there is reason to believe that the persistence of this state of affairs over prolonged periods is consistent with the maintenance of apparent health. At G is represented the final stage of complete restitution, with the disappearance of all infecting

bacteria, and the persistence in the tissues or body fluids of protective antibodies. It is probable that a proportion of infections may be terminated at one of the initial stages, A, B, C or D, without ever passing to E, the stage of clinical illness; and there are reasons for believing that such abortive infections, especially if repeated, may lead to the acquirement of an effective degree of immunity to reinfection. With suitable modifications our diagram could be adapted to other bacterial infections, differing in regard to the primary focus of infection and the direction and extent of the secondary spread.

SUMMARY

(1) The mechanism brought into play in the removal of bacteria from the tissues is, in its main outlines, identical with that employed in the removal and storage of inert particles of the same order of size.

(2) When large numbers of bacteria are introduced directly into the blood stream they are in part taken up by the sessile histiocytes in such reticulo-endothelial depôts as the liver and the spleen, in part phagocytosed by polymorphonuclear cells and by macrophages in the lungs, in part flocculated into aggregates which are filtered off in the lung capillaries and later phagocytosed.

(3) With avirulent bacteria, that have little power of multiplication within the tissues, this mechanism results in a rapid and permanent clearing of the circulation. With virulent bacteria an initial partial clearing is followed by a stage of bacterial multiplication in the foci to which the bacteria have been removed, resulting in a secondary invasion of the blood stream. The fate of the host is determined by the balance between this bacterial multiplication and the efficiency of the clearing mechanism. With slightly or moderately virulent strains there is a secondary bacteræmia followed by a secondary clearing. With highly virulent strains the secondary bacteræmia increases and the animal dies of an acute septicæmia. Even when secondary clearing occurs, and the animal remains in apparent health, foci of infection may remain in the various histiocyte depôts, and living bacteria may persist in these situations over long periods of time.

(4) When bacteria gain access to the body by other routes they are dealt with in part by local mechanisms of the same general character as that described under (3). The extent to which invasion of the blood stream occurs depends upon the anatomy and functional activity of the tissues first invaded, and upon the virulence of the invading organism. The blood stream is invaded very rapidly after intraperitoneal inoculation, less rapidly after intramuscular inoculation, and still less rapidly after subcutaneous inoculation. In all cases the passage from the primary site of infection to the blood stream is mainly by way of the lymphatics, and the histiocyte depôts in the regional lymphatic glands play a prominent part in the removal of the invading bacteria. When relatively avirulent bacteria gain access to the tissues at some site from which passage to the blood stream is slow, infection may never pass beyond the first line of defence in the regional lymphatic glands; but here, as in the case where

a generalized infection has become established, localized foci of infection may persist over long periods of time.

(5) In addition to this process of removal by phagocytic cells, a direct bactericidal action of the serum plays a part in ridding the tissues of bacteria. The importance of this purely humoral immunity appears to differ widely in different bacterial infections; but it is probably always subsidiary to the phagocytic mechanism, and is seldom, if ever, the main factor in defence.

(6) The efficiency of the clearing mechanism of a normal animal may be increased, *vis-à-vis* a particular bacterial parasite, by active immunization with a killed culture of that particular organism. The immunity so produced is specific in the serological sense. The actively immunized animal deals with a virulent strain of the bacterium against which it has been immunized in the same way as a normal animal deals with an avirulent or slightly virulent strain of the same bacterial species.

(7) Immunity of this type may be passively transferred by the inoculation into a normal animal of the serum obtained from an animal that has been actively immunized.

(8) The antibodies by which this passive immunity is conferred are those that unite with the surface antigens of the virulent bacteria; and in order to induce an effective active antibacterial immunity the immunizing agent employed must contain these surface antigens.

(9) The mechanisms described above come into play during the later stages of any bacterial infection that is associated with the development of an antibacterial immunity.

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CHAPTER IX

THE RESPONSE OF BACTERIA TO THE DEFENCE MECHANISMS OF THE HOST

WE have considered in Chapter III the ways in which bacteria injure the tissues of the infected host, and in Chapters VII and VIII the defence mechanisms by which the host opposes the activities of the invading parasites. We may now turn back for a little to the reactions of bacteria and their products, and consider how they affect the defence mechanisms that we have described.

The available data are, as is so commonly the case, sadly confused by the fact that we have been forced to work with the crudest and most complex of reagents. The easiest way of disentangling, to some extent, the confused threads of this particular problem is to take as our starting-point a series of observations recorded by Bail and his colleagues (Bail 1900, 1902-3, 1904a, b, c, 1905, Bail and Pettersson 1902, 1903, Bail and Weil 1906, 1911, Weil 1905a, b). Working with the anthrax bacillus, with organisms of the *Pasteurella* group, with the cholera vibrio and with the typhoid bacillus, they found that exudates produced in experimental animals by the inoculation of these bacteria had characteristic properties, which Bail attributed to the presence of special bacterial products to which he gave the name *aggressins*; these aggressins were, he believed, produced in the tissues of the infected host, but seldom, if ever, *in vitro*.

The properties of aggressins, as described by Bail, may be summarized as follows:

(1) Sub-lethal doses of bacteria are rendered lethal by the addition of aggressins.

(2) A lethal dose of bacteria, which alone would produce a slowly fatal infection, causes a rapidly fatal infection associated with characteristic lesions when aggressins are added to the inoculum.

(3) The addition of cholera aggressins neutralizes the bactericidal action of an anticholera serum, as tested by intraperitoneal injection in the guinea-pig.

(4) By the injection of aggressins an immunity is produced which is far more effective than that produced by the inoculation of bacteria and is different in kind. This immunity can be passively transferred.

According to Bail, the aggressins are not themselves toxic. They produce their effects, which are in the main specific, solely by aiding the rapid multiplication of bacteria within the tissues. He states also that they are extremely thermolabile, being completely inactivated by 30 minutes' exposure to a temperature of 55° C. and markedly affected by any temperature above 44° C.

Bail's contention, that the results of his experiments required for their interpretation the assumption of the existence of a hitherto unrecognized bacterial product of peculiar importance in infection and immunity and deserving the new title of "aggressin," did not long remain unchallenged. Wasserman and Citron (1907) recorded their reasons for believing that the aggressins were simply products of bacterial growth, or of bacterial autolysis, which could be prepared in the test-tube by suitable methods. Citron (1906) recorded experiments which showed that the addition of bacterial extracts to living bacteria increased their virulence; that by the use of such extracts it was possible to immunize animals against experimental infection; and that the serum of such immunized animals conferred passive immunity on others. Bail and Weil (1906) claimed that the immunity produced by Citron with his bacterial extracts was far less effective than that produced by natural aggressins, and that it was different in kind; though it is not entirely clear on what differential criteria they relied in distinguishing anti-aggressin immunity from the varieties previously recognized.

Doerr (1906) criticized the aggressin theory on somewhat similar grounds, but emphasized particularly the fact that large doses of dead bacilli may cause death in experimental animals with the lesions described by Bail as characteristic of the aggressin effect. He also noted that if sub-lethal doses of killed cultures were added to sub-lethal doses of living bacteria the combined inoculum might cause a fatal infection of the aggressive type. This additive effect did not appear to be in any way specific, but some killed cultures were more effective than others. For instance, dead typhoid or dysentery bacilli were more aggressive than dead staphylococci. In addition, Doerr demonstrated that aggressive exudates did, in fact, contain ordinary bacterial antigens; since a typhoid exudate gave well-marked precipitation with an ordinary antityphoid serum.

Sauerbeck (1907) has also criticized Bail's hypothesis. He concludes, from the results of a large series of experiments, that aggressive exudates are not lacking in toxicity, since two or three times the amount that produces the characteristic aggressive effect will kill an animal within a few days in the absence of living bacteria; that the activity of a given aggressive exudate is in no way proportional to the virulence of the organism that produced it, but rather to its toxigenicity; that characteristic aggressive effects can be produced with toxic bacterial products that have been prepared in the test-tube; and that aggressive action, whatever its cause, is entirely non-specific. His findings as regards the heat-stability of the aggressive bacterial products also differ from Bail's, since he states that they are reduced in potency, but not completely inactivated by heating at temperatures between 55° C. and 70° C.

The detailed studies of Dudgeon (1912) on the properties of exudates obtained from natural infections in man and from experimental infections in animals made clear one of the mechanisms involved in the total aggressin effect.

The method he adopted in the majority of his experiments was as follows. He obtained exudates from natural infections in man or from experimental infections in animals. The exudate, after the separation of as much material as possible by centrifugation, was mixed in a capillary tube with an equal volume of normal serum, or of serum from an immunized man or animal. After 1 hour's incubation at 37° C. leucocytes and bacterial suspension were added to each mixture, the tubes were re-incubated for 15 minutes and films were prepared from the contents, the degree of phagocytosis being determined in the usual way. In control tubes the exudate was replaced by saline. In

each case the opsonic effect of the serum, and of the serum-exudate mixture, was determined against the bacterium causing the infection and against some related organism. The results shown in Table XVI illustrate an effect that was commonly observed. The exudate came from a subcutaneous staphylococcal abscess; the immune serum was from the infected patient; the normal serum was from an uninfected control. The sera and the serum-exudate mixtures were tested against the strain of staphylococcus grown from the abscess and against a strain of *Bact. coli*.

TABLE XVI

Mixture.	Number of Bacteria ingested by 50 Leucocytes.	
	<i>Staph. aureus.</i>	<i>Bact. coli.</i>
Normal Serum + Saline	240	229
Immune Serum + Saline	294	203
Normal Serum + Exudate	139	200
Immune Serum + Exudate	139	237
Exudate + Saline	61	21

The exudate alone had a slight opsonic effect, but it specifically reduced the opsonic action of normal or of immune serum on *Staph. aureus*, leaving unaffected their opsonic action on *Bact. coli*.

The substances responsible for this specific anti-opsonic effect were found, in many cases at least, to be markedly thermostable. Table XVII shows the effect of adding a boiled exudate, obtained from an experimental infection with *Bact. enteritidis*, to the serum of the infected animal.

TABLE XVII

Mixture.	Number of Bacteria ingested by 50 Leucocytes.	
	<i>Bact. enteritidis.</i>	<i>Bact. coli.</i>
Serum + Saline	248	130
Serum + Boiled Exudate	27	102

In further experiments Dudgeon submitted exudates to boiling, removed the coagulum by centrifugation, added excess of alcohol to the clear supernatant fluid, collected the precipitate by centrifugation, and redissolved it in saline. The preparation so obtained still showed specific anti-opsonic action.

These results bore an obvious analogy to an earlier series of observations (Dudgeon, Panton and Wilson 1910). Extracts were prepared from various bacteria by grinding or by alternate freezing and thawing, and these extracts were shown to have a specific anti-opsonic effect. The anti-opsonic substances were markedly thermostable, remaining active after boiling for 30 minutes. A considerable series of control tests showed that they had no direct action on the leucocytes.

In addition to these heat-stable specific anti-opsonic substances, Dudgeon found that many infective exudates contained directly toxic substances, as judged by injection into experimental animals. In this respect, however, they varied widely, according to the infecting bacterium. It may be noted that certain highly toxic exudates, such as those obtained in experimental infections with *Bact. enteritidis*, retained their toxicity after boiling.

Further light has been thrown on the probable nature of the anti-opsonic substances in aggressive exudates during the prolonged and detailed study of the factors involved in antipneumococcal immunity.

Rosenow (1907) found that extracts or autolysates of virulent pneumococci had the power of inhibiting the phagocytosis of relatively avirulent pneumococci, and he suggested the name *virulins* for the active substances concerned.

Cole (1917) observed that empyema fluids from pneumococcal infections contained soluble substances that specifically neutralized the protective antibodies in an antipneumococcal serum, and that similar substances might be present in the blood of rabbits suffering from experimental pneumococcal infection. He showed that, when an antipneumococcal serum was injected intravenously into a rabbit previously infected with the same type of pneumococcus, the protective antibodies disappeared very rapidly from the blood, and that a similar phenomenon could be demonstrated in patients severely ill with lobar pneumonia.

The more recent studies on the antigenic components of bacterial cells that we have outlined in Chapter VI, and the demonstration that sensitization to the phagocytic action of polymorphonuclear cells, and presumably of macrophages, depends upon the union of antibody with an antigenic component situated at the surface of the bacterial cell, offer the obvious suggestion that the substances responsible for the specific anti-opsonic effects of aggressive exudates are dissolved bacterial antigens. Landsteiner's demonstration of the inhibition of antigen-antibody reactions by simple haptens (see p. 59), and the *in vitro* reactions of specific bacterial polysaccharides that fail to stimulate antibody production *in vivo*, indicate that bacterial haptens, as well as complete antigens, may be expected to exert this anti-opsonic effect.

There is good evidence that this is actually the case. Sia (1926) has studied the action of the type-specific polysaccharides of Type II and Type III pneumococci on the growth of these organisms in serum-leucocyte mixtures from the rabbit and from the cat. The pneumococci were of relatively low virulence and developed very poorly in either of the serum-leucocyte mixtures employed. The addition of minute amounts of Type II polysaccharide greatly increased the growth of Type II pneumococci, but had little effect on the growth of the Type III organisms. The addition of minute amounts of Type III polysaccharide greatly increased the growth of Type III pneumococci, but not of Type II.

Felton and Bailey (1926) have demonstrated the aggressive action of Type II polysaccharide by injecting it into mice together with relatively avirulent pneumococci of the same type, and inducing a fatal infection.

Ward (1932) has recently shown that the inhibitory action of extracts from pneumonic lungs on the bactericidal action of whole blood is greater than that of the specific polysaccharide as prepared in the laboratory. It may be that the polysaccharide hapten as developed in the tissues is in a more active state, or it may be that more than one hapten or antigen is involved. In any case it seems highly probable that dissolved antigenic components play an important part in neutralizing the natural antibodies, or the antibodies formed by the host during the course of an infection, and so in

reducing the effectiveness of the defence mechanisms. Cole (1917) and Park and Cooper (1928) record evidence suggesting that a fatal outcome in cases of lobar pneumonia in man is frequently associated with the presence in the blood of specific soluble antigen in amounts greater than can be neutralized by the antibodies that the patient has produced.

In addition to these dissolved haptens or antigens it is clear that some infective exudates will contain bacterial toxins that interfere with the defence mechanism of the host by an attack delivered against the cellular rather than against the humoral defences. Two pathogenic species at least, the pyogenic staphylococcus and the hæmolytic streptococcus, produce a specific leucocidin that attacks the polymorphonuclear cells. We know little as yet with regard to the action of bacterial toxins on the cells of the reticulo-endothelial system; but it is quite probable that we shall find that certain bacterial species are capable of producing toxins that have a selective action on the macrophages.

To sum up: it would seem that we are justified in recognizing the following factors as involved, to a varying degree, in the defence rupture that is produced by the injection of sterile infective exudates, of dead bacteria, or of bacterial extracts, in association with non-lethal doses of living bacilli.

(1) *Dissolved bacterial haptens or antigens, or similar components still attached to bacterial protoplasm.* These act specifically by uniting with the corresponding antibody, and so preventing the effective sensitization of the living bacterial cells. Many of these haptens are of the nature of polysaccharides. Many of them appear to be thermostable. It is probably only those haptens or antigens that are situated at the surface of the bacterial cell that are able to function in this way; since it is the surface components that are concerned in sensitization.

(2) *Bacterial toxins that selectively attack the cells concerned in tissue defence.* Their action is non-specific *qua* the infecting bacterium; since the injury to the phagocytic cells will reduce the efficacy of the defence mechanism against all types of invading bacteria. Many of these toxins are thermolabile; others, perhaps, are thermostable.

(3) *Other toxic bacterial products, bacterial enzymes, etc.,* that may act in any of the ways that we have considered in Chapter III. In many such instances the effect may be merely additive—the tissues may have to deal simultaneously with a toxæmia and an invasive infection which have no direct connection with one another. This is hardly defence rupture, or aggressive action, in the usually accepted sense. In other cases the bacterial toxins, enzymes or hormone-like products may interfere with the defence mechanism of the host in ways of which we are as yet ignorant. In any case such action is probably non-specific in the immunological sense, though it is quite likely that a particular bacterial product may favour one type of infection more than another. Of the nature of the bacterial products concerned we as yet know little or nothing, except that some of them are thermostable while others are probably thermolabile.

To which of these bacterial products, if any, should we attach the label *aggressin*? There seems little advantage in using it at all; it describes an

effect, not an entity. There is, perhaps, something to be said for the term *aggressive action*, if we can so define it as to make it fill a gap in our terminology. It would seem to be most usefully employed as indicating an action on the cells or body fluids that is not directly injurious, or of which the directly injurious effects are minimal, but which so interferes with the defence mechanism of the host as to favour, specifically or non-specifically, the multiplication of bacteria within the tissues.

Used in this sense, it may be compared with the term *defence rupture*, employed to describe the action of certain chemical agents such as calcium and silica (see pp. 248-250).

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CHAPTER X

THE ORIGIN OF THE NATURAL ANTIBODIES AND THEIR RÔLE IN SPECIFIC IMMUNITY

WE have seen in Chapter VIII that active or passive immunity, experimentally induced in a naturally susceptible animal, is dependent, in the main, on an integrated system of cellular reactions conditioned by the presence of specific sensitizing antibodies in the blood and tissue fluids, or on a directly bactericidal effect conditioned by the same or similar antibodies; and in Chapter VII that induced antitoxic immunity is dependent on the presence in the blood and tissue fluids of specific antitoxins.

It is necessary to inquire whether natural immunity to potentially virulent bacteria and their products is conditioned by the presence of similar specific antibodies, or whether it depends on some different mechanism.

NATURAL ANTIBACTERIAL IMMUNITY

That the phagocytic clearing mechanism plays an important part in the defence of the naturally immune host there can be no doubt. The reaction to the injection of dye suspensions or of carbon particles is a quite general phenomenon; and a normal animal that is susceptible to a virulent bacterium deals with an avirulent strain of the same species much as it deals with inert, non-living particles. It would appear that any bacterium that is sensitive to the flocculating action of the blood plasma and to the phagocytic action of the reticulo-endothelial cells and leucocytes is *ipso facto* relatively avirulent. The point at issue is this: given a bacterium that is sensitive to the defence mechanism of host A but is insensitive to the defence mechanism of host B, is this difference, which makes A immune and B susceptible, due in whole or in part to the fact that A possesses natural sensitizing antibodies for the bacterium in question, while B does not?

The evidence at present available suggests that such antibodies often play a part in natural specific antibacterial immunity; but it does not justify any final or generalized conclusion.

Kyes (1916) showed that, when virulent pneumococci were inoculated into the naturally immune pigeon, the organisms were rapidly withdrawn from the blood stream and phagocytosed by macrophages in the liver and spleen. Heist, Solis-Cohen and Solis-Cohen (1918) found that the whole blood of the pigeon was actively bactericidal for the pneumococcus while the whole blood of the susceptible mouse or rabbit was not. The serum of the pigeon alone was not bactericidal, indicating that the killing power depended on

the leucocytes as well as on the constituents of the serum. That serum constituents are in fact concerned, and that among these constituents are specific antibodies, would seem to follow from the observations of Bull and McKee (1921), who studied the passive transfer of immunity from the normally resistant chicken to the normally susceptible mouse. They found that chicken serum would protect mice against virulent pneumococci of Types I, II and III; and that absorption of the chicken serum with Type I pneumococci removed its protective power against Type I but not against Type II. Similarly absorption with Type II removed the protective power against this type, leaving the protective power against Type I.

Robertson and Sia (1924) found a marked inhibition of the growth of pneumococci in serum-leucocyte mixtures from the resistant cat or dog, but no such inhibition in similar mixtures from the susceptible rabbit or guinea-pig. More recently (Robertson and Sia 1927) they have reported experiments which suggest that this bactericidal action is due to sensitization followed by phagocytosis. They sensitized pneumococci by prolonged contact with large amounts of the serum under test, and then determined the degree of phagocytosis by the leucocytes of various resistant and susceptible animals. They found that sensitization with serum from the resistant cat, sheep, pig and horse rendered the pneumococci susceptible to phagocytosis by the leucocytes of these animals, or by those of the susceptible rabbit or guinea-pig; while treatment with serum from such susceptible species as the rabbit, guinea-pig or man, did not sensitize the pneumococci for the leucocytes of these animals, or for those from the resistant cat, sheep, pig or dog.

The actual number of sera from each species examined by Robertson and Sia was not large, and it would seem that their original conclusions require some modification, at least in regard to man. Thus, Robertson and Cornwell (1930), using serum-leucocyte mixtures, studied the presence of antibodies for various types of pneumococci in the serum of normal persons. All the sera examined showed the presence of antibodies for one or more of the serological types employed (I, II, III and V), but any given serum might be inactive against some types though active against others. It would seem, moreover, that the presence of antibodies against a particular type does not afford complete protection against infection with that type, since Robertson and others (1930) found that the serum of patients in the first 48 hours of an attack of lobar pneumonia often contained sensitizing antibodies for the infecting type of pneumococcus. There was, however, throughout the course of the disease an inverse correlation between the sensitizing power of the serum and the degree of bacteraemia. In the presence of a well-marked sensitizing effect pneumococci were not found in the circulating blood.

The presence in normal sera of specific sensitizing antibodies for the various types of pneumococci is in no sense an isolated or peculiar phenomenon. Bürgi (1907) tested sera from the dog, guinea-pig, rabbit, goose, hen, sheep, goat, horse, ox and man against suspensions of various bacteria, including *V. cholerae*, *Bact. typhosum*, *Bact. coli*, *Past. aviseptica*, *Staph. aureus* and *Proteus vulgaris*, and noted well-marked agglutination in many instances, often to a relatively high titre. He found, however, that the various sera tended to be arranged in very much the same order of activity, against whichever species of organism they were tested; and, in further experiments, that this order was in general maintained when the sera were tested for their ability to flocculate suspensions of mastic.

Gibson (1930) carried out similar experiments with sera from the ox, rabbit, guinea-pig, horse, sheep, pig, rat, cat and man, tested against *Proteus* X19, *Ps. pyocyanea*, *Bact. typhosum*, *Bact. paratyphosum A*, *Bact. paratyphosum B*, *Bact. enteritidis* (Gärtner), *Bact. dysenteriae Y*, *Bact. shiga*, *Bact. morgani*, *Bact. friedländeri*, several strains of *Bact. coli* and *V. cholerae*. He found, as Bürgi had done, that the sera tested tended to fall in a definite order with regard to their power of agglutinating a wide variety of bacteria. Ox sera were the most active; pig and horse sera were somewhat less active; sheep serum was next in order; while human, cat, rabbit and guinea-pig sera, in that succession, showed progressively weaker effects. Rat serum was the weakest of all. Similarly the

different bacterial species showed a definite order of sensitivity to agglutination. *Bact. dysenteriae* Y, *Bact. friedländeri* and *Ps. pyocyanea* were the most sensitive, while certain strains of *Bact. coli* were least sensitive. By absorption experiments, Gibson was able to show that this agglutination was dependent on the presence of specific antibodies; but since any one organism, in addition to removing completely the specific agglutinin acting on itself, might reduce the titre of the serum for other organisms, and a similar general reduction in titre might be produced by adsorption with some non-specific agent such as charcoal, he concluded that another non-specific serum constituent was concerned in the full effect obtained with the untreated sera. In later experiments (Gibson 1932), in which the H and O antigens and antibodies were differentiated, he obtained results suggesting that the specificity was due mainly to the H agglutinins, while the O agglutinins for any one bacterium might be greatly reduced by absorption with an antigenically unrelated organism.

Timmerman (1930) tested sera from the guinea-pig, rabbit, hare, goat, sheep, buffalo and man against *Bact. typhosum* and was able to demonstrate the presence of both somatic (O) and flagellar (H) agglutinins in one or more specimens of serum from each species, except in the case of the guinea-pig, in which 2 of 20 animals showed O but no H agglutinins.

Lovell (1932) tested the sera of 202 normal swine, 23 cattle, 46 sheep and 20 horses for flagellar agglutinins against 7 different types of *Salmonella* bacilli. Normal agglutinins against one or more of these types were present in 190 swine, 13 cattle, 36 sheep and 18 horses, usually to relatively low titre. Absorption experiments indicated that these agglutinins were in most cases specific. In further (unpublished) experiments Lovell has shown that the sera of these species also contain O agglutinins against the bacilli of this group, though to rather lower titre, and that these too can be specifically absorbed. Mackie and Finkelstein (1930, 1931, 1932) have tested the serum of a number of normal animals, such as the ox, sheep, horse, pig, rabbit, rat, cat, guinea-pig, pigeon and man, against a variety of bacteria, including *V. cholerae*, *Bact. typhosum* and other members of the *Salmonella* group, dysentery bacilli, proteus bacilli, influenza bacilli *Br. abortus*, *Br. melitensis*, meningococci and so on. They have also (Mackie *et al.* 1932) tested the bactericidal action of whole blood and of serum-leucocyte mixtures. They were able to demonstrate complement fixation and bactericidal action in a large number of cases, and to show that the bactericidal effect depended on the joint action of complement and a heat-stable factor that could be removed by absorption. This absorption was specific, in the sense that it was often possible to demonstrate the removal of bactericidal power against the absorbing organism while the absorbed sera was still bactericidal for other bacteria.

Gordon and Carter (1932) record results which lead them to a different conclusion. Testing rabbit and guinea-pig sera against cholera vibrios, typhoid and other *Salmonella* bacilli, and dysentery bacilli, they were able to demonstrate a well-marked bactericidal action that depended on the interaction of complement and some heat-stable factor; but they failed to demonstrate any specific absorption of the latter. Their results suggested that the bactericidal activity against the less sensitive organisms could be removed by absorption with many different bacteria, leaving in each case an effective residuum of bactericidal action against those organisms, such as the cholera vibrio and *Bact. dysenteriae*, that are particularly sensitive to serum lysis.

To summarize these findings: the serum of most normal animals contains substances that react with bacteria in the test-tube giving agglutination, complement fixation and bacteriolysis (bactericidal action). The sera of some animals, such as pigs and cattle, seem to react with a wider variety of bacteria than do the sera of other animals, such as the rabbit and guinea-pig. Different species of bacteria also vary in their sensitiveness to normal sera. Some species are agglutinated, or lysed, by normal serum from many different animals,

others are sensitive only to a few. The specificity of the flagellar agglutinins in normal sera seems to have been clearly established, and the evidence is definitely in favour of the specificity of some, at least, of the agglutinins acting on the antigens at the surface of the bacterial cells. Those agglutinins, flagellar and somatic, are, it should be noted, usually present to low titre only (1:5—1:40 or thereabouts). The evidence with regard to the specificity of the bactericidal antibodies is more conflicting; but results indicating specificity have been recorded, and if specific antibodies are present we should expect them to have a bactericidal effect in the presence of complement. There is no doubt at all that specific sensitizing antibodies are frequently present in normal sera. It is, at the moment, rather more doubtful whether we are entitled to assume the presence of a specific antibody in every case in which we can demonstrate agglutination to low titre, or the bactericidal action of undiluted, or slightly diluted, serum.

Whether the presence of specific sensitizing antibodies affords an effective resistance against the corresponding bacterium we do not know. We should clearly expect that an individual possessing an antibody acting on the surface antigen of a particular bacterial parasite would be more resistant to this organism than another individual from whose blood this antibody was absent. But direct evidence is very scanty. In the particular case of the pneumococcus there is suggestive, but not conclusive, evidence that sensitizing antibodies are distributed among different animal hosts in accord with demonstrable differences in resistance; but in most other instances we have no evidence at all. We know, for instance, that some pigs have O agglutinins for *Bact. suispestifer* while others have not. We do not know whether the former are more resistant to infection than the latter. We know (see pp. 304, 305) that many normal persons have O agglutinins acting on *Bact. typhosum* and on *Bact. paratyphosum* B. We do not know whether these persons are more resistant to typhoid or paratyphoid fever than others who do not possess these antibodies.

NATURAL ANTITOXIC IMMUNITY

In the case of antitoxic immunity we are on firmer ground. There is a great deal of evidence indicating that natural immunity of this type, apart from exceptional instances of natural insensitivity to a particular toxin, depends on the presence of specific antitoxin in the blood and tissue fluids. This evidence has, in the main, been collected during the intensive study of two human diseases—diphtheria and scarlet fever. That normal human beings may contain diphtheria antitoxin in their blood has long been known (Abel 1894, Wassermann 1895), but no extensive data on the distribution of antitoxin among the population at large were collected until after the publication of Römer's convenient technique for the measurement of small amounts of toxin and antitoxin (see p. 382). This was soon followed by the studies of Karasawa and Schick (1910), Schick (1911) and von Groer and Kassowitz (1915, 1917, 1919) which gave us a fairly detailed knowledge of the frequency of the presence

of antitoxin among different age groups. The elaboration of the Schick test (Michiels and Schick 1913, Schick 1913) in which the presence of circulating antitoxin is detected by the insensitiveness of the skin to the intradermal injection of a small amount of toxin (the *negative* Schick reaction), has provided a still simpler method of detecting the presence of antitoxin above a certain level (1/100 to 1/30 unit of antitoxin per c.c. of blood).

The direct experimental evidence that the presence of this amount of antitoxin confers immunity is scanty; but, so far as it goes, it is demonstrative.

Guthrie, Marshall and Moss (1921) collected 8 volunteers and determined their reaction to the Schick test; 4 reacted positively and 4 negatively (including 2 pseudo-reactions). The throat of each of these 8 volunteers was swabbed with a virulent culture of the diphtheria bacillus. The four positive reactors developed clinical diphtheria; the 4 negative reactors, possessing natural antitoxin at or above the Schick-immune level, developed no illness, though 3 of them became carriers.

The main body of evidence on which we rely in concluding that natural antitoxin confers natural immunity has, however, been collected during the course of extensive clinical and epidemiological studies (see Chapters XVII and XIX). There is no doubt that a person whose blood contains antitoxin at or above the Schick-immunity level is very unlikely to contract diphtheria, even when exposed to serious risk of infection. If he does so, the attack will almost always be of a mild and clinically atypical type.

In the case of scarlet fever the Dick test (Dick and Dick 1924, 1925) has given us a similar method of detecting the presence of antitoxin against the rash-producing toxin of the hæmolytic streptococcus.

The nature of the evidence that associates natural antitoxic immunity with effective resistance to scarlatinal infection is of the same general kind as in the case of diphtheria.

THE NATURE AND ORIGIN OF THE NATURAL ANTITOXINS

It is necessary, as an integral part of the discussion of their significance, to consider briefly the available evidence with regard to the origin of the natural antibodies, and it will be convenient to reverse our previous order and to consider antitoxins before antibacterial sensitizers.

The proportion of persons who possess diphtheria antitoxin in the circulating blood varies in a striking and characteristic way as we pass from birth, through childhood and adolescence to maturity and old age. Table XVIII sets out the extensive data recorded by Zingher (1923).

At about 6 months of age just under half the infants tested react negatively to the Schick test. The percentage of negative reactors then falls rapidly and between 8 months and 3 years fluctuates about an average value of approximately 12 per cent. After this period there is a continuous rise, at first rapid then more gradual; between the 6th and 7th year the negative reactors number 49.6 per cent., by 12-13 they have risen to 73.4 per cent., by 20-30 to 88.3

per cent., and at the allotted three-score years and ten to approximately 95 per cent.

TABLE XVIII

SHOWING PERCENTAGE OF SCHICK-POSITIVE REACTORS (*i.e.*, PERSONS CONTAINING LESS THAN $\frac{1}{30}$ UNIT OF DIPHTHERIA PER C.C. OF BLOOD) IN VARIOUS AGE GROUPS.

Age Group.		Number Tested.	Per cent. Positive.
6-7	months	53	56.6
7-8	"	41	63.4
8-9	"	62	83.8
9-10	"	58	93.1
10-11	"	61	87.0
11-12	"	34	91.1
1-3	years	1,727	83.2
4-6	"	1,328	58.6
6-7	"	13,754	50.4
7-8	"	16,180	43.5
8-9	"	17,126	36.6
9-10	"	18,065	32.2
10-11	"	18,057	29.3
11-12	"	17,994	28.2
12-13	"	16,258	26.6
13-14	"	14,138	23.1
14-15	"	9,650	19.7
15-16	"	4,861	17.8
16-17	"	369	18.4
20-30	"	1,253	11.7
30-40	"	1,488	10.6
40-50	"	1,220	8.2
50-60	"	920	6.4
60-70	"	662	5.4
Over 70	"	181	5.5

The most generally accepted view with regard to the succession of immunological events that underlie this observed fluctuation in skin-sensitivity to diphtheria toxin is as follows. At birth the majority of infants are endowed with circulating antitoxin which they have acquired passively from their mothers—mainly by passage of this antibody through the placental membrane, in part perhaps with the colostrum in the early days of suckling. This passive congenital immunity, like all other types depending on the passive acquirement of antibodies, will be short-lived, since the antitoxin will gradually disappear from the tissue fluids and will not be replaced by any activity of the infant's own tissues. From infancy onwards, however, the child will be exposed to the risk of infection, increasing from year to year during the earlier part of school life. The result of this exposure will be that some children will contract the typical disease, while others will escape with mild and atypical infections, or with infections so slight that they fall wholly within the bacteriological as opposed to the clinical sphere. Each of these infections will stimulate the production of antitoxin, so that there will result a gradual process of natural immunization, the transitory passive immunity of early infancy being thus replaced, after a period of susceptibility, by a lasting

active immunity conditioned by the reception of external environmental stimuli.

It should be noted that this description of the replacement of passive by active immunity after a phase of susceptibility does not rest entirely on the results obtained by Schick tests at different ages. The observations carried out by Römer's technique, and particularly the extensive series of observations carried out by von Groer and Kassowitz (1915), have placed on a firm basis the assumption that the results of the skin test afford a reliable indication of the frequency of circulating antitoxin in the different age groups. Thus 48 per cent. of infants at birth were found to have diphtheria antitoxin in their blood in a concentration of 1/200 A.U. or over, at ages 9-18 months the proportion had fallen to 32 per cent. and by 2½-3½ years to 28 per cent. It then rose steeply and by 17-18 years was back at the birth figures of 84 per cent.

There is, moreover, direct evidence that antitoxic immunity to diphtheria in infants results from transference of antitoxin from mother to child. Von Groer and Kassowitz tested the blood of 143 mothers and their new-born children. In 96 per cent. of cases antitoxin was present in both mother and child, or absent in both. It should be noted, however, that in some instances discrepancies have been noted between the result of the skin test in infancy and the presence of antitoxin in the blood, a proportion of Schick-negative infants showing little if any circulating antitoxin (Kellogg 1926-7, Friedberger and Heim 1929, Okell 1932, see also Cooke and Sharma 1932).

The distribution of scarlatinal antitoxin among the different age groups, as judged by the Dick test, appears to be essentially similar to that of diphtheria antitoxin. Table XIX gives the results obtained by Zingher (1924) in testing 7,700 persons.

TABLE XIX

PERCENTAGE OF POSITIVE DICK REACTIONS IN DIFFERENT AGE GROUPS (ZINGHER).

Age Group.	Number of Persons Tested.	Per cent. Positive.
0-6 months	29	44.8
6-12 „	52	65.3
1-2 years	233	71.6
2-3 „	204	64.2
3-4 „	241	60.5
4-5 „	264	48.4
5-10 „	1,955	33.6
10-15 „	2,965	22.8
15-20 „	981	16.8
Over 20 „	776	14.4

A negative Dick reaction in infancy would seem, however, to be a very unsafe criterion of congenital passive immunity. Cooke and others (1927, 1928) tested the skin sensitivity to scarlatinal toxin of 200 mothers and their offspring. In some cases they also examined the blood of mother and child for circulating antitoxin. The results confirmed the general observation that a positive Dick reaction is very rare in young infants; but the correlation between the skin reaction in mother and child was by no means complete; and a number of infants who gave negative skin reactions had

no detectable antitoxin in their blood. These infants were retested at various intervals (6 weeks to 3 months) when it was found that many of them had become Dick-positive. After a further interval of time several became Dick-negative again; and this change was always associated with the appearance of scarlatinal antitoxin in the circulating blood. It would appear that the frequency of Dick-negative reactions in young children must be attributed, in part at least, to an insensitiveness or lack of reactivity of the skin. Kobak and Pilot (1931) record analogous observations in regard to skin sensitivity to the toxin of *Staph. aureus*.

A note may be interpolated with regard to the nature and mechanism of congenital passive immunity. The transference of antiabrin and antiricin from a mother to her young, by the transplacental route or in the colostrum, was demonstrated by Ehrlich (1892). It would seem that the relative importance of these two routes of transference depends upon the number of tissue layers that separate the maternal and foetal circulation in different animal species. Table XX, taken from a recent paper by Mason, Dalling and Gordon (1930), sets out this relation in summary form.

TABLE XX

THE RELATIVE IMPORTANCE OF THE PLACENTAL AND MAMMARY ROUTES IN THE PASSIVE TRANSMISSION OF ANTIBODIES FROM MOTHER TO OFFSPRING. (After Mason, Dalling and Gordon.)

Species.	Layers of Tissue between Maternal and Foetal Circulation.	Importance of Placental Transmission.	Importance of Transmission by Colostrum.
Pig	5	—	+++
Ruminants	4	—	+++
Carnivores	2	+—	+
Rodents, apes, man	1	+++	+—

Returning to the course of events from infancy onwards, are we justified in accepting the view that the acquirement of antitoxic immunity with increasing age is entirely due to natural active immunization resulting from the reception from without of specific stimuli in the shape of clinical or subclinical infections? This hypothesis has certainly not been placed beyond dispute. It has indeed been vigorously disputed, particularly by Hirszfild (1926) and his colleagues.

Hirszfild believes that all the normal antibodies should be regarded as "biochemical organs," with which the individual is endowed as the result of his evolutionary history. These "biochemical organs" ripen, or come to maturity, at different periods in his own individual development; just as the various organs and tissues of the body come to full maturity at different ages. As we speak of a "morphogenesis" through which each individual passes, so we should think of a "serogenesis" which forms an integral part of normal development. The time at which any particular antibody normally makes its appearance may be regarded as the period of "immunological crisis" for that particular antibody, and marks the establishment of a "biochemical reflex" on which the production of that antibody depends. Until the biochemical reflex has become established attempts at immunization with the particular antigen concerned will have little effect. Hirszfild does not deny that specific, or non-specific, stimuli from without may play some part in accelerating the production of particular antibodies, or in increasing their concentration; but he would relegate such influences to a very secondary place. He would not regard

the presence in the blood stream of antibodies acting on a particular bacterium as valid evidence of past or present infection with the bacterium in question. Above all, he would not accept the view that the antibody-forming apparatus is a *tabula rasa*, on which environmental stimuli can write at will. It is a complex mechanism with its possible activities fixed and ordered by the phylogeny of each animal species; and it will therefore respond to the appropriate stimuli and to no others.

It will be simpler to discuss the more general aspects of Hirszfeld's views when we have dealt with the origin of antibacterial sensitizers, and to confine our immediate attention to antitoxic immunity, noting in passing that the problem is in no sense a purely academic one but has important bearings on many problems of diagnosis and prevention.

Perhaps the most significant data, supporting Hirszfeld's contention, are to be found in observations (Hirszfeld, Hirszfeld and Brokman 1924) which suggest a linkage between the inheritance of the power to produce antitoxin and the power to produce the iso-agglutinins which determine the human blood-groups (see p. 155). From these records Hirszfeld draws the following conclusions. Where both parents are Schick-negative the children will be Schick-negative, except during the first few years of life. Where both parents are Schick-positive the children will be Schick-positive, and will remain so throughout life. Where one parent is Schick-negative and the other Schick-positive the children who belong to the same blood-group as the Schick-negative parent tend to be Schick-negative, those who belong to the same blood-group as the Schick-positive parent tend to be Schick-positive. The validity of these observations has, however, been questioned by Rosling (1928) who examined 50 children over one year of age, whose parents belonged to different blood-groups and reacted differently to the Schick test. He found that 23 children gave the same Schick reaction as the parent to whose blood-group they belonged, while 27 reacted differently.

The exact part played by environmental stimuli in the natural formation of antitoxin is extremely difficult to determine, for naturally occurring environmental stimuli are extremely difficult things to measure; we can, however, select environments in which the frequency of such stimuli are likely to be very unequal and compare the Schick or Dick reactions of samples of children drawn from each group.

Zingher (1923) noted that the proportion of Schick-positive children in different New York schools fluctuated very widely (14.62-70.13 per cent.), and that the schools with the highest percentage of positive reactors were those attended by the children of the more fortunate classes, who would have been less likely to have contracted the infection during early life, while the lowest percentage of positive reactors were found in schools attended by children of the poorer classes, and particularly in orphan asylums. The effect of institutionalism on the frequency of the Dick reaction is similar. Table XXI gives a series of observations recorded by Dyer and others (1926).

We might hope to obtain data of the same kind by comparing the frequency of Schick-positive reactions in countries where diphtheria is common with their frequency in countries where it is rare; but the presence or absence of a particular disease among native races, and especially among their children, is less easy to determine than might be supposed, and such evidence as we have is conflicting.

Smits (1926) states that diphtheria is a very rare disease among the natives in the Dutch Indies, and it might therefore be expected that a high proportion of the native population would give a positive Schick reaction. This, he found, was not the case. Among 600 Javanese natives positive results were obtained in only 2 per cent. of the men

and 8 per cent. of the women, while 41 per cent. of the children reacted positively. If the statement of the rarity of diphtheria could be accepted, this striking decrease in the proportions of Schick-positives with age would clearly support the view that antitoxin may be elaborated in the absence of any external specific stimulus. Kirschner (1929), however, records a detailed study of the prevalence of diphtheria in the Preanger district of Java during the period 1921-27, and notes the great increase in the number of recorded cases which followed the provision of facilities for bacteriological diagnosis in 1923. He produces evidence that the disease is an important cause of mortality among native children and that a mild form is relatively common. We cannot, therefore, accept the findings with regard to the Schick test as supplying valid evidence in support of Hirsfeld's views.

TABLE XXI

PERCENTAGE OF DICK-POSITIVE CHILDREN IN CERTAIN SCHOOLS IN WASHINGTON. (After Dyer, Caton and Sockrider.)

School.	Age in Years.	No. Tested.	Per cent. Positive.
Suburban	3-15	75	77.3
Suburban	6-15	59	74.5
33 Rural Schools	4-21	1,147	72.5
33 Rural Schools	6-15	1,039	73.6
Episcopal Home	6-15	47	38.3
Orphan Asylum	3-14	80	32.5
Orphan Asylum	5-15	148	8.1

Smits' observations do not stand alone. Heinbecker and Irvine-Jones (1928) performed Schick tests on 49 Eskimos; only 12 gave positive reactions, and of these 11 were under 12 years of age. Antitoxin was demonstrated in some of the negative reactors. Yet diphtheria is said to be unknown among the Eskimos.

Kleine and Kroó (1930) tested 101 East African natives by the Schick reaction; 95 of these were children between the ages of 6 and 15, the remaining 6 were adults. In no case was a positive reaction obtained. The toxin employed was subsequently retested and found to have lost none of its activity. Specimens of serum were obtained from 11 of the natives and tested for antitoxin; 10 of these contained antitoxin in considerable amount (0.05 A.U. to more than 1 A.U. per c.c.). It is clear therefore that the East African native frequently forms diphtheria antitoxin early in life; yet, according to Kleine and Kroó, this population is not, so far as is known, exposed to infection with diphtheria. (See also Parr and Avery 1928).

The great difficulty in all these cases is that we cannot be sure that the absence of a disease in its clinically typical form means the absence of latent infections. Before we can attach any significance, from this point of view, to a survey of the Schick reactions of a particular population, we need a simultaneous bacteriological survey of the same population, to give us accurate figures with regard to the frequency of the diphtheria bacillus in the throats of apparently normal persons.

A particularly instructive survey of Schick immunity in a population with a low incidence of diphtheria has recently been recorded by Dungal (1932). It concerns the course of events in Reykjavik since 1917. From 1926 onwards diphtheria has been very rare, not only in Reykjavik but in Iceland as a whole; but in the preceding years there was a moderate prevalence, reaching its peak

in Reykjavik in 1921 with an attack rate of 6.9 per 1,000, and subsiding rapidly during the following 5 years. In December 1931 and during the early months of 1932 Dungal tested 814 school children in Reykjavik between the ages of 8 and 14 years. Of these, 81.9 per cent. gave a positive Schick reaction, a figure differing widely from that recorded in cities such as New York or London, where diphtheria is endemic. Still more significant, the curve describing the development of Schick immunity with age was of a very different kind. There was a trivial rise in the proportion of negative reactors from 13.97 per cent. in the 8-year age group to 17.24 per cent. in the 11-year age group; but there was a sudden rise to 26.36 per cent. among the 12-year-olds, and a further rise to 37.50 per cent. at 13 years. This clearly corresponds with the inclusion of children who had experienced the epidemic prevalence of 1921 and the neighbouring years. Fig. 13, reproduced from Dungal's paper, illustrates these facts very plainly. Such an experience as this is clearly a weighty piece of evidence in favour of the dominant rôle played by latent infection in the stimulation of antitoxin production.

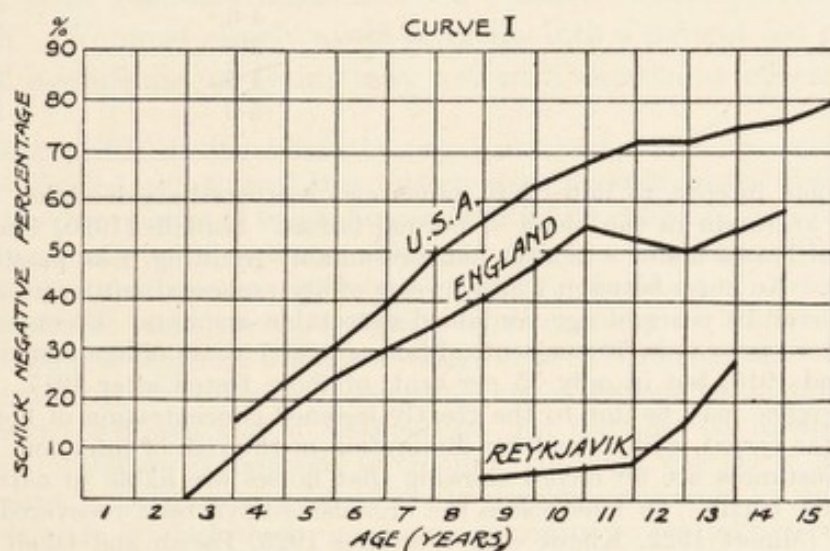


FIG. 13.

Another very instructive way in which we may obtain evidence with regard to the influence of environment on antitoxin production is to study the rate of change from the Schick-positive to the Schick-negative state under different conditions.

A good example is given by Zingher (1923), who records the percentage of Schick-positive children in different age groups among the inmates of a particular institution, dividing them into those who had been resident there for more or less than 3 months. The figures are set out in Table XXII. The numbers examined in some of the groups were small so that the corresponding percentages must be accepted with caution; but there can be no doubt as to the significance of the marked difference between the recently admitted inmates and the older inhabitants. The institutional environment had clearly induced a swing from positive to negative reactivity, with a velocity far higher than could be accounted for by changing age.

Perhaps the most conclusive evidence of the effect of environment and

infection is provided by such studies as those of Dudley (1923, 1926), who observed a small semi-isolated community of boys through a period of several years during which it was visited by three waves of diphtheria. By testing the Schick reactions at different periods he was able to demonstrate a wave of antitoxic immunization associated with each wave of disease (p. 278).

TABLE XXII

PERCENTAGE OF CHILDREN GIVING POSITIVE SCHICK REACTIONS AMONG THOSE WHO HAD RESIDED IN A CERTAIN INSTITUTION, (a) FOR MORE THAN 3 MONTHS, AND (b) FOR LESS THAN 3 MONTHS, ACCORDING TO AGE GROUP. (After Zingher.)

Age Group.		Over 3 Months.	Under 3 Months.
4-5	years.	23.8	66.6
5-6	„	27.3	33.3
6-7	„	17.0	43.0
7-8	„	13.9	43.7
8-9	„	4.6	33.3
9-10	„	3.8	9.0
10-11	„	1.6	33.3
11-12	„	5.0	25.0

It is of some interest to note that numerous observers have recorded the presence of diphtheria antitoxin in the blood of normal horses. Sordelli (1920) found antitoxin in the blood of horses under 9 months old, presumably resulting from passive congenital immunization. No horse between 1 and 5 years of age possessed antitoxin, but the blood of all horses over 10 years of age contained detectable amounts. Glenn (1925) found 0.1 A.U. or more per c.c. in 70 per cent. of horses over 7 years of age tested between the years 1907 and 1915, but in only 35 per cent. of those tested after 1917. He suggests that this difference may be due to the greatly lessened concentration of horses in towns during the later period, and a resulting diminution in the risk of infection. A few well-documented instances are on record showing that horses are liable to natural infection with diphtheria bacilli. In most cases the organisms have been recovered from superficial wounds (Minett 1922, Kliewe and Westhues 1925, Parish and Okell 1926); only twice have they been demonstrated in the throat (Cobbett 1899, Ramon 1925). We have, however, no adequate evidence with regard to the frequency of such infections, so that we cannot tell whether the frequency of antitoxin in the blood, as recorded by Glenn, can reasonably be attributed to external stimuli of this kind.

Summing up, we may say that some of the available evidence, particularly that dealing with the frequency of negative Schick and Dick reactors in areas where diphtheria and scarlet fever are said to be unknown, accords ill with the view that active immunization by latent infection is an essential step in the natural production of antitoxin; but none of this evidence is decisive, since none of it has yet proved the existence of natural antitoxic immunity in a population free from latent as well as from overt infection. There is, on the other hand, no doubt at all that the frequency of negative Schick and Dick reactors in any population or community in which diphtheria and scarlet fever are prevalent, is determined very largely by the degree of exposure to infection. Active immunization certainly plays a large part in natural antitoxin production under such conditions. Whether or not antitoxin would be produced in the absence of this specific stimulus is another question.

THE ORIGIN OF THE NATURAL ANTIBACTERIAL SENSITIZERS

It would take us altogether too far afield to discuss in detail the mass of evidence that bears on the possible mode of origin of the natural agglutinins, lysins, opsonins and complement-fixing antibodies that have been demonstrated in the serum of normal men and animals.

There is no doubt that the antibody-forming apparatus functions in the absence of external stimuli, or that its activities are in part determined by genetic laws. Mankind, and certain other animal species, are divided into a well-defined series of blood-groups, the sera of certain of those groups containing sensitizing antibodies for the red blood corpuscles of certain others. Without entering into recent refinements and complexities, the main facts may be summarized here, partly because of their bearing on the points at issue, partly because of their practical importance in relation to the procedure of blood-transfusion. We must clearly avoid injecting into a patient red cells on which his normal agglutinins or lysins may act with disastrous effects.

Landsteiner (1901) distinguished the principal blood-groups into which the human race may be divided, and showed that this grouping depends on the distribution among human red blood corpuscles of two antigenic constituents, which may be present together, or separately, or may both be absent. There are two corresponding antibodies, and these are so distributed that neither antibody is present in the blood of an individual whose red cells contain the corresponding antigen. By determining the antigens present in any sample of blood it is therefore possible to assign it to its correct group. It happens that two classifications of the four main blood-groups are in common use in different countries, that of Jansky (1908) and that of Moss (1910). The fact that the numbering differs, so that Group I of Jansky corresponds to Group IV of Moss, has led to much confusion. For our present purpose we may adopt the terminology suggested by von Dungern and Hirschfeld (1910-11), in which the antigens are represented by capital letters, the antibodies by Greek letters, and the absence of both antigens or of both antibodies by the cipher O. The groups may then be conveniently named by their antigenic constituents, a method which can cause no confusion. The constituent factors of the four groups can then be set out as follows:

Antigens	O	A	B	AB
Antibodies	$\alpha\beta$	β	α	O

The serum of a Group O blood will agglutinate the corpuscles of Groups A, B and AB, for it contains the antibodies α and β ; but the corpuscles of this group will not be agglutinated by the serum of any group, since they contain neither of the antigens A and B. A person belonging to this group is a "universal donor"; his blood can be transferred with safety to any recipient, but he can only receive with safety the blood of a person belonging to the same group as himself. The interactions of the other groups will be obvious, and it will be noted that a member of Group A can receive blood from Groups O and A, a member of Group B from Groups O and B, while a member of Group AB can receive blood from any group, since his serum contains no antibodies, but can donate blood only to a member of his own group.

There are marked racial differences in the relative frequency of the different groups. Thus among Europeans Group A is far commoner than Group B, while among Asiatics, and particularly among the Indians, the reverse is the case. There is evidence (von

Dungern and Hirszfeld 1910a, b, Hirszfeld 1926) that the antigenic constituents A and B are inherited along Mendelian lines, the presence of each factor being dominant to its absence.

In addition to these isoagglutinins and isolytins—antibodies acting on the corpuscles of other individuals within the same species—the serum of all animal species that have been examined has been found to contain antibodies acting on the red cells of other animal species. The kind, number and activity of these antibodies vary from one species to another in the same way as the bacterial sensitizers described earlier in this chapter. The pioneer researches of Ehrlich and Morgenroth (1899, 1900a, b) established quite clearly, by means of absorption tests, the specificity of the antibodies concerned.

In this connection and because it has a direct bearing on the problem with which we are mainly concerned—the origin and nature of the natural antibacterial sensitizers—brief reference must be made to an antigen-antibody reaction of a curious and interesting kind which was first described by Forssman (1911), who observed that the injection into rabbits of organs obtained from the guinea-pig, or from certain other animals, led to the production of hæmolysin for sheep's red corpuscles. These observations have been confirmed and extended by many subsequent workers (see Taniguchi 1919–20, 1921, 1922, Browning 1931); and it is now known that the so-called *heterophile* or *Forssman* antigen, which is responsible for this reaction, is widely distributed among different animal species, and in a curiously random fashion. Thus the organs of the guinea-pig, horse, dog, cat, mouse, fowl and tortoise stimulate the production of heterophile antibody when injected into the rabbit, while the organs of man, rabbit, ox, sheep, rat, goose, pigeon, eel and frog do not. The organs and tissues that have been examined with regard to the presence or absence of heterophile antigen include kidney, liver, lung, adrenal, testicle, heart, skeletal muscles, lymph glands and serum, though not all of these have been tested in the case of each of the species enumerated above. It is noteworthy that the organs of the sheep, freed of all red corpuscles, do not stimulate the production of heterophile antibody; and it appears to be a general rule that those species that contain heterophile antigen in their red corpuscles do not contain it in their tissues, at least in the full and effective antigenic form.

It soon became clear, from absorption experiments of the usual type, that the hæmolysin produced by the rabbit in response to the injection of guinea-pig's kidney was not identical with that produced in response to injection with sheep's corpuscles. The hæmolytic serum in the latter case contains a mixture of hæmolysins, only one of which corresponds to the heterophile antigen.

It appears (Iwai 1917, Georgi 1919, Taniguchi 1921) that the specific component (hapten) of the heterophile antigen is contained in the lipoid constituents of the tissues, especially in those which are soluble in alcohol and ether and insoluble in acetone—the so-called “lecithin fraction.” The heterophile antibody, in addition to acting as a hæmolysin for sheep's red cells, forms a precipitate and fixes complement when mixed with an emulsion of the lecithin fraction derived from a tissue containing the heterophile antigen. The lipoid extract does not stimulate the formation of the heterophile antibody when injected into the rabbit, but when mixed with a suitable foreign protein it regains its full antigenic property (Landsteiner and Simms 1923). It should be noted that only animals of the rabbit type—those not containing heterophile antigen in their tissues—are capable of producing heterophile antibody in response to the injection of guinea-pig's kidney or other suitable tissue. It is also of particular interest from our present point of view that certain normal antibodies are of the heterophile type—for instance, the natural hæmolysin for sheep's red cells that is found in the serum of rabbit and man (Friedemann 1917).

The natural hæmolysins and hæmagglutinins, like diphtheria antitoxin, are absent from the blood of very young animals, and are developed during the process of normal growth (Hirsfeld 1926, Friedberger, Bock and Fürstenheim 1929). The same is apparently true of the natural antibacterial agglutinins (Kraus and Low 1899, Lüdke 1905, Braun 1909, Gibson 1930. See also Mackie and Finkelstein 1928, 1930). Observations made by Bailey (1923) add a point of considerable significance. In studying the development of the normal hæmagglutinins of the fowl, he noted their absence in the young chick and their appearance during the normal process of growth, but he also found that chickens failed to respond to immunization with foreign red cells until they had spontaneously developed some agglutinating capacity.

It has been noted, in a previous chapter, that all the available evidence indicates that the serum antibodies are closely associated with the globulin fractions of the serum proteins. It is therefore of particular interest to find that the serum of new-born animals is markedly deficient in globulin, particularly in euglobulin, that this deficiency is temporarily made good in certain species by the transfer of globulin from mother to young in the colostrum, and that the capacity for producing euglobulin from its own tissues is developed by the young animal at a later stage of growth (Toyama 1919, Howe 1922, Lewis and Wells 1922). The appearance of the natural antibodies and the increase in serum globulin are closely correlated with one another (see Famulener 1912). Orcutt and Howe (1922) have observed the passage of natural agglutinins for *Br. abortus* from a cow to her calf in the globulins of the colostrum; and Timmerman (1931) has demonstrated the presence of agglutinins for the typhoid bacillus in human colostrum during the first 5 days of suckling.

Accepting the fact that the antibody-forming apparatus functions independently of any external stimulus, so far as certain of its activities are concerned, we may ask ourselves whether the balance of evidence suggests that the naturally occurring antibacterial sensitizers are produced in this way, as a by-product of normal growth and development, or whether their formation depends on a series of responses to specific stimuli on the part of a mechanism that is absent, or imperfectly developed at birth, but develops rapidly thereafter and apparently attains its full potentialities at a relatively early period of life. If we adopt the latter view, we must inquire a little more closely into the nature of the specific stimuli concerned. Are we to assume that they consist, of necessity, in typical, atypical and symptomless infections with each and all of the bacteria on which the natural antibodies act, or are there other ways in which effective stimuli might be applied?

It will simplify the issue to take the hypothesis of infection first. What evidence is there that natural antibacterial sensitizers arise in this way? That they may so arise, the evidence is overwhelming. There are, for example, abundant observations on the production of agglutinins—flagellar and somatic—during an attack of enteric fever, and their persistence, in some cases at least, well beyond the period of clinical convalescence. There is also the

highly significant observation (see pp. 320, 321) that the frequency of particular agglutinins among different human populations appears to be highly correlated with the frequency of infection with the corresponding bacteria.

It is needless to multiply instances from human medicine, and a single illustrative example from the field of experimental studies will suffice.

Bailey (1927) examined 81 rabbits taken from a laboratory stock among which cases of snuffles were occurring, due to infection with *Pasteurella lepi-septica*. Three rabbits were suffering from acute snuffles; cultures from the nose gave a copious growth of *Past. lepi-septica*, and agglutinins and complement-fixing antibodies were demonstrated in the blood stream. Nine had chronic snuffles; all of these showed the presence of *Past. lepi-septica* in the nares and agglutinins and complement-fixing antibodies in the serum. Three had suppurative lesions from which *Past. lepi-septica* was isolated; agglutinins and complement-fixing antibodies were demonstrated in their serum. Six showed no clinical symptoms of infection, but *Past. lepi-septica* was isolated from the nares; in all 6 agglutinins and complement-fixing antibodies were found. Ten showed no symptom of infection and cultures from the nares yielded no *Past. lepi-septica*; in none of these could agglutinins or complement-fixing antibodies be demonstrated. Six rabbits were then taken, 1 of which yielded a scanty growth of *Past. lepi-septica* and showed the presence of complement-fixing antibodies to low titre, while the other 5 showed no evidence of infection. All 6 were infected with *Past. lepi-septica* by nasal instillation. All 6 became carriers without any symptoms of snuffles. The rabbit which was already infected showed a rise in titre of complement-fixing antibodies, and these antibodies appeared in each of the other 5 rabbits during the course of the succeeding 3 months.

There is then no doubt that the members of a herd, or population, submitted to a constant or recurrent risk of infection with a particular bacterium will tend to form antibodies acting on that particular organism in response to the stimuli provided by infection of varying severity. The question is whether such a mode of formation can be accepted as a reasonable explanation of the origin of *all* the antibacterial sensitizers found in the blood of normal men and animals.

If we turn back to the observations of Bürgi, of Gibson and of Lovell on normal agglutinins recorded on pages 144, 145, we seem at first sight to be met with insuperable difficulties if we attempt to adhere to the infection hypothesis. Can we really believe that such animals as the ox and the pig are infected during their relatively short lives with such a variety of organisms as *Proteus* X19, *Bact. typhosum*, *Bact. paratyphosum A*, *Bact. paratyphosum B*, *Bact. enteritidis*, *Bact. dysenteriae* (Flexner), *Bact. dysenteriae* (Shiga), *V. cholerae* and, doubtless, a host of other pathogenic organisms that are only absent from the list because they have not been tested? It is proverbially difficult to prove a negative; and it would be rash to assert that even so extensive an infection experience as this is impossible. Perhaps the best answer is that it seems altogether unlikely—so unlikely that any other alternative hypothesis would be welcome. If we confine ourselves to the Salmonella group, for instance, we find (Lovell 1932 and unpublished data) that agglutinins for a variety of the flagellar and somatic antigenic components of the members of this group may be demonstrated in the blood of a high percentage of young swine, and that these agglutinins can, in the majority of cases, be specifically absorbed;

yet cultures of the faeces and organs of the animals from which the specimens of blood were obtained have in no instance yielded a growth of the corresponding organisms. Moreover the cultural results recorded by other observers are almost all of the same negative kind, at least in those instances in which the strains isolated were adequately identified.

If we tentatively abandon the view that all the normal antibacterial sensitizers are produced in response to specific infection, must we at the same time jettison the hypothesis that their formation is conditioned by some external specific stimulus, and fall back on the conception of an innate tendency to produce antibodies of a particular type? Our present knowledge of the factors determining antigen-antibody reactions lends no support to such an attitude. Antigenic specificity depends, as we have seen, on chemical structure. Its biological or, in the narrower sense, its bacteriological significance is determined simply by the actual distribution of the specific chemical groupings—the haptens—among existing species of micro-organisms. Our knowledge of this distribution is scanty in the extreme. We know something of the detailed antigenic structure of certain groups of closely related bacteria, but almost nothing as regards the possible sharing of antigenic components by species that show no other obvious relationship. What little we do know suggests that such a possibility cannot be ignored (see pp. 105, 106).

We must, then, accept the possibility that the antibodies which we detect in the blood of a high proportion of animals of a particular species, and which act specifically on bacteria *A*, *B* and *C*, may have been produced in response to infection with some other organisms, *X*, *Y* and *Z*. Lovell (unpublished data) has explored this possibility in the case of the natural agglutinins of young swine, and has found in two instances that coliform bacilli isolated from the mesenteric glands will induce in rabbits the formation of agglutinins acting on certain bacilli of the *Salmonella* group. Though these observations can, at the moment, be regarded as no more than suggestive, they indicate the desirability of further work along similar lines. Nor should we limit the possibilities to actual infection with an unrelated bacterium possessing a common antigenic grouping. In defining an antigen we excluded administration by the digestive tract when demanding that it should give rise to the production of antibodies on injection into a suitable animal, because the antigen might be chemically altered while in the digestive tract, and we should in any case have no assurance that it would pass from the intestine into the tissues in an active form. But recent studies on oral immunization (see pp. 177, 178) have made it clear that the antigenic components of dead bacterial cells do, in some cases at least, pass from the intestines to the tissues and stimulate antibody formation. It is possible that among the unlimited variety of other antigenic materials which gain access to the intestinal tract, some, under favourable conditions, may leave an immunological imprint as they pass. Were this the case, there would be little cause for surprise if some few of the multiplicity of reactive groupings produced by an antibody-forming apparatus subjected to an infinite succession of widely varied stimuli should

happen to show affinities to the antigenic groupings of certain species of bacteria. Nor need we limit the possibilities to the intestinal tract. There is good evidence that inhalation of antigenic material may result in an effective antibody response; and under suitable conditions similar material may gain access to the tissues through the skin.

Even if we exclude external stimuli, there may well be cross reactions between the antibodies produced under the influence of genetic factors and the antigenic components of certain bacterial cells. There is indeed definite evidence that this is so. There can be little doubt of the genetic origin of the heterophile antigen of Forssman, yet (see p. 156) this antigen, or an antigen with a similar grouping, seems to be present in many bacteria.

We must then accept the view that the naturally occurring antibacterial sensitizers, and perhaps also the natural antitoxins, may owe their origin to any one of four mechanisms: actual infection with the corresponding bacterium; infection with some other organism that shows a common antigenic material component; the entrance to the tissues *via* the intestinal tract, or possibly by other routes, of non-living antigenic material capable of stimulating the production of an antibody with the active group in question; or the formation of such antibodies as a by-product in the normal functioning of the antigen-forming apparatus altogether apart from any specific external stimulus.

It would seem that we should regard an animal host as endowed at birth with a complex of antibodies, many of them with protective value, derived from the maternal blood-proteins, either by way of the placental blood vessels, or by way of the colostrum, or both. As infancy passes, the young host will lose this congenital endowment, and must equip itself with a new set of antibodies, produced by the activity of its own tissues. A period will elapse, between early infancy and adolescence, during which its stock of antibodies will be at a low ebb, because its own antibody-forming apparatus will not yet have developed full functional activity. As this apparatus reaches maturity it will form and pass into the blood stream a complex of antibodies, some at least of which will be developed in accordance with innate tendencies and altogether apart from external environmental stimuli. Many of these will be primarily directed against antigens contained in the red blood corpuscles or tissue cells of other animal species, or of other members of the same species; but among them may be some, either chance by-products or formed in accord with some evolutionary and heritable tendency, which act on certain bacterial cells or on their products. However this may be, it is certain that the adolescent animal is sensitive to stimuli from without, and that in response to such stimuli an additional complex of antibodies will be formed. The possible nature of such stimuli we have discussed above. In the adult stage, one animal will differ from another of the same species as the result of differences in immunological experiences during early life. It would be rash to say that this experience is the sole significant factor in determining the immunity of the adult host; but there can be no doubt that it is an exceedingly important factor, and its importance is increased from the medical point of view by the fact

that our ability to control infective disease must depend, in large part, on determining and then exploiting the various factors on which this type of acquired immunity depends.

SUMMARY

(1) Antibodies of various kinds—antitoxins, agglutinins, bactericidal antibodies and so on—are frequently found in the serum of normal men and animals. The origin of these naturally occurring antibodies is still a matter in dispute; but it seems possible that they may arise in several different ways.

(2) The new-born animal, when born of a naturally immune mother, is endowed with a congenital passive immunity, due to transference of specific antibodies from the maternal blood through the placenta, or by way of the colostrum during the early days of suckling. This, like other types of passive immunity, is of relatively short duration.

(3) This congenital passive immunity is followed by a period of relative susceptibility, until the young animal develops an active and lasting immunity conditioned by the formation of specific antibodies by its own tissues. It is the mode of formation of these antibodies that is still a matter of uncertainty.

(4) There are reasons for believing that the production of antibodies is dependent on the activity of an antibody-forming apparatus that is imperfectly developed at birth, but reaches functional maturity at a relatively early period of life.

(5) It is certain that this apparatus, apart from any external stimuli, will form a particular group of antibodies—the normal hæmagglutinins or hæmolysins—which distinguish one animal species from another. It is believed by some that the antibodies acting on bacteria or their products may be formed in the same way, as the result of a normal serogenesis determined by purely genetic factors.

(6) The more commonly accepted view is that these antibodies arise as the result of the response of the antibody-forming apparatus to external environmental stimuli, and that such stimuli usually consist of overt or latent infections with the bacterium in question. This view has been upheld particularly in relation to natural antitoxic immunity against diphtheria and scarlet fever.

(7) In the case of specific antibacterial immunity there are greater difficulties in accepting this hypothesis in its entirety. Samples of sera collected from a given animal species may contain antibodies active against an enormous number of different bacteria, and it seems difficult to believe that a single animal can have been infected with so many different organisms, some of which are not known to be natural parasites of the species to which it belongs.

(8) It is possible that other forms of external stimuli may be concerned, such as (a) infection with a different species of bacterium which shares a common antigenic factor with the bacterium on which the antibody in question has been shown to act, or (b) the absorption from the intestinal canal or

through the lungs or skin of some antigenic material containing a similar reactive chemical group.

Whatever the true facts may be, there is no doubt that typical and atypical infections play a part, and probably a very important part, in determining the actual distribution of antitoxins and antibacterial sensitizers among any sample of an adolescent or adult population.

(9) The presence of natural antitoxin in the circulating blood usually confers an effective protection against clinical infection, at least in the case of diphtheria and scarlet fever.

(10) The relation between the presence of antibacterial sensitizers and resistance to infection with the corresponding bacteria is very uncertain. We should expect those natural antibodies that act on the surface antigens of virulent bacteria to exert a protective action, but there is as yet little direct evidence bearing on this point.

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CHAPTER XI

THE ANTIBODY-FORMING APPARATUS AND ITS REACTIONS

IN the preceding chapters we have referred freely to the serum antibodies without any attempt to describe the mechanism by which they are produced. Any adequate description is, indeed, impossible. We know too little. It is, for instance, quite clear that the formation of antibodies is associated in some intimate way with the formation of the serum proteins, but we are quite ignorant as to how these proteins are produced or where. The scanty data at our disposal with regard to the cells and tissues probably involved and the way in which they respond to specific and non-specific stimuli are, however, well worth considering ; since they throw light on several problems of practical as well as theoretical importance.

THE SITE OF FORMATION OF ANTIBODIES

Ehrlich's conception of antibodies was that they were shed "cell-receptors." The antigen gained effective contact with the cell by becoming anchored, through specific chemical groupings of its own, to specific chemical groupings on "side-chains," carried by the tissue cells. This theory seemed to imply, by logical extension, the view that antibodies were produced by those cells to which particular antigens, by reason of their chemical structure, were most likely to become attached—that an antitoxin, for instance, was produced by the cells that were most susceptible to the corresponding toxin. A further implication seemed to be that any cell in the body was endowed with the potentiality of forming antibodies should antigens with suitable side-chains present themselves to it. It would be rash to say that the possibility of such a generalized distribution of function has been excluded, but the trend of opinion, especially in recent years, has quite clearly been towards a different conception, which would regard the antibody-forming apparatus as an integrated system of cells, together constituting a functional though not a structural entity. The evidence on which this view is based may be summarized as follows.

The Local Formation of Antibodies.—Experiments designed to demonstrate the local formation of antibodies, at or in the immediate neighbourhood of the site of inoculation, have given negative or equivocal results.

Römer (1901) recorded observations which he regarded as demonstrating the formation of anti-abrin in the rabbit's conjunctiva ; and von Dungern (1902) injected crab-plasma

into the anterior chamber of one eye of each of several rabbits and recorded, in one instance, the appearance of a precipitin for crab-plasma in the aqueous humour of the injected eye before its appearance in the blood serum. But these observations have never been confirmed; and, at least in the case of Römer's results, they are open to criticism on technical grounds (see Hektoen 1911). Similarly, the results recorded by Wassermann and Citron (1905), who injected typhoid bacilli into rabbits intrapleurally and intraperitoneally, and compared the antibody titre of the local exudate and of the blood serum at various times thereafter, afford no convincing evidence of any formation of antibodies in the serous cavities. Hektoen (1911) conducted a very careful series of experiments on the immunization of dogs with rat or goat corpuscles. He was unable to obtain any evidence of antibody formation in the anterior chamber of the eye, in the pleural or peritoneal cavity, or in the connective or muscular tissue of the leg. In every case antibodies appeared in the blood, but there was no evidence that these were produced at the site of inoculation. Thus, amputation of a leg 48 hours after a suspension of red cells had been injected into the muscles made no difference to the time at which antibodies appeared in the blood, or to the titre which they attained.

Trawinski (1932) records experiments indicating that the skin is concerned in agglutinin production, irrespective of the route by which the bacterial antigen is injected; but this he attributes to the presence of numerous reticulo-endothelial cells, so that his results are in accord with the views developed in later sections of this chapter.

The Distribution of Antibodies in the Tissue Fluids.—There is a considerable body of evidence indicating that the distribution of antibodies in the tissue fluids follows a very definite order, and that this order is independent of the particular method of immunization employed.

Pagano (1894), Falloise (1903) and Batelli (1904) found that the concentration of hæmolysin was lower in the thoracic lymph than in the blood serum. Hughes and Carlson (1908), working with normal dogs, horses and cats, found that the concentration of hæmolysin for rabbit corpuscles formed a descending series, in the order—serum, thoracic lymph, neck lymph, pericardial fluid, aqueous humor. Becht and Greer (1910) studied the distribution of normal and immune antibodies in dogs. They also studied the rate of transference of antibodies from the circulating blood to the tissue fluids after passive immunization by the intravenous route. Their findings with regard to the distribution of normal and immune antibodies were in accord with those of Hughes and Carlson. In the actively immunized animals they observed a marked rise in the antibody titre of the serum, thoracic lymph and neck lymph, the relative concentrations in the different fluids maintaining the same order as in the normal animals. After passive immunization, by transfusion of blood from an immunized to a normal dog, the characteristic distribution of antibodies was established within 4½ hours. These findings were confirmed in all essentials by Hektoen and Carlson (1910).

The Rôle of the Reticulo-Endothelial System in the Formation of Antibodies.

Hektoen and Carlson (1910) carried out other experiments which supplied data of great significance. They injected dogs with rat or goat corpuscles and bled them dry from the carotid artery during the latent period of immunization, before any rise had occurred in the antibody titre of the serum; immediately thereafter they restored their blood volume by transfusion from a normal dog. This procedure appeared to have, if anything, a stimulating effect on the subsequent antibody production, indicating that the injected antigen was rapidly removed from the blood. This suggestion was confirmed in other experiments in which normal dogs were bled dry and transfused with blood from other dogs in the latent stage of immunization (3 to 48 hours after

inoculation of the antigen). When the dose of red cells injected into the donor had been of the usual optimal amount the recipient showed no rise in antibody titre, indicating that no appreciable amount of antigen remained in the circulating blood even after so short an interval.

We need only recall the description of the clearing mechanism given in Chapter VIII to evoke the suggestion that the cells of the reticulo-endothelial system, which we know to be concerned in the removal of foreign particulate material from the blood stream, are also concerned directly or indirectly in the formation of antibodies.

The view that certain of the organs or tissues which we now group together under this system might be specially concerned in antibody formation is by no means new. Pfeiffer and Marx (1898) stated that in rabbits which were undergoing immunization against cholera vibrios bacterial antibodies might be present in the spleen, the bone-marrow, and perhaps the lungs, in higher concentration than in the blood (see also Tsurumi and Kohda 1913, Cary 1922).

Deutsch (1899) studied the effect of splenectomy on the production of agglutinins and protective antibodies after intraperitoneal injection of typhoid bacilli into guinea-pigs, and found that splenectomy before the injection of the bacterial antigen had little or no effect on the subsequent antibody production, while splenectomy carried out 3 to 5 days after the immunizing injection led to a significant decrease in the concentration of antibodies. He added the interesting observation that, when the spleen was extirpated from a recently immunized guinea-pig and transferred to the peritoneum of a normal guinea-pig, antityphoid agglutinins appeared in the blood of the latter though to a relatively low titre. Luckhardt and Becht (1911) studied this question in greater detail. Dogs were injected intravenously with rat or goat corpuscles. Shortly afterwards the spleen was removed, emulsified, and injected into the peritoneum of a normal dog. The recipients showed the typical rise in the antibody titre of the blood serum. The animals from which the spleen had been removed after injection of the red cells produced hæmolysins, hæmagglutinins and hæmopsonins less rapidly and to a lower titre than did the control animals not submitted to splenectomy.

Some recent experiments on agglutinin formation in rabbits (Topley 1930) have given analogous results, and afford the additional suggestion that, after minimal doses of antigen, the reticulo-endothelial cells do not release the antigen in an unaltered form. Rabbits received intravenous injections of killed paratyphoid bacilli in a dose of 10^5 bacilli per kilo. body weight. At varying intervals thereafter, 24 hours to 216 days, they were bled and killed. The spleen, emulsified in saline, was in each case injected into a normal rabbit. The serum from a rabbit killed after 24 hours contained no agglutinins. The rabbit that received the spleen tissue from this animal developed agglutinins to low titre, but the time relations of the response differed from those of the typical response to active immunization (see pp. 170-172) in several ways. The highest titre was reached earlier, and the agglutinins disappeared far more rapidly from the blood stream. Reactions of the same general type were noted in the normal rabbits injected with the spleens removed from the immunized animals after longer intervals. Moreover, these rabbits, when injected

21 to 62 days after receiving the spleen tissue with a minimal dose of the same bacilli, showed no evidence of the energetic secondary response (see pp. 173-175) which might have been expected if they had received a previous stimulus with unaltered antigen. These results are more compatible with the view that the histiocytes of the spleen themselves form antibodies or some intermediate product than with the view that they merely retain the antigen and afterwards liberate it in a form which provides an antigenic stimulus to other antibody-forming cells.

Scarff (1931) has recorded experiments suggesting that bacteria and red blood corpuscles are dealt with in a different way by the splenic tissue; and it may be that we should consider different antigenic materials—foreign cells, bacteria and antigens in solution—in relation to different depôts of histiocytes rather than antigens in general in relation to reticulo-endothelial cells in general.

The rôle of the reticulo-endothelial system in the production of antibodies has also been investigated by the method known as "blockade," alone or combined with splenectomy. In this method large amounts of Indian ink or colloidal iron are injected intravenously, with the object of engaging the activities of as large a quota as possible of the reticulo-endothelial system, and so rendering it less able to deal with a subsequent injection of antigenic material. The results recorded show some divergence. Bieling and Isaac (1921, 1922*a*, *b*) reported an inhibitory effect of blockade on hæmolysin production, and several workers have obtained analogous results (Siegmond 1922, Gay and Clark 1924, Vanucci 1924, Jungeblut and Berlot 1926, Lewis and Loomis 1926, Meyer 1926). Others, however, have failed to detect any difference between the behaviour of normal and blockaded animals (Rosenthal and Fischer 1922, Fränkel and Grunenbergl 1924, Ross 1926). Such discrepancies need cause no surprise. In the case of a system of cells so widely distributed throughout the body, the effectiveness of any method of blockade must vary according to the conditions obtaining in each particular case. Blockade that falls short of completeness may, as some authors have suggested (Standenath 1923), have a stimulating rather than a depressing effect.

Other methods of injuring or disabling the cells of the reticulo-endothelial system have been utilized in attempts to study the part played by these cells in antibody production—the action of X-Rays (Benjamin and Sluka 1908, Låwen 1909, Hektoen 1915, 1918, 1920), of thorium X (Hektoen and Corper 1920), and of radium emanation (Hektoen and Corper 1922), of benzene (Rusk 1914, Simonds and Jones 1915, Hektoen 1916*a*), of toluene (Hektoen 1916*b*) and of mustard gas (dichlorethyl sulphide) (Hektoen and Corper 1921). The results of these experiments, taken as a whole, lend support to the view that any procedure which severely injures the cells of the reticulo-endothelial system will markedly, or sometimes completely, inhibit the formation of antibodies. Their significance is, perhaps, rendered a little dubious by the fact that the doses required to produce a clear-cut inhibitory effect were of an order which makes it unlikely that their action was confined to the cells of the reticulo-endothelial system and the hæmopoietic tissues.

The Rôle of Other Organs or Tissues in the Formation of Antibodies.

It may be noted that the effect on antibody formation of the removal of other organs—unconnected with the reticulo-endothelial system—has been studied by various observers. The removal of the thyroid has apparently no inhibitory effect (Fjeldstad 1910, Hektoen and Curtis 1915, Ecker and Goldblatt 1921, Houssay and Sordelli 1921, Clevers 1921,

Také 1923). An increase in antibody production has occasionally been recorded as a result of this procedure (Ecker and Goldblatt 1921, Houssay and Sordelli 1921, Clevers 1921). Hektoen and Curtis (1915) state that complete removal of the stomach or of the small intestine does not interfere with the formation of hæmopsonins or hæmagglutinins; simultaneous removal of the spleen and pancreas appears to have the same depressing effect on antibody production in dogs as removal of the spleen alone; adrenalectomy in dogs, at the height of the antibody curve, does not alter the antibody content of the blood serum; in rats, removal of one half of the liver appears to have no effect on the production of hæmolysin.

The Formation of Antibodies in Tissue Cultures.—A method which offers obvious possibilities for the study of antibody formation is that of tissue culture.

Carrel and Ingebrigsten (1912) cultivated fragments from the bone-marrow and lymph glands of guinea-pigs in the homologous blood plasma, and added to the cultures small amounts of red cells from the goat, against which the serum of the guinea-pig showed no lytic action. Hæmopsonins appeared in the culture on the 3rd day, as judged by direct observation of the degree of phagocytosis. Hæmolysins were detectable on the 4th day, and had increased markedly in activity on the 5th. The hæmolysins could be specifically absorbed from the fluid by the usual technique. Lüdke (1912) injected killed cultures of typhoid or dysentery bacilli into rabbits, removed fragments of the spleen or bone-marrow after 1 to 5 days, and cultivated them in homologous plasma. After 2 to 5 days' incubation he was able to detect lysins and agglutinins in the culture fluid. In a similar way he succeeded in producing hæmolysin for ox or sheep corpuscles. His results were for the most part negative when he added the antigenic materials directly to the tissue cultures. Przygode (1913, 1914), Reiter (1913) and Schilf (1926) have also recorded positive results. Meyer and Loewenthal (1928) record experiments in which they were able to demonstrate a significant rise in agglutinins in tissue cultures prepared from rabbits that had been inoculated with typhoid bacilli. The tissues examined were the spleen, the lymph glands and the milk spots from the omentum. The latter observation is highly significant, since the omental milk spots, in addition to fibroblasts, contain only cells of the reticulo-endothelial type.

Conclusions with regard to the Site of Formation of Antibodies.—Taking the data as a whole the conclusions to be drawn do not seem to be in doubt in spite of minor discrepancies. There is ample evidence that the organs and cells which have been shown to be concerned in the clearing mechanism of the tissues are also concerned in some way with antibody formation. There is no clear evidence that any other organ or tissue plays a similar rôle.

If we inquire more closely as to the part which the cells of the reticulo-endothelial system play in antibody formation, we must be content with vague hints rather than detailed answers. We know that they remove from the blood stream particulate antigenic material, and probably similar material in colloidal solution. Do they themselves produce the corresponding antibodies, or do they merely retain the antigen temporarily, yielding it up in an altered or

unaltered state to the tissue fluids, and thus passing it on to some other cells in which the actual antibody synthesis occurs?

Of the experiments outlined above, some of those dealing with the rôle of the spleen in antibody production, and the results obtained by Meyer and Loewenthal with cultures from the milk spots of the omentum, seem to offer the suggestion that the histiocytes are capable of producing antibodies in the fully formed state.

THE MODE OF PRODUCTION OF ANTIBODIES

As to how antibodies are formed we have no positive knowledge that is based on direct experiment; though it would seem that one possibility can be excluded.

In the early days of immunology the hypothesis that the injected antigen was in some way incorporated in the antibody was tentatively advanced. It had the obvious merit of offering a possible explanation of specificity, but was soon abandoned because of the enormous quantitative disparity between the antigen injected and the antibody formed. More recently it has been revived in a less direct form; but it is difficult to accept in any form at all. In certain of the experiments recorded above it was calculated that the dose of active antigenic material injected into a 2,000 gm. rabbit was of the order of 0.000,000.5-0.000,005 mg., yet this dose might result in the production of agglutinin to a titre of 1:500. Hooker and Boyd (1931) have calculated that, taking such relationships into account, one molecule of active antigenic substance must give rise to the amount of antibody globulin sufficient to agglutinate 600 bacteria; and, since the surface relationship between one molecule of globulin and 600 bacteria is of the order of 1:25,000,000, there seems no room for any hypothesis which assumes the permanent incorporation of any fraction of the antigen in the antibody complex. Berger and Erlenmeyer (1931) and Hooker and Boyd (1931, 1932) have examined this question experimentally, using a linked synthetic antigen containing arsenic, and testing for the presence of arsenic in the antibody-containing serum. These tests were entirely negative and the limits thus set to the amount of antigen present in the antiserum appear to render it impossible that a molecule of antibody can contain any grouping derived from the antigen. If the antigen is incorporated in the antibody at any stage in the formation of the latter, it must apparently be released again, perhaps leaving its impress on the complex of which it once formed a part, and by repeated reassimilation modifying successive fractions of the serum globulin (see Topley 1930).

A working hypothesis of the method of antibody formation, that has a reasonable *a priori* probability and accords well with our present knowledge of the structure of antigens and antibodies, has been put forward independently by Breinl and Haurowitz (1930), Alexander (1931) and Mudd (1932). This hypothesis, ignoring minor differences in the views of its sponsors, assumes that any antibody is globulin, and that it is *new* globulin, synthesized under the directing influence of the antigen. This influence is exerted through a combination of the peptides, or amino-acids, with the active groupings of the antigen during the globulin synthesis. Mudd lays special stress on the point that this synthesis occurs at the interface between the antigen and the surrounding fluid, a point of particular interest in view of the observation (see p. 100) that the antibody content of an antibacterial serum seems to be largely determined by the nature of the active groupings at the cell surface. The globulin thus formed is assumed to be dissociated from the antigen after a certain stage

has been reached, and the latter is thus able to leave its stereochemical imprint on successive molecules of antibody globulin, synthesized under the same conditions. Breinl and Haurowitz would locate this synthesis in the reticulo-endothelial cells, the antibody globulin being released when these cells disrupt. This hypothetical localization would, at least, accord quite well with our knowledge of the activity of the reticulo-endothelial system.

THE RESPONSE OF THE ANTIBODY-FORMING APPARATUS TO SPECIFIC STIMULI

The Primary Response to a Specific Stimulus.—It will simplify discussion to consider first the response to the injection of an antigenic material directly into the blood stream. It will also be convenient to select an example in

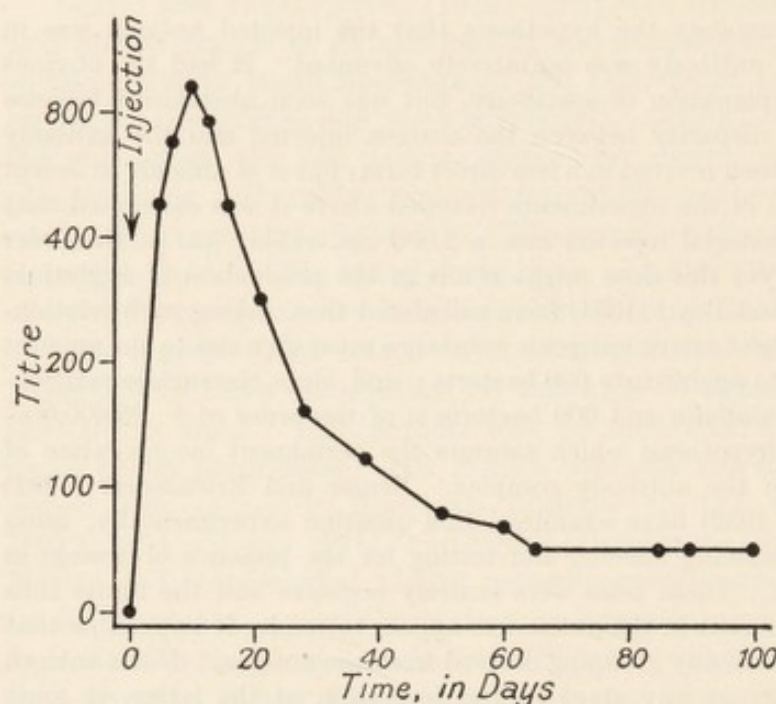


FIG. 14.

Production of agglutinins in rabbit after single injection of *Bact. paratyphosum B.*

which the antibody concerned is not present *ab initio* in the blood of the experimental animal employed. The production of flagellar agglutinins by the rabbit in response to a single intravenous injection of a killed suspension of *Bact. paratyphosum B* affords a convenient illustrative example. Fig. 14 shows a typical reaction.

There is an initial induction phase, not shown in this particular diagram because the first reading after the injection of the antigen was taken on the fifth day. This induction phase usually lasts for 24 to 48 hours, by the end of which time agglutinin is usually detectable to very low titre (1 : 5 to 1 : 20). The titre then rises rapidly as shown in the figure, reaching its maximum about the 16th day—the time limits between which the maximum titre is usually attained may be put at about the 10th to 22nd day. It then falls, at first rapidly, then more slowly, agglutinins still being present in the blood 100 days after the inoculation. It will be noted that, in this chart, the titres are plotted as ordinates on a logarithmic scale, time as abscissæ on an arithmetical scale. Equal rises and falls in the curve represent not equal increments or losses of agglutinins but equal proportionate increases or decreases—for example, doublings or halvings of the amount present.

The Effect of Dosage.—As regards the relation between the dose of antigen

administered and the amount of antibody produced, Table XXIII sets out some figures for the particular case we are considering.

The minute dose of 10,000 bacilli per kilo. body weight, administered to rabbits varying from 1,380 to 3,750 gm., results in no detectable formation of agglutinins. When we increase the dose ten times we pass the threshold and obtain an average titre of 1:330. Over the range 10^5 to 10^8 bacilli a thousandfold increase in dose results in about a tenfold increase in titre—the critical student glancing at the numbers in the second column will not place too much reliance on the mean values recorded in the third. If we increase our dose still further we shall get still higher titres; but we shall be reaching the point where our inoculum attains an appreciable toxicity, and we shall, apart from this, be subject to the law of diminishing returns. However high we push our dose, up to the limit that will kill our rabbit, we are unlikely, after any single inoculation, to reach a titre of more than 1:10,000–1:20,000.

TABLE XXIII

SHOWING THE MEAN VALUES OF THE HIGHEST TITRE OF FLAGELLAR AGGLUTININS IN SMALL GROUPS OF RABBITS INJECTED INTRAVENOUSLY WITH DIFFERENT DOSES (PER k.b.w.) OF *Bact. paratyphosum B.*

No. of Bacilli injected per k.b.w.	No. of Rabbits tested.	Highest Titre. (Mean Value).
10^8	3	3,540
10^7	3	1,860
10^5	6	330
10^4	4	0 ¹

We may sum up in general terms by stating that there is a minimal threshold value, below which no response is obtained, that above this value the titre attained varies with the dose administered but in such a way that the increase in titre is relatively much smaller than the increase in dose required to produce it, and that a point is reached at which further increase in dose results in little or no increase in antibody production.

Variations in Response to Different Antigens.—Fig. 15 shows the effect of injecting into an animal (a dog) a particular antigen (rat corpuscles) against which it possesses a normal antibody (hæmolysin).

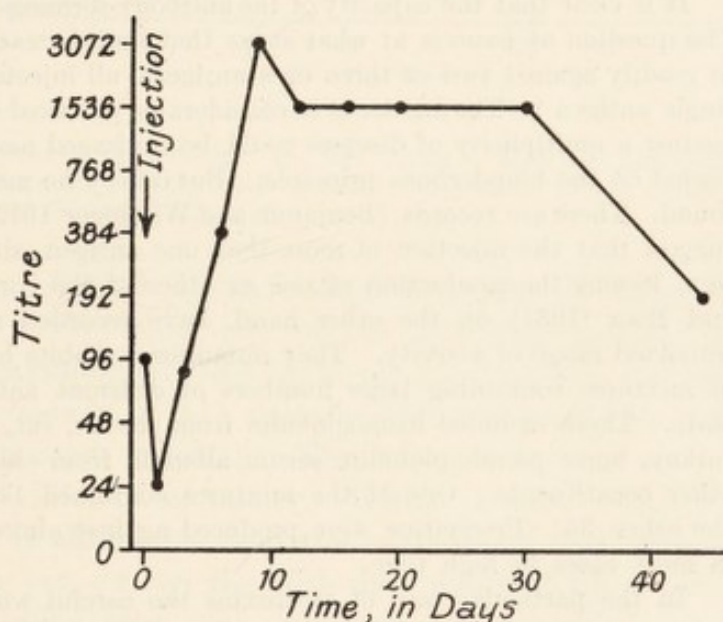


FIG. 15.

Production of hæmolysin in dog after single injection of rat corpuscles. (After Hektoen and Carlson.)

¹ = no agglutination at a dilution of 1:4.

It will be noted that the immediate effect of the injection is to cause a fall in the normal antibody titre, followed by a rapid rise of the type depicted in Fig. 14. This *negative phase* is a characteristic result of injecting any large amount of antigen into an animal that possesses the corresponding antibody in its circulating blood.

The response to a single dose of an antigen in colloidal solution is illustrated in Fig. 16, which shows the course of precipitin formation in the rabbit following a single injection of horse serum.

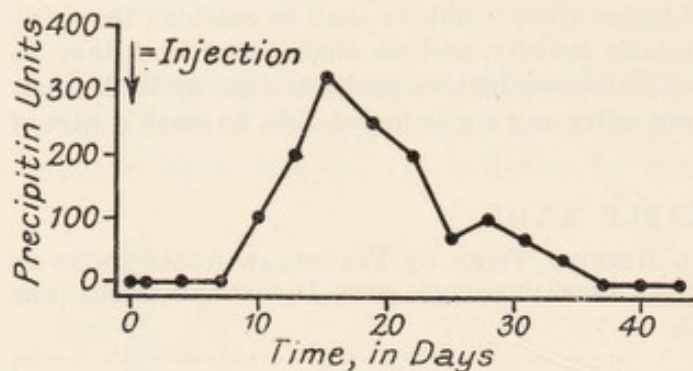


FIG. 16.

Precipitin production: response of rabbit to single injection of horse serum. (After Dean and Webb.)

chapter since the titres are plotted as an arithmetical not as a geometrical or logarithmic series. To make the curve comparable with those describing agglutinin or hæmolysin production the logarithms of the number of precipitin units would have to be plotted as ordinates.

The Response of the Antibody-forming Apparatus to the Simultaneous Injection of Several Antigens.

It is clear that the capacity of the antibody-forming apparatus must have some limit. The question at issue is at what stage that limit is reached. Will antibodies be formed as readily against two or three or n antigens, all injected at the same time, as against a single antigen? The answer is of considerable practical interest, since mass immunization against a multiplicity of diseases could be envisaged more cheerfully if syringes could be loaded on the blunderbuss principle. But it is by no means certain that that principle is sound. There are records (Benjamin and Witzinger 1912, Huntoon and Craig 1921) which suggest that the injection of more than one antigen, simultaneously or in rapid succession, lessens the production of one or other of the corresponding antibodies. Hektoen and Boor (1931), on the other hand, have recorded results which indicate a far less restricted range of activity. They immunized rabbits by repeated intravenous injections of mixtures containing large numbers of different antigens, each in a relatively pure state. These included hæmoglobulin from the ox, cat, dog, hog, horse, man, sheep and turkey, horse pseudo-globulin, serum albumin from chicken and man, egg albumin and other constituents. One of the mixtures contained 14 different antigenic constituents, the other 35. Precipitins were produced against almost all the antigens injected, and in most cases to high titre.

In the particular case of antitoxins the careful work of Glenny and his colleagues (Glenny 1925a, Glenny, Hopkins and Waddington 1925, Glenny and Waddington 1926, 1928) makes it clear that the simultaneous injection of several antigens may considerably reduce the titre of antitoxin obtained. It seems likely, as Glenny suggests, that this "crowding out" effect is particularly prone to occur when any one antigen is present in great excess.

With optimal doses of each constituent and a total inoculum which is not unduly

large, it seems probable that an effective response can be obtained against a reasonable number of different antigens, even if the titre of some or all of the corresponding antibodies is relatively reduced. From the practical point of view the problem will clearly differ according as we are attempting to induce an active immunity in man or animal, or to produce a high titre serum for passive immunization. In the former case a reduction in the antibody titre attained might be of minor importance, in the latter it would be a grave disadvantage. Each case, as it arises, should be subjected to experimental trial.

The Difference in Response to Primary and Secondary Stimuli.—Having considered the response of the antibody-forming apparatus to a single injection of antigen we may inquire how it responds to repeated stimuli, and it will be convenient to take as our first instance the response of a horse to the injection of diphtheria toxin, selecting an animal whose blood, at the time of the initial injection, is devoid of antitoxin. This particular reaction has been studied in great detail by Glenny and his colleagues (Glenny 1925*b*, 1931, Glenny and Südmersen 1921) and forms the basis of his insistence on the fundamental difference between a primary and a secondary response. Fig. 17 shows a typical example.

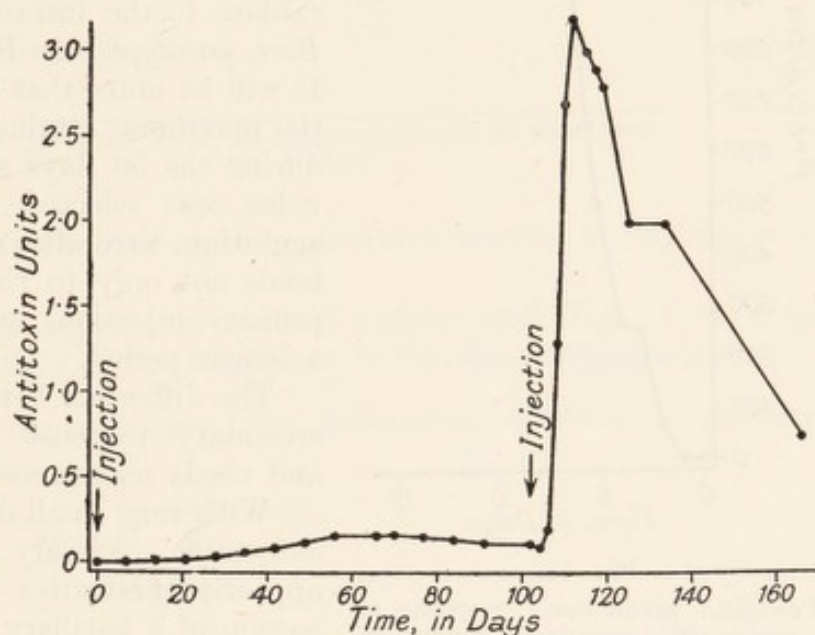


FIG. 17.

Antitoxin production in the horse: showing difference between primary and secondary response. (After Glenny and Südmersen.)

The primary injection is followed by an induction period of some 14 days, after which there is a very slow rise in the antitoxin content of the blood, reaching a low maximum of 0.16 units per c.c. between the 9th and 10th weeks, and sinking slowly to 0.11 units by the middle of the 15th week, at the time of the second injection. The response to this second injection is of an altogether different character. Within 4 days a distinct rise in the titre of antitoxin is observable, and the curve then rises steeply to a maximum of 3.2 units per c.c. on the 10th day, followed by a fairly rapid decline. It may be added that with repeated and increasing doses the antitoxin content of a horse's serum may be raised to well over 1,000 units per c.c.

This difference between the primary and subsequent injections of the same antigenic material is not limited to the production of diphtheria antitoxin,

though it is particularly well marked in that case. Fig. 18, showing the response to an injection of horse serum in a rabbit which had previously been injected with that antigen, but whose precipitin titre had since sunk to zero, may be compared with Fig. 16 above.

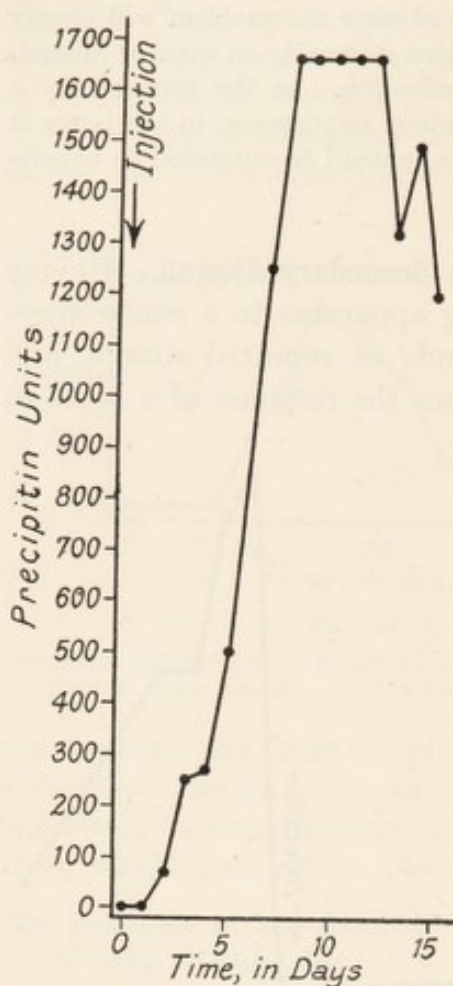


FIG. 18.

Precipitin production: response of previously sensitized rabbit to single injection of horse serum—secondary response. (After Dean and Webb.)

The same holds true, in regard to the comparative titre attained, though not in regard to the prolonged induction period after the primary stimulus, when the antigenic material is particulate, as in the case of a bacterial suspension. Table XXIV sets out the primary and secondary response of rabbits to the intravenous injection of 10^5 *Bact. paratyphosum B* per kilo. body weight. It will be noted that the titres given are not the maximum attained but the average titre during the 50 days after inoculation. This value was selected because the curve of agglutinin titre after a secondary inoculation tends not only to rise higher than after a primary injection, but to remain high over a longer period.

The difference between the primary and secondary response is sufficiently obvious and needs no comment.

With very small doses there may be no detectable primary response, antibodies appearing first after the second or later injection of a bacillary suspension. An illustrative example is given in Table XXV.

The tendency for the antibody titre to be maintained at a high level for a longer

time after secondary and subsequent responses than after a primary response is illustrated in Fig. 19, showing the response of a rabbit to three widely spaced injections of *Bact. typhosum*, Fig. 20, showing the response of an un-

TABLE XXIV

SHOWING THE AVERAGE TITRE OVER A PERIOD OF 50 DAYS AFTER PRIMARY AND SECONDARY INJECTIONS INTO RABBITS OF 10^5 *Bact. paratyphosum B* PER K.B.W.

Rabbit.	Primary Average Titre.	Interval between 1st and 2nd Injections.	Secondary Average Titre.
R11	280	147 days	2,720
R19	37	147 „	1,720
R25	130	147 „	430
R30	130	133 „	1,230
R31	91	133 „	1,230
R33	110	133 „	2,010

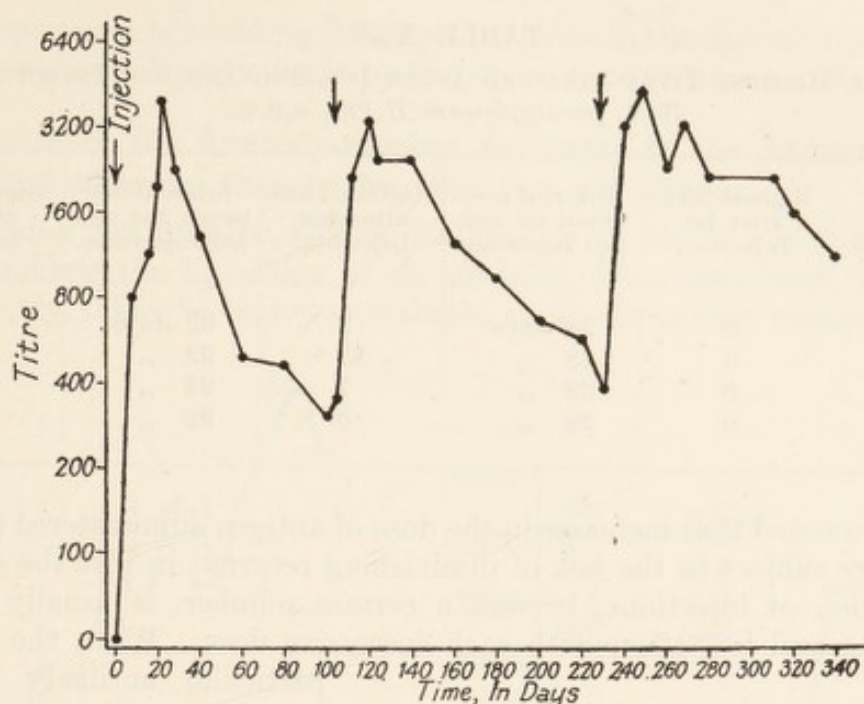


FIG. 19.

Agglutinin production: response of rabbit to widely-spaced injections of *Bact. typhosum*.

inoculated person to an injection of typhoid vaccine, and Fig. 21, showing the response of a previously inoculated person to the same antigenic material.

It must not be supposed that the differences between a primary and secondary response are always so clear-cut as those depicted here. The curves may, however, be regarded as giving a true picture of the kind of differences observed. It will be noted in Figs. 19 and 20 that the primary response itself is quite brisk and results in a high titre of agglutinins. This is the usual experience as regards the production of H agglutinins in response to the injection of an optimal dose of flagellated bacilli.

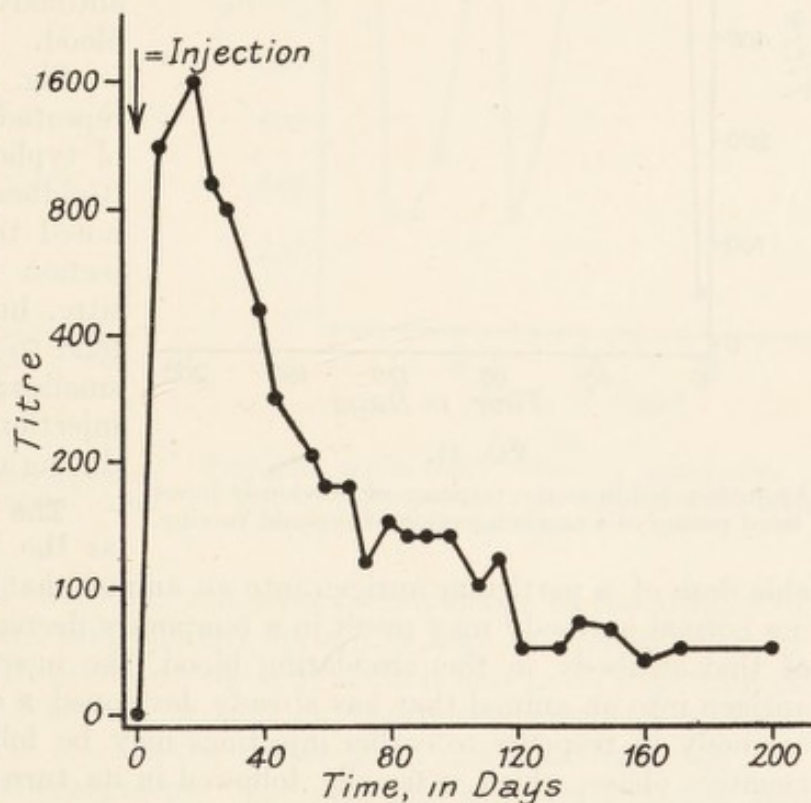


FIG. 20.

Agglutinin production: response of uninoculated person to a single injection of typhoid vaccine.

TABLE XXV

SHOWING THE HIGHEST TITRE ATTAINED AFTER 1ST, 2ND AND 3RD INJECTIONS OF 10^4
Bact. paratyphosum B PER K.B.W.

Rabbit.	Highest Titre after 1st Injection.	Interval be- tween 1st and 2nd Injection.	Highest Titre after 2nd Injection.	Interval be- tween 2nd and 3rd Injections.	Highest Titre after 3rd Injection.
R16 . .	0	28 days	8	92 days	40
R32 . .	0	28 "	32	92 "	319
R46 . .	0	28 "	7	92 "	20
R47 . .	0	28 "	0	92 "	0

We have noted that increases in the dose of antigen administered at a single injection are subject to the law of diminishing returns: in just the same way the repetition of injections, beyond a certain number, is usually found to produce less and less effect with each successive dose. When the titre of a

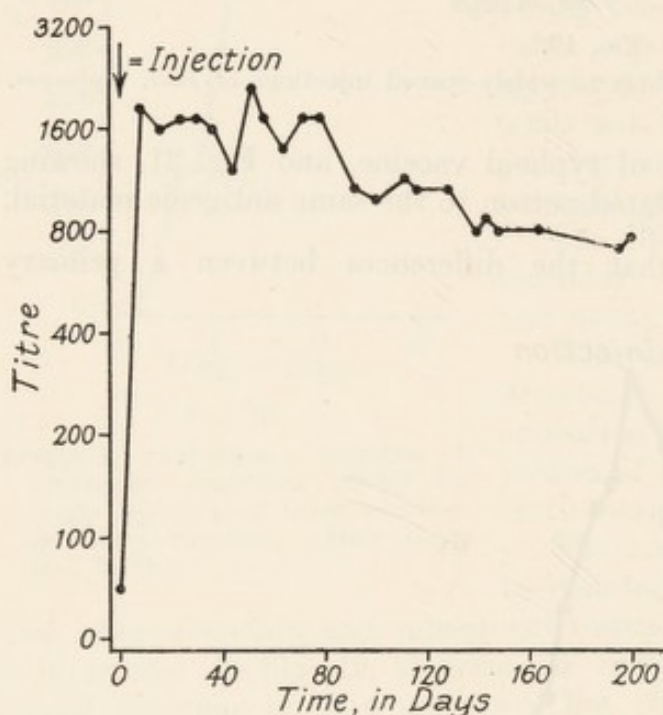


FIG. 21.

Agglutinin production: response of previously inoculated person to a single injection of typhoid vaccine.

particular antibody has been forced up to a certain level, which varies widely with the nature of the antigen employed and the responsiveness of the animal injected, it becomes impossible to induce any further rise in the concentration of antibody in the circulating blood.

Fig. 22 shows the effect of repeated subcutaneous injections of typhoid bacilli in the rabbit (Goldberg 1901). It will be noted that each successive injection raises the agglutinin titre, but that the increments tend to become proportionately smaller and smaller, the last injection on the 35th day producing a relatively trivial effect.

The Negative Phase.—Just as the injection of a considerable

dose of a particular antigen into an animal that possesses a corresponding normal antibody may result in a temporary decrease in the concentration of that antibody in the circulating blood, the injection of a large dose of antigen into an animal that has already developed a certain concentration of antibody in response to earlier injections may be followed by a well-marked negative phase, which is usually followed in its turn by a rise in titre above the previous level. An illustrative example is afforded by the response of an immunized rabbit to two injections of horse serum, given intravenously at an interval of 5 days (Fig. 23).

The importance of avoiding this negative phase in therapeutic immunization has been emphasized particularly by Wright and his co-workers (Wright 1909).

The Response of the Antibody-forming Apparatus to the Administration of Antigens otherwise than by Injection into the Tissues.

In defining the properties of an antigen on p. 55, it was stated that it should stimulate the formation of an antibody when introduced *parenterally* into the body. This caveat was included in the definition because adminis-

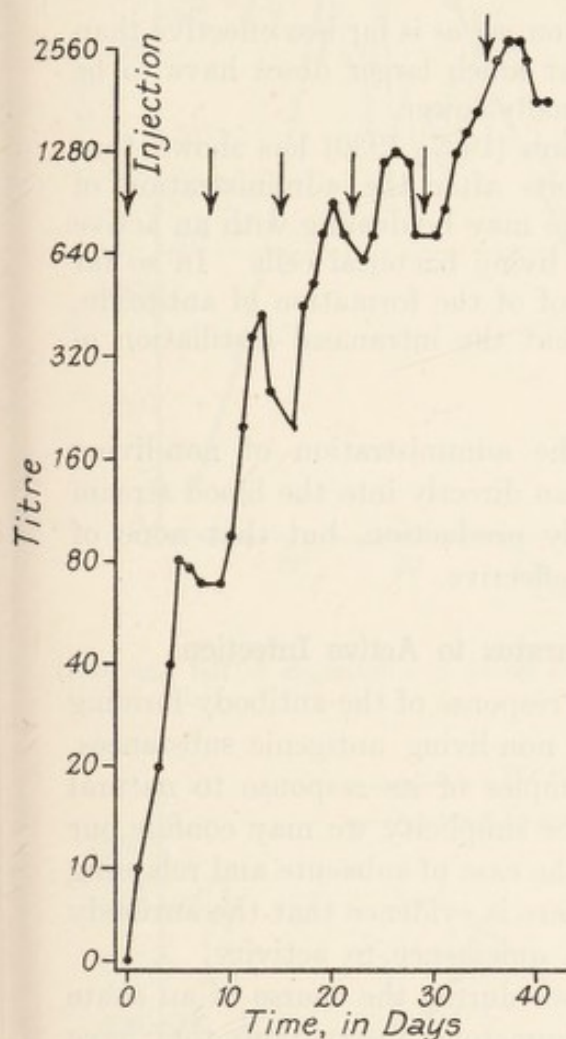


FIG. 22.

Effect of repeated injections of *Bact. typhosum* on agglutinin production in rabbit. (After Goldberg.)

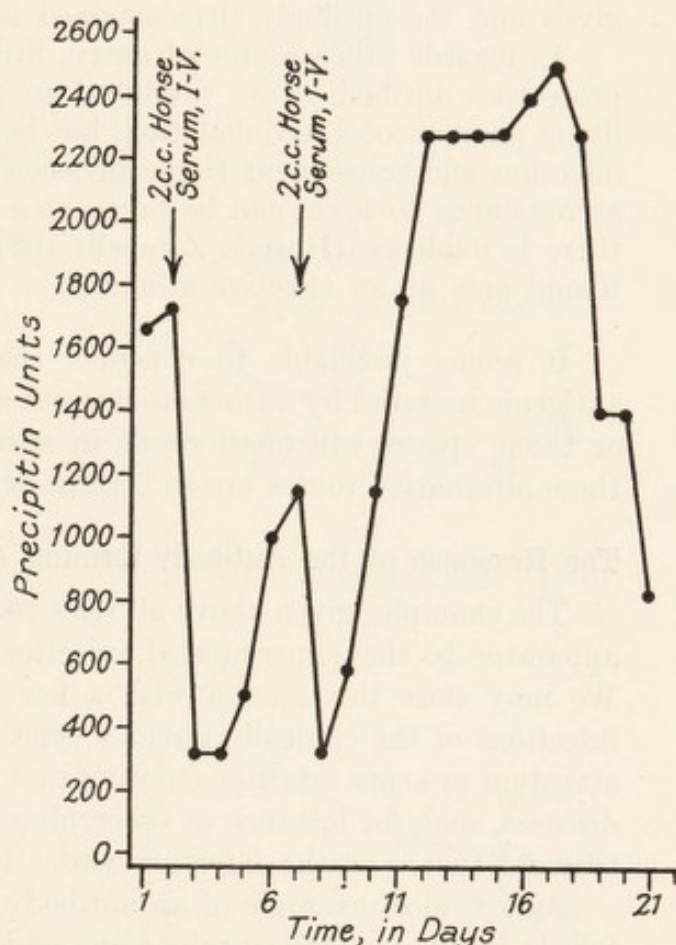


FIG. 23.

Precipitin production: effect of intravenous injection of horse serum into an immunized rabbit. (Dean and Webb, 1928.)

tration *via* the intestinal tract does not ensure the passage of the antigen to the tissues in an unaltered and effective form. It may be broken down by the digestive enzymes. If it is not acted on by them it may never pass through the intestinal mucosa. Whether, in fact, any particular antigenic material administered by this route reaches the tissues in an unaltered form can only be determined by direct experiment. The available evidence suggests that some bacterial antigens, but not all, induce the formation of specific antibodies when administered by the mouth. Thus, the administration *per os* of killed suspensions of flagellated bacilli of the typhoid-paratyphoid group results in

the production of somatic (O) agglutinins, but flagellar (H) agglutinins are either not produced at all, or only to low titre (Pijper and Dau 1930, Greenwood, Topley and Wilson 1931). The same difference between the formation of H and O agglutinins appears to hold in the case of the cholera vibrio (Pfeiffer and Lubinski 1930). Dysentery bacilli, which are non-flagellated, induce the formation of somatic agglutinins when administered by the mouth (Kanai 1921, Otten and Kirschner 1927); and it is probable that similar effects are produced by many other bacterial cells (see for instance Ross 1926, 1930, 1931, 1932).

In all cases it would seem that administration *per os* is far less effective than inoculation into the tissues, in the sense that much larger doses have to be given and the antibody titre attained is usually lower.

As regards other routes of entry, Stillmann (1927, 1930) has shown that protective antibodies are produced in rabbits after the administration of living pneumococci by inhalation, but here we may be dealing with an active infection and consequent tissue invasion by living bacterial cells. In so far as resistance to toxin can be taken as a proof of the formation of antitoxin, there is evidence (Hosmer-Zambelli 1931) that the intranasal instillation of toxoid acts as an effective stimulus.

It seems justifiable to conclude that the administration of non-living antigenic material by various routes other than directly into the blood stream or tissue spaces will often result in antibody production, but that none of these alternative routes are so certain or so effective.

The Response of the Antibody-forming Apparatus to Active Infection.

The examples given above all refer to the response of the antibody-forming apparatus to the experimental injection of non-living antigenic substances. We may close this section with a few examples of its response to natural infections of the clinically obvious type. For simplicity we may confine our attention to acute infections, noting that in the case of subacute and relapsing diseases, such for instance as tuberculosis, there is evidence that the antibody titre fluctuates as the infection varies from quiescence to activity.

As a typical example of an antibody curve during the course of an acute febrile infection we may take some of the numerous observations that have been made on patients suffering from typhoid fever. Figs. 24 and 25, constructed from data recorded by Jörgensen (1905), show the fluctuations in typhoid agglutinins during a typical attack of the disease, and during the terminal stage of a primary attack and the two relapses which in this case immediately followed it. The clinical course of the disease is, in each case, indicated by the fluctuations in temperature.

The Relation of Antibody Production to Other Reactions in an Infected Host.

It is of interest to trace the relation between the response of the antibody-forming apparatus and other events in the infected host; and, here again, typhoid fever provides us with a useful illustrative example. Quantitative data based on repeated agglutination tests, blood cultures and faecal cultures in

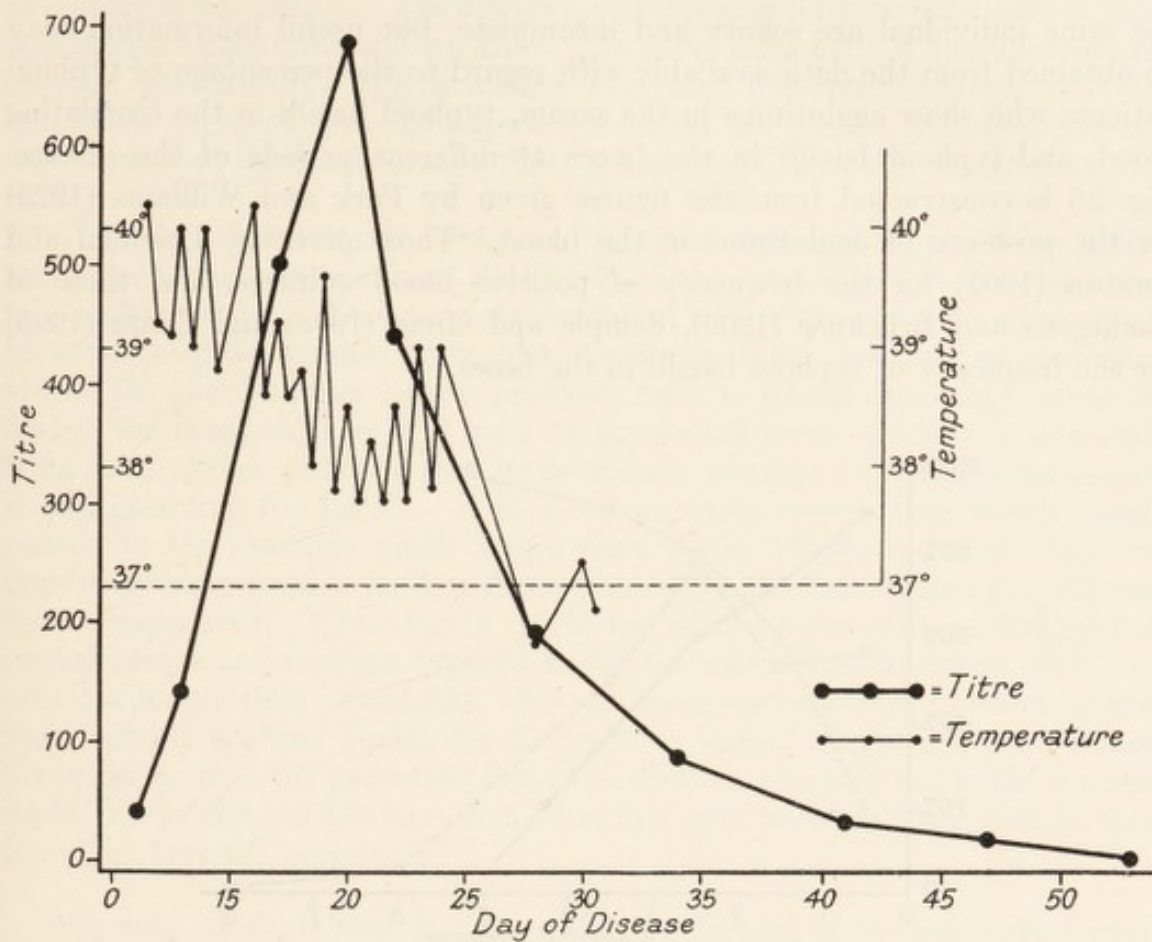


FIG. 24.

Rise and fall of agglutinins in blood of patient during an attack of typhoid fever. (After Jörgensen.)

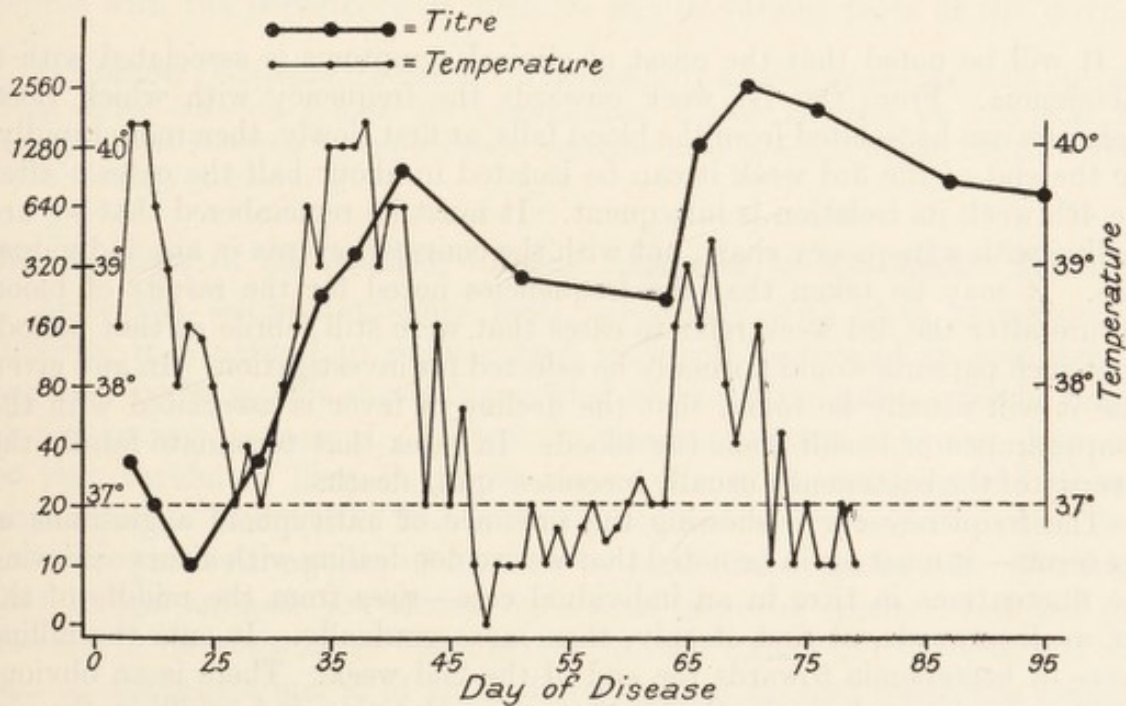


FIG. 25.

Fluctuations of agglutinins in blood of patient during attack of typhoid fever, followed by two relapses. (After Jörgensen.)

the same individual are scanty and incomplete, but useful information may be obtained from the data available with regard to the percentage of typhoid patients who show agglutinins in the serum, typhoid bacilli in the circulating blood, and typhoid bacilli in the faeces at different periods of the disease. Fig. 26 is constructed from the figures given by Park and Williams (1925) for the presence of agglutinins in the blood. Those given by Coleman and Buxton (1907) for the frequency of positive blood cultures, and those of Gaetgens and Brückner (1909), Semple and Greig (1908) and Lentz (1905) for the frequency of typhoid bacilli in the faeces.

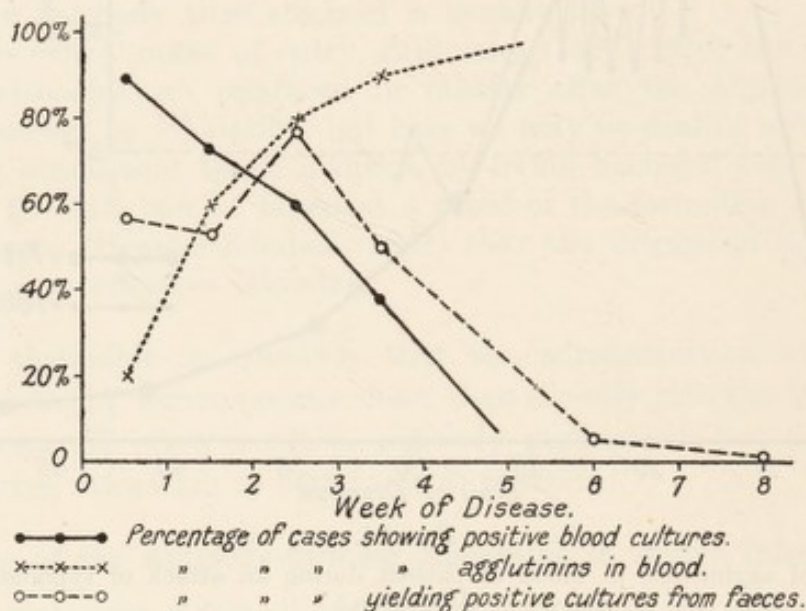


FIG. 26.

It will be noted that the onset of clinical symptoms is associated with a bacteraemia. From the 1st week onwards the frequency with which *Bact. typhosum* can be isolated from the blood falls, at first slowly, then more rapidly. By the end of the 3rd week it can be isolated in about half the cases; after the 4th week its isolation is infrequent. It must be remembered that we are dealing with a frequency chart, not with the course of events in any individual case. It may be taken that the frequencies noted for the results of blood culture after the 3rd week refer to cases that were still febrile at that period, since such patients would normally be selected for investigation. In any given case it will usually be found that the decline of fever is associated with the disappearance of bacilli from the blood. In cases that terminate fatally the severity of the bacteraemia usually increases until death.

The frequency curve showing the presence of antityphoid agglutinins in the serum—it must again be noted that we are not dealing with a curve showing the fluctuations in titre in an individual case—rises from the middle of the 1st week onwards, at first steeply, then more gradually. It cuts the falling curve of bacteraemia towards the end of the 2nd week. There is an obvious inverse correlation between the presence of agglutinins and bacilli in the circulating blood, and we shall not be stretching inference if we adopt the view that these relations probably stand to each other as cause and effect. One

caveat must be entered. The figures for agglutinins refer, in large part at least, to flagellar agglutinins and we know that these have little if any effect on the clearing mechanism. The available evidence, however, suggests that the frequency curve of somatic agglutinins would not depart in any significant way from that depicted, except that its rise would probably occur somewhat earlier.

The curve describing the frequency of *Bact. typhosum* in the faeces, unlike the curve of bacteraemia, rises slightly from the 1st to the 3rd week and then falls rather slowly. The exact relationship of these curves to each other cannot be accurately determined from such figures as these, because, as mentioned above, the blood-culture figures probably refer to febrile cases only, while the figures for faecal cultures will refer to unselected cases, since it is important from the hygienic point of view to determine whether a typhoid convalescent is still excreting the bacilli. It is, however, quite certain that bacilli usually remain in the intestine much longer than in the blood, and that they may persist in that situation for months or years after all clinical signs or symptoms have disappeared. Thus, Lentz (1905) recorded 4.5 per cent. of 400 typhoid convalescents as excreting typhoid bacilli for more than 10 weeks, and 3 per cent. for longer than 13 months. Several cases are on record in which persons have carried typhoid bacilli for 25 years or more. There are good reasons for believing that the persistent focus of infection is usually not in the intestine itself, but in the gall-bladder, and other foci may be established such as those found in typhoid periostitis.

We may conclude that the successful functioning of the antibody-forming apparatus during an acute infective disease is associated with the effective clearing of the organisms from the blood by the mechanism discussed in earlier chapters; but, as we there saw, this effective clearing is quite compatible with the persistence of infective foci in various parts of the body.

As a further example of the response of the antibody-forming apparatus to the stimulus of an active infection and of the consequences of that response we may note the course of events during an experimental pneumococcal infection in the cat (Robertson *et al.* 1928) (Fig. 27).

The Response of the Antibody-forming Apparatus to Non-Specific Stimuli.

Since the serum antibodies react specifically with their corresponding antigens, it is natural to suppose that they will be formed *de novo* only in response to the specific stimulus provided by the introduction into the tissues of the antigen concerned. In making this supposition we must, however, be very careful to limit its implications. We must recollect, as has been emphasized in Chapter V, that we are concerned with chemical entities, and not with particular species of bacteria or particular types of cell. We know that certain antigens are shared by several bacterial species; and the production, in response to the introduction into the tissues of one bacterium containing antigens *a* and *x*, of antibodies acting on another bacterium containing the antigens *b* and *x* is, in so far as it depends on the production of antibody X, a strictly specific response in the only sense with which we are here

concerned. With these limitations, our supposition is in accord with all the recorded facts. The available literature contains no instance in which the introduction into the tissues of a substance of known chemical constitution has led to the production *de novo* of an antibody reacting specifically with a second substance, the chemical constitution of which bears no relation to that of the first.

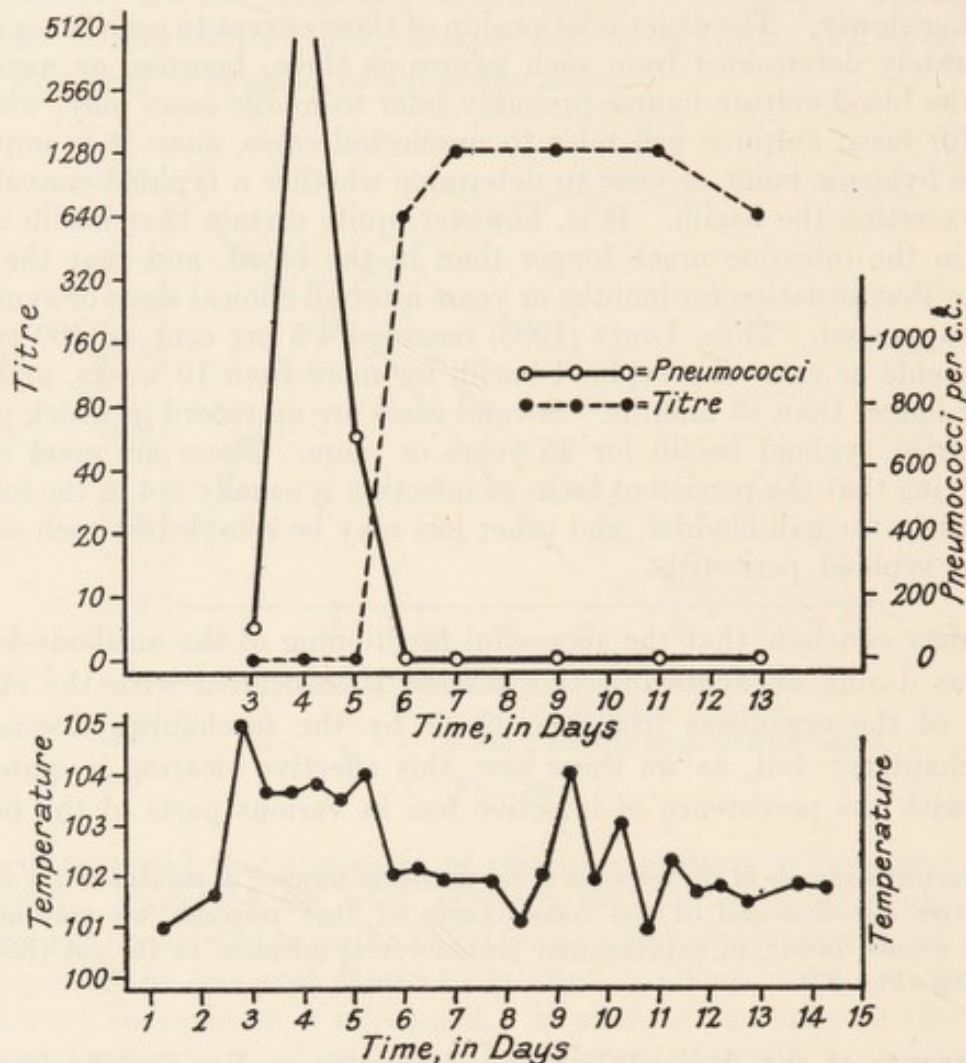


FIG. 27.

Showing rise and fall of bacteræmia in relation to appearance of pneumococcicidal antibodies in blood of cat, during experimental pneumococcal infection. (After Robertson, Woo, Nan Cheer and King.)

If we turn to the production of a normal antibody in increased amount, a rise in the titre of an antibody previously produced in response to a specific stimulus, or the reappearance of such an antibody after the titre has fallen to zero, it is clear that our supposition no longer holds on *a priori* grounds. There would be nothing remarkable in an antibody-forming apparatus that had once produced, and possibly stored, a particular antibody turning that antibody into the circulation anew, or in greater amount, in response to some non-specific stimulus which increased the activity of the apparatus without

imposing on it any general direction. Whether or not this type of non-specific stimulation occurs can be settled only by empirical trial.

In this connection we may note a terminological confusion which has sometimes arisen in the literature. The production, in response to an antigenic stimulus, of an antibody that has been produced in the tissues on some previous occasion, is often referred to as an *anamnetic reaction* (*anamnesis*—recollection). Sometimes this term is used in connection with the type of non-specific stimulus to which we have just referred—the tissues are stimulated to general activity, in which is included the renewed production of a particular antibody that had been formed at some time in the past. Sometimes, perhaps more accurately, it is employed in reference only to those instances in which the particular chemical stimulus concerned formed part of the earlier experience of the antibody-producing cells. In this limited sense it is clearly synonymous with the secondary response, in the sense defined above, and has no reference to the problem with which we are here concerned.

Several observers have recorded a stimulation of antibody production as the result of excessive or repeated bleeding.

Friedberger and Dorner (1905) stated that the withdrawal of considerable amounts of blood, either shortly before an immunizing injection, or within a few days thereafter, had a definitely stimulating effect on the subsequent antibody production. Hektoen and Carlson (1910), in the experiments already referred to, found that bleeding a dog dry during the initial latent stage of active immunization and immediately transfusing it with the blood of a normal dog appeared to increase the production of antibodies. Hahn and Langer (1917) have reported experiments in which they observed a marked increase in agglutinin titre in 4 rabbits as the result of repeated daily bleedings. Trommsdorf (1921) has recorded an increase in agglutinins for the diphtheria bacillus as the result of daily bleedings. Other workers (Klinger 1918, Landau 1918) have however failed to confirm these results. It may be noted that an opposing factor is brought into play when an immunized animal is subjected to massive repeated bleeding. The mere withdrawal of considerable quantities of blood would, of itself, tend to lower the titre of the circulating antibodies if the antibody-forming apparatus did not share in the subsequent activity of the hæmopoietic tissues. That immunized animals may suffer extensive bleedings without any detectable and lasting fall in antibody titre has long been known (Roux and Vaillard 1893).

It may be accepted that bleeding does act as a stimulus to antibody production in the same sense that it acts as a stimulus to the production of the serum proteins in general; that is, there is an active response tending to make good the loss sustained. Whether this particular stimulus suffices to force the titre, transitorily or for longer periods, far above the pre-existing level is more doubtful.

Another procedure that has been stated to supply a non-specific stimulus to the antibody-forming apparatus is the injection of various metallic salts, colloidal solutions or other substances. We may ignore, from our present point of view, those instances in which the effect of such injections is limited to an increase in the bactericidal power of the blood. Such effects will be more conveniently considered in Chapter XIII.

Walbum and his collaborators (Walbum and Morch 1923, Walbum and Berthelsen 1925, Walbum 1925, 1926, Schmidt 1926, Ørskov and Schmidt 1928) have recorded numer-

ous experiments along these lines. They conclude that the inoculation of salts of certain metals, especially those of cobalt, manganese and beryllium, causes an increase in the production of antitoxin, or of agglutinins, if given early in the course of immunization, and leads to a secondary rise in titre, if injected when the antibody titre has commenced to fall. Several investigators have, however, failed to confirm these observations (McIntosh and Kingsbury 1924, Horgan 1925), and the protocols included in some of Walbum's reports suggest that the response is very irregular.

Steaben (1925) studied the effect of various colloids on antibody production. Using agar, gelatin, a silver sol and an iron sol, she found that the injection of these colloids had no power to increase the antibody titre when this had become steady, though the injection of such a colloid at the same time as the bacterial antigen might increase the antibody response.

Obermayer and Pick (1904) recorded that animals immunized 3 months previously showed a fresh formation of precipitin after the injection of a 5-10 per cent. solution of peptone. Fleckseder (1916) stated that the injection into human beings of deutoalbumose or nucleic acid gave rise to an increase in the agglutinins for typhoid bacilli which were already present in detectable amount in the subjects in question. Weichardt and Schröder (1919) noted a definite increase in agglutinin titre after the injection of deutoalbumose, sodium nucleinate or milk; and Matsuda (1924) reported that the injection of deutoalbumose was followed by a secondary increase in the disappearing viricidal antibodies in the blood of vaccinia-immune animals. An excellent summary of the data with regard to the effect of protein substances as non-specific stimuli is contained in a review by Weichardt (1922). Here again we may note the irregularity of the results obtained, and the absence of any large increase in antibody content such as usually follows a specific stimulus.

Finally we may consider those cases in which the non-specific stimulus is provided by a complex antigen, similar in type to that providing the initial specific stimulus but possessing no known common antigenic factor. So far as this antigenic dissimilarity is in fact complete the case does not differ in its essentials from those we have considered above.

Dreyer and Walker (1909) recorded a secondary rise in agglutinins for *Bact. coli*, following the intraperitoneal injection of killed staphylococci into previously immunized rabbits. Conradi and Bieling (1916) state that rabbits immunized against *Bact. typhosum* show a secondary rise in agglutinins after various non-specific stimuli, such as those provided by the injection of *Bact. coli*, dysentery bacilli and *C. diphtheriae*. Tsukahara (1921) records a series of experiments which are highly suggestive. He injected rabbits with typhoid, paratyphoid and dysentery bacilli, using one organism as the primary specific stimulus and another as the secondary non-specific stimulus, and noted many instances of a secondary rise in agglutinins. A careful perusal of his protocols however indicates very clearly that, where the secondary response was well marked, there was some antigenic relationship between the two kinds of bacteria employed, indicated by the fact that a primary inoculation with one of them was followed by a slight but definite production of antibodies acting on the other. This fact, of course, excludes these particular examples from the category which we are considering.

Rosher (1924) was unable to detect non-specific stimulation of antibody production in any of the following instances: primary specific antigen *Bact. coli*, secondary non-specific inoculum *Staph. aureus*; primary specific antigen *Bact. coli*, secondary non-specific inoculum sheep's red cells; primary specific antigen *Bact. paratyphosum A*, secondary non-specific inoculum suspension of mixed unrelated bacteria; primary specific antigen *Bact. typhosum*, secondary non-specific inoculum *Bact. paratyphosum B*; primary specific antigen *Bact. enteritidis*, secondary non-specific stimulus infection with living tubercle bacilli. In all cases in which this point was examined, the secondary inoculum acted as an effective primary specific stimulus, inducing the production to a relatively high titre of antibodies reacting with its own antigenic components, while leaving the titre

of antibodies acting on the previously injected antigen unaltered. Analogous results had been noted by many of the observers referred to above.

To summarize, we may say that there is some evidence that non-specific stimuli—extensive bleeding or the injection of metallic salts or colloids or protein solutions or unrelated bacteria—may induce (a) an increase in the titre of a normal antibody, (b) an increase in the production of immune antibodies when administered during the early stages of immunization, or (c) a secondary rise in titre when administered after the effect of the preliminary specific immunization has reached its zenith, or declined from it. The responses falling within this category are, however, exceedingly irregular, usually trivial, and never comparable to those which follow the reinjection of the specific antigen. It is quite certain also that the injection of a given antigen may stimulate the antibody-forming apparatus to the energetic production of the corresponding antibody, without any detectable production or mobilization of antibodies acting on an unrelated antigen to which it had previously responded.

The Effect of Dietary Factors on the Efficiency of the Antibody-Forming Apparatus.

—In view of the discussion as to the effect of diet on resistance to infection, which will be found in a later chapter, it is sufficient to note that most of the available evidence is definitely against any known type of vitamin deficiency affecting the capacity of an animal to produce antibodies in response to the usual stimuli (see Zilva 1919, Werkman 1923, Werkman *et al.* 1924, Cramer and Kingsbury 1924).

The Fate of Antibodies when Injected into a Normal or Immunized Animal.

This is a convenient place in which to note a series of observations which, while not immediately related to the phenomena that we have been discussing, are of considerable practical importance.

When we inject an antibody-containing serum into a normal animal, the rate at which it reaches the blood stream and the maximum concentration which it there attains are in great part determined by the route of injection. Obviously we shall obtain the most rapid effect by injecting our antiserum intravenously. Injection by any other route will involve a long period before the maximal concentration is reached in the circulating blood; and since the antibody-containing serum is being slowly eliminated throughout the whole period the concentration in the blood and tissue fluids in general will reach a lower level.

Smith (1907) records a series of experiments that serve to illustrate these points.

Fig. 28 shows the disappearance of agglutinins for *Bact. coli* from the blood of a rabbit after an intravenous injection of the serum of an immunized goat.

Fig. 29 shows the effect of inoculating the same serum subcutaneously. It will be noted that the maximal concentration of agglutinin in the circulating blood is not reached until 53 hours after the injection.

Fig. 30 shows the concentration of antitoxin in the circulating blood of a human subject at varying intervals after the subcutaneous injection of an

antitoxic serum. It will be noted that the peak is not reached until the 3rd or 4th day.

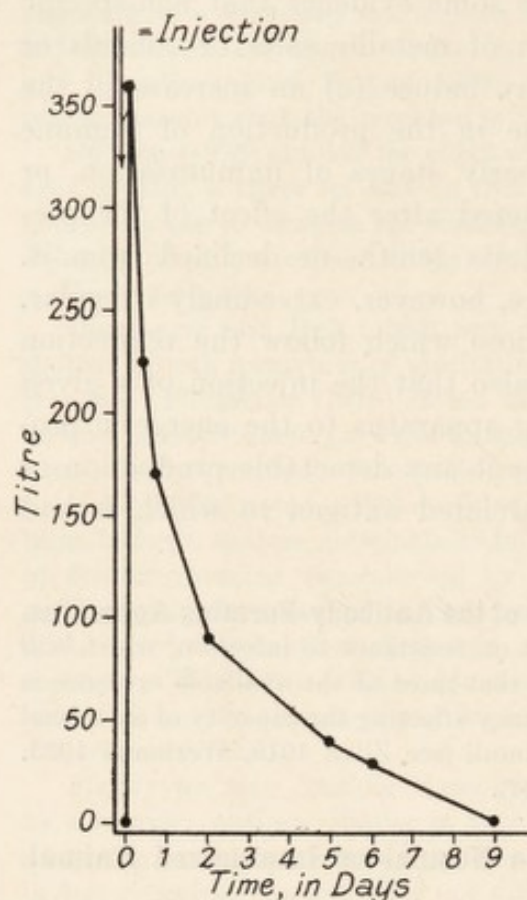


FIG. 28.

Disappearance of agglutinins from circulation of rabbit after intravenous injection of agglutinating serum. (After Smith.)

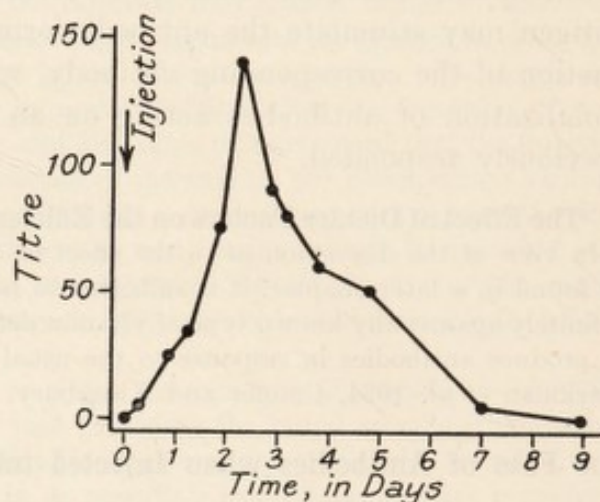


FIG. 29.

Rise and fall of agglutinins in blood of rabbit after subcutaneous injection of agglutinating serum. (After Smith.)

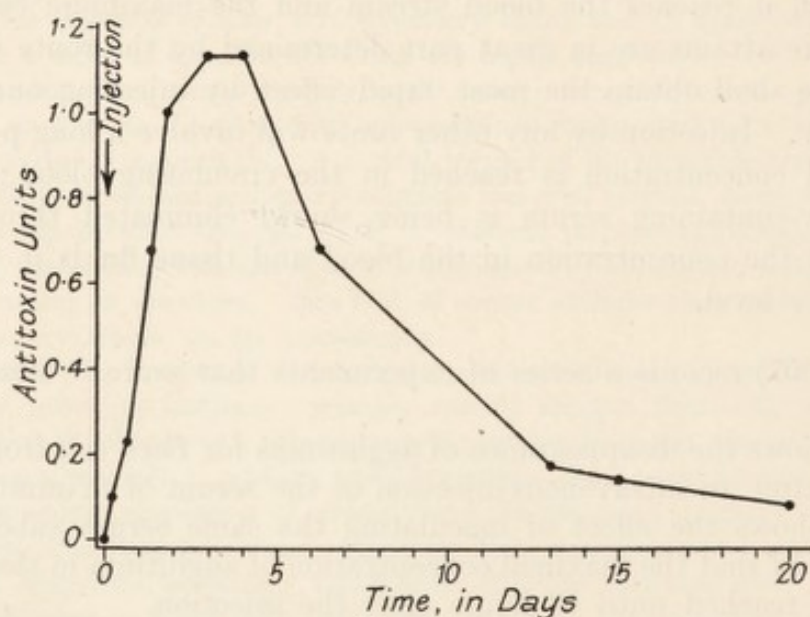


FIG. 30.

Rise and fall of antitoxin in blood of human subject after subcutaneous injection of antitoxic serum. (After Smith.)

Another point of some importance in connection with passive immunization is illustrated in Figs. 31 and 32 (Glenny and Hopkins 1922).

In Fig. 31 is recorded the rate of disappearance of antitoxin from the circulation of 3 normal rabbits after the intravenous injection of 0.5 c.c. of diphtheria antitoxin, derived from the horse. Fig. 32 gives a similar record in the case of 3 rabbits that had received previous inoculations of horse serum.

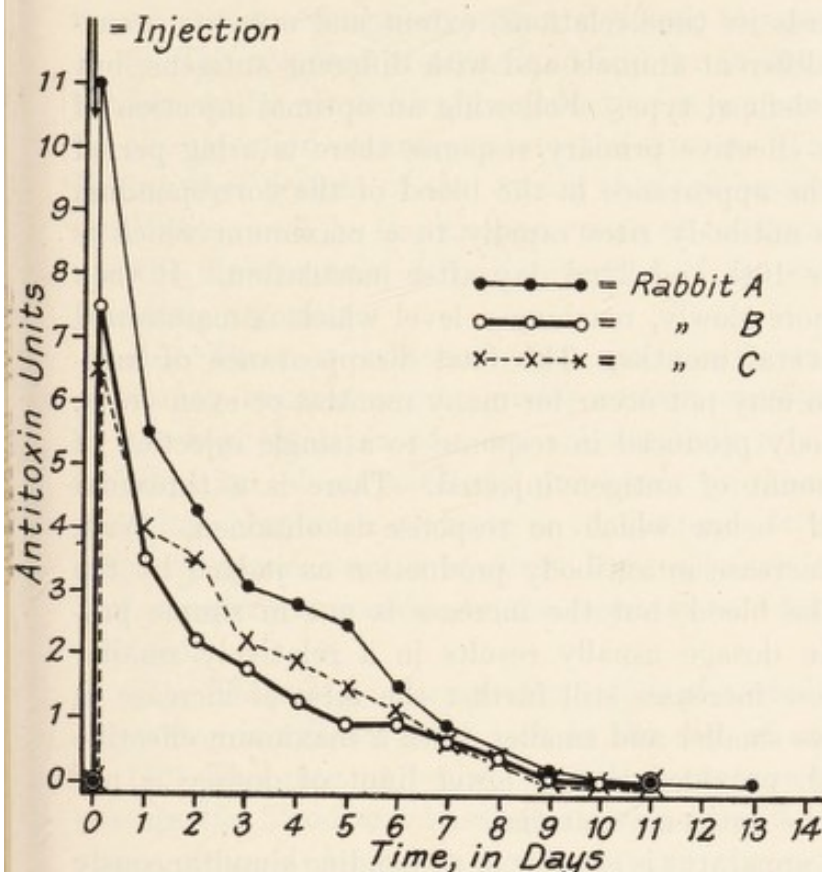


FIG. 31

Disappearance of antitoxin from blood of three normal rabbits, after intravenous injection of antitoxic horse serum. (After Glenny and Hopkins.)

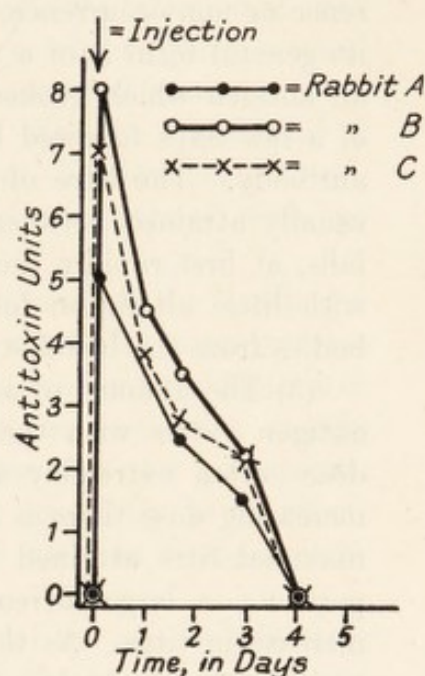


FIG. 32.

Disappearance of antitoxin from blood of three rabbits, previously sensitized to normal horse serum, after intravenous injection of antitoxic horse serum. (After Glenny and Hopkins.)

These were "immune" or "sensitized" to horse serum—the relation of the immune to the sensitized state is discussed in Chapter XII—and in consequence eliminated the antibody from their circulation much more rapidly than did the normal rabbits. The same phenomenon will occur when a person who has previously received an injection of horse serum is given an antitoxic or antibacterial serum derived from that animal.

SUMMARY

(1) The available evidence indicates that the cells of the reticulo-endothelial system, which are known to remove injected antigenic material from the blood and tissue fluids, play an essential part in the production of antibodies. Whether these cells themselves produce the antibodies in their final and complete form, or whether their rôle is confined to the temporary retention

of antigen and its subsequent liberation in an altered or unaltered state, the actual synthesis of antibodies occurring elsewhere, cannot yet be determined. The present indications are that the part played by the reticulo-endothelial system is not confined to the retention and release of unaltered antigens.

(2) The antibody-forming apparatus responds in a characteristic way to a specific stimulus provided by the introduction of an antigen into the tissues. This response differs as regards its time relations, extent and even its occurrence or non-occurrence, in different animals and with different antigens, but its general form is of a well-defined type. Following an optimal injection of an antigen which evokes an effective primary response there is a lag period of a few days followed by the appearance in the blood of the corresponding antibody. The titre of this antibody rises rapidly to a maximum which is usually attained between the 10th and 22nd day after inoculation. It then falls, at first rapidly then more slowly, reaching a level which is maintained with little alteration for several months. The final disappearance of antibodies from the blood stream may not occur for many months, or even years.

(3) The amount of antibody produced in response to a single injection of antigen varies with the amount of antigen injected. There is a threshold dose—often extremely small—below which no response is obtained. With increasing dose there is an increase in antibody production as judged by the maximal titre attained in the blood, but the increase is not in simple proportion; a large increase in dosage usually results in a relatively smaller increase in titre. As the dose increases still further the ratio of increase in titre to increase in dose grows smaller and smaller, until a maximum effective dose appears to be attained, provided that a lower limit of dosage is not imposed by the toxicity of the antigenic material.

(4) The antibody-forming apparatus is capable of responding simultaneously to the injection of several different antigens, but the multiple response tends to be less effective against any one antigen than if it were responding to that antigen alone.

(5) The antibody-forming apparatus displays a characteristic difference in its response to primary and subsequent injections of the same antigen. Following a secondary stimulus antibody production is usually more rapid and more copious than after the primary stimulus, and the decline in titre of circulating antibodies after a maximum has been reached is usually delayed. An immunized differs from a normal animal, not only in the possession of a particular antibody, but in its capacity to produce that antibody rapidly and in large amount in response to the specific stimulus.

(6) Repeated inoculations are on this account more effective in raising the antibody titre to a high level than a single injection, even of a very large dose. Just as there appears to be a limit to the titre that can be attained by increasing the size of a single dose of antigenic material so there is a limit beyond which repeated injections fail to force the titre.

(7) The injection of a large dose of antigen into an animal in whose blood the corresponding antibody is present frequently results in a temporary fall

in the concentration of that antibody, followed in most cases by a secondary rise.

(8) Antibody formation may be induced by the administration of antigens by the mouth, by inhalation, or by intranasal instillation. Administration by these routes is, in general, less effective than direct inoculation into the tissues. In the case of administration *per os* there is evidence that antibodies may be produced against certain antigenic components but not against others.

(9) The response of an animal host to an acute bacterial infection is very similar, so far as antibody production is concerned, to the response evoked by the injection of antigen into the tissues. There are good reasons for believing that certain of the events that are associated with the appearance of antibodies, such as the disappearance of bacteria from the circulating blood, are the direct result of the interaction of the antibodies so formed with the infecting organism.

(10) There is evidence that non-specific stimulation of the antibody-producing apparatus may under certain conditions result in some increase in the concentration of a normal antibody or in the renewed production or mobilization of an antibody previously produced in response to a specific stimulus; but such evidence is conflicting. It is clear that this non-specific response occurs very irregularly, is usually trivial or transitory in its effect, and is never comparable to the response to a further injection of the specific antigen.

(11) There is no clear evidence that the efficiency of the antibody-forming apparatus is affected by a lack in the diet of any of the known vitamins.

(12) The rate at which antibody, contained in any foreign antiserum, reaches the blood and tissue fluids of a normal animal and the concentration there attained depend in great part on the route by which the antiserum is injected. If the animal or man has received a previous injection of any serum of the same animal origin the antibody will be eliminated at a greatly increased rate.

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CHAPTER XII

ANAPHYLAXIS, HYPERSENSITIVENESS AND ALLERGY

IN the preceding chapters we have considered a series of reactions most of which have as a common factor the property of affording specific protection against bacteria or their products. Numerous observations both clinical and experimental have afforded instances of the contrary condition, in which an individual is more susceptible to some noxious agent than are the generality of his own species. In some cases an individual may be highly sensitive to an agent which is entirely innocuous to his normal fellows. The phenomenon of hypersensitiveness is presented in its clearest and least ambiguous form when the exciting agent is some non-living material, so that there is no question of multiplication within the body, or of the complex and variable tissue reactions that are associated with bacterial infection. It will be convenient to consider first the data relating to this type of abnormal sensitivity, and then to discuss its bearing on the phenomena of bacterial disease. It will also simplify matters to start with the most striking and clear-cut case of hypersensitiveness—the phenomenon of acute anaphylactic shock.

ANAPHYLACTIC SHOCK

The detailed study of this curious reaction dates from the beginning of the present century, though several observations are on record in the earlier literature which with our fuller knowledge we can recognize as undoubted examples of anaphylaxis. The observations of Richet and his co-workers (Portier and Richet 1902, Richet 1907) first drew general attention to the experimental facts; though the issue was at this stage obscured by the chance that the agent selected for study was itself toxic for the experimental animal employed. Richet observed that dogs which had survived a single injection of an extract of certain actinians might succumb rapidly, and with a curious and characteristic symptom complex, to a second injection of the same material given after days or weeks. It was this substitution of increased susceptibility for the increased resistance which usually follows the injection of sublethal doses of a toxic material that led him to coin the term *anaphylaxis*.

At about the same time Theobald Smith in America had noted the abnormal sensitiveness of guinea-pigs to widely spaced injections of toxin-antitoxin mixtures. He communicated these observations to Ehrlich in 1904; and Otto in 1905 published the results obtained in a detailed study of this phe-

nomenon. He showed that the horse serum in the mixtures, not the toxin, was the essential agent; that an interval of 10 days elapsed after the sensitizing dose before hypersensitiveness was established; and that no hypersensitiveness resulted when large injections of serum were given at short intervals.

The same phenomenon was studied independently by Rosenau and Anderson (1906, 1907), who established the strict specificity of the reaction. They found that guinea-pigs sensitized to horse serum showed little if any hypersensitiveness to the serum of other animals, such as the rabbit, cat, dog, pig, sheep, chicken or man. Similarly, guinea-pigs sensitized to horse serum did not respond in any abnormal way to the subsequent injection of egg-white, vegetable protein or milk. They noted also that the amount of serum required to produce sensitization was extremely small; as little as 0.000,001 c.c. sometimes induced typical hypersensitiveness. The amount required to produce anaphylactic shock in a sensitized animal was, however, considerably greater (0.01–0.1 c.c.). They confirmed the existence of a latent period of 10–12 days between the sensitizing injection and the establishment of the anaphylactic state, and noted that, once established, this state lasted for an indefinite period, extending over several months at least.

These observations have been repeatedly and consistently confirmed by later workers. Animals may be actively sensitized by the injection of any substance that is antigenic in the full sense. The sensitizing injection may be given by any route. Often a single sensitizing dose suffices. In some animals, and with some antigens, it is necessary to give more than one sensitizing injection, and to use doses far larger than the minute amount that suffices in the particular case of the sensitization of the guinea-pig to horse serum; but the same principle holds—the injections must not be too large, nor too often repeated.

As regards the shock-inducing, or “assaulting” injection, either the antigen used for sensitization or certain types of separated haptens containing the specific reacting grouping (see p. 196) are effective in eliciting the characteristic symptoms. The route of injection is important. Intravenous inoculation gives the most constant results; in some animals it is the only effective route for this purpose. In the guinea-pig, which is peculiarly susceptible in this respect, shock may be produced by intraperitoneal inoculation, but larger doses are required.

A curious and significant fact emerged early in the widespread investigation which followed the opening-up of this new field of research. The symptoms and lesions of anaphylactic shock were found to be constant for a given animal species, irrespective of the nature of the sensitizing antigen; while these symptoms and lesions differed sharply from one animal species to another. Thus the guinea-pig reacts in the same way when anaphylactic shock is produced with horse serum, egg albumin, milk or various bacterial antigens; but the reactions of the sensitized guinea-pig, rabbit and dog to horse serum differ sharply and characteristically from one another.

Anaphylactic Shock in the Guinea-pig.—After a sensitized guinea-pig has received an intravenous injection of the specific antigen, it at first remains quiet; but within a minute marked restlessness develops. The hair begins to bristle over the head and neck, and the animal usually rises on its hind legs, rubs its nose vigorously, and emits loud spasmodic sneezes. Often it gives a jump, or a series of jumps. Within a few minutes tonic and clonic convulsions set in, and the animal falls on its side. The respiration becomes slow, the mouth opening with each violent inspiratory effort; and breathing ceases within a few minutes. The heart continues to beat for several minutes after breathing has ceased.

The post-mortem findings were first described in any detail by Gay and Southard (1908); but it is to Auer and Lewis (1910) that we owe the first adequate account of the physiological reactions that lead up to the fatal issue. In guinea-pigs examined immediately after death from fatal anaphylactic shock, these observers confirmed the presence of intestinal congestion and the occurrence of active peristaltic movements that had been previously noted. They observed the frequency of hæmorrhages on the under side of the diaphragm, and noted, as Gay and Southard had done, the distension of the lungs; but they showed that this distension was not due to simple emphysema. The lungs remained distended long after the thorax was freely opened. Even when removed from the body they showed no sign of collapse; and portions cut off from the pulmonary lobes, if not too small, remained in their condition of dilatation. More detailed examination showed that the distension was caused by contraction of the bronchioles, which retained the inspired air in the dilated alveoli. The death of the animal was clearly referable to suffocation consequent on this obstruction to respiration. Auer and Lewis showed that the bronchiolar contraction was not prevented by section of the vagi or by the administration of curare, and was therefore of muscular or immediately neuro-muscular origin. They showed also that the blood pressure during anaphylactic shock at first rose considerably and then fell slowly but steadily till death, as one would expect in asphyxia.

Anaphylactic Shock in the Rabbit.—The rabbit, like the guinea-pig, suffers from acute anaphylactic shock if the sensitization is adequate, and if a sufficient amount of antigen is subsequently administered by the intravenous route. The reaction is not, however, so readily elicited. The symptoms of shock differ in several important respects. The preliminary signs of irritation, the coughing and the violent inspiratory efforts are absent. The animal lies with outstretched legs or falls on its side, gives a series of convulsive movements often associated with the passage of urine and fæces, and dies. In many cases irregular respiratory movements continue for a brief period after the heart has ceased to beat—a reversal of the order of cessation observed in the guinea-pig. At necropsy the pulmonary dilatation, which is so striking a feature in the guinea-pig, is absent in the rabbit. The most characteristic abnormality found in the latter is the extreme dilatation of the right side of the heart. Coca (1919) showed that this dilatation was associated with marked obstruction to the pulmonary circulation, evidenced by the increased pressure required to drive fluid from the pulmonary artery to the left auricle. He concluded that acute anaphylactic death in the rabbit was due to a spasmodic constriction of the branches of the pulmonary artery, followed by rapid dilatation of the right side of the heart and acute heart failure. His observations and conclusions have been confirmed by other workers (Drinker and Bronfenbrenner 1924).

Anaphylactic Shock in the Dog.—A sensitized dog, after receiving an intravenous injection of the specific antigen, shows marked signs of restlessness and excitement. Within a few minutes the animal vomits and usually passes urine and fæces, the latter sometimes blood-stained. It then collapses and lies prone. The muscles of the limbs are relaxed, and the dog appears extremely weak. Respiration is slow, and often deep and laboured. Stridor may be heard, and a little froth may exude from the lips. In rapidly fatal shock the weakness progresses, renewed diarrhoea and vomiting set in, and coma, occasionally

associated with epileptiform convulsions, leads on to death. It has been pointed out by Dean and Webb (1924) that the reaction almost always occurs in two stages, the initial acute symptoms being followed by some signs of recovery. In fatal cases these soon give place to the second stage of increasing collapse. In other instances severe initial shock is followed by a rapid return to normal.

As pointed out by Biedl and Kraus (1909), anaphylactic shock in the dog is associated with a progressive fall in blood pressure; later investigations (Pearce and Eisenbrey 1910, Dale 1920*b*) have made it clear that this is not due to any failure of output from the heart or to any influence of the central nervous system, but to the collection and stagnation of the blood somewhere in the tissues. The studies of Dale and his colleagues (Dale 1920*b*) render it highly probable that capillary dilatation is the essential factor.

Manwaring (1910) performed a large number of experiments in an attempt to localize the site of reaction; and, having found that shock did not occur when the abdominal vessels were ligated, proceeded to explore the rôle of the various abdominal viscera by a series of operative experiments designed to exclude each in turn. He found that the exclusion of the liver inhibited acute shock in dogs; and his observations have been amply confirmed by subsequent workers (Voegtlin and Bernheim 1911, Denecke 1914). These results accord well with the findings at necropsy. The congestion of the abdominal viscera has been noted by many observers; and Weil (1917) points out that in dogs dying of anaphylactic shock in its acutest form the single outstanding feature at necropsy is the enormous distension and congestion of the liver.

We may briefly note certain additional phenomena, which seem to be general to anaphylactic shock as displayed by any of the species so far investigated, though not developed to an equal degree in all of them. There is commonly a marked leucopænia; and this, as Webb (1924) has shown, is associated with an aggregation of polymorphonuclear cells in the capillaries of the lungs, a phenomenon clearly analogous to that which follows the intravenous injection of bacteria in immunized animals. There is a decrease in the coagulability of the blood, most marked in the dog. There appears to be a decrease in the amount of complement in the circulating blood. There is a marked fall in body temperature.

Anaphylactic Shock in Other Animals.—Although the guinea-pig, rabbit and dog have been studied more extensively than any other species in regard to their anaphylactic reactions, a considerable number of experiments have been carried out on other animals; and it is instructive to note that, in addition to striking differences in behaviour and physiological reaction during anaphylactic shock, various species differ widely from one another in the ease with which they can be sensitized. Thus, among rodents, the rat appears to occupy a peculiar position in that it is extremely refractory to sensitization (Longcope 1922, Parker and Parker 1924, Spain and Grove 1925); though an anaphylactic response can be demonstrated under suitable conditions (Kellaway 1930). Birds, so far as they have been examined, may be sensitized by the usual procedures (Friedberger and Hartoch 1909); and the anaphylactic reaction in the pigeon has been studied in some detail (Gahringer 1926, Hanzlik and Stockton 1927). Sensitization to foreign proteins, associated with phenomena which are anaphylactic in kind but do not include typical shock, has been described in cold-blooded animals such as the frog (Goodner 1926). In monkeys, and this is of particular interest for the student of human pathology, sensitization appears to be induced irregularly and with considerable difficulty. Zinsser (1920) reports his failure to sensitize rhesus or ringtail monkeys with a single injection of horse serum; and he could produce only mild anaphylactic symptoms after treatment with repeated doses of antigen.

The problem of human anaphylaxis will be most conveniently considered in relation to the problem of natural protein hypersensitiveness.

Passive Anaphylaxis.

It was noted by some of the earliest workers in this field that female guinea-pigs which had been actively sensitized gave birth to young that were themselves hypersensitive. Otto (1907) first demonstrated that the anaphylactic state could be passively transferred by injecting the serum from an anaphylactic into a normal animal, and in so doing placed anaphylactic shock in the category of the *in vivo* antigen-antibody reactions. His findings were soon confirmed and extended by other workers, in particular by Doerr and Russ (1909), and the numerous observations which have since been recorded place the following propositions on a firm basis of experimental fact, so far at least as the guinea-pig is concerned. A normal animal that has received an injection of the serum of an actively sensitized animal will respond to the subsequent injection of the corresponding antigen by developing acute anaphylactic shock. Passive sensitization may be effected with equal or even greater certainty by an injection of the serum of an animal that has been "immunized" against a given antigen by repeated injections, so that it is not itself sensitive to the antigen in question. In either case the injection of the serum is not followed by the *immediate* sensitization of the recipient. An interval of some hours must elapse between the injection of the sensitizing serum and the injection of the corresponding antigen if typical shock is to be regularly obtained. For maximal sensitization the interval is even longer—4 to 6 days (Kellaway and Cowell 1922).

The efficacy of an antiserum in the induction of passive anaphylaxis appears to be closely related to its content in specific antibody as recorded by precipitin tests (Doerr and Russ 1909), and there seems no reason for supposing that the reagents involved differ from those with which our other immunological studies have made us familiar. We need not, therefore, burden our vocabulary and confuse our minds with such terms as "anaphylactogens" or "anaphylactic antibodies." We may, however, reasonably inquire what rôle partial antigens or haptens play in anaphylactic reactions.

The Rôle of Haptens in Anaphylaxis.—Tomesik and Kurotchkin (1928) immunized rabbits against *Bact. aerogenes*, the pneumobacillus, and a yeast. In each case they were able to obtain a high-titre precipitating serum for the polysaccharide hapten extracted from the corresponding organism. Guinea-pigs injected with these rabbit antisera developed acute anaphylactic shock when subsequently injected with the corresponding hapten. Lancefield (1928) sensitized guinea-pigs with the sera of rabbits immunized against hæmolytic streptococci, and induced acute shock by the injection of the separated haptens. Enders (1929) obtained similar results with a polysaccharide fraction from the tubercle bacillus. Avery and Tillett (1929) studied the anaphylactic action of the pneumococcal polysaccharides. These haptens will not induce active sensitization in the guinea-pig, but they will induce acute anaphylactic shock in animals passively sensitized by the injection of precipitating antisera prepared in the rabbit, and these reactions are type-specific. They add the interesting and significant observation that it is impossible to

induce passive sensitization in guinea-pigs by the injection of an antipneumococcal serum prepared in the horse—although such a serum gives a typical precipitin reaction—and in this connection it may be noted (see Friedberger and Hartoch 1909, Scott 1931) that guinea-pigs cannot be passively sensitized with the serum of fowls, or pigeons with the serum of rabbits.

Tillett, Avery and Goebel (1929) have tested the anaphylactic response to synthetic sugar-proteins. They prepared *p*-aminophenol glucoside and *p*-aminophenol galactoside and linked these to serum globulin and to egg albumin. Preliminary precipitation tests showed that antisera produced in rabbits reacted specifically *in vitro* with the carbohydrate groupings. Guinea-pigs passively sensitized by the injection of an anti-glucoside-globulin serum developed typical anaphylactic shock when subsequently injected with glucoside-albumin. Guinea-pigs sensitized with anti-galactoside-globulin serum reacted similarly when injected with galactoside-albumin. In each case the reaction was shown to be specific. Active sensitization with the globulin compounds, followed after 11 days by injection of the corresponding albumin compounds, gave similar results. The unconjugated glucoside or galactoside did not induce shock when injected into sensitized animals, but each inhibited anaphylactic shock when injected immediately prior to the injection of the corresponding carbohydrate-protein compound. Klopstock and Selter (1929) carried out similar experiments and obtained similar results, using compounds of protein with atoxyl, or with the sodium salt of metanilic acid. Landsteiner and Levine (1930) sensitized guinea-pigs to the *d*- and *l*-isomers of *p*-amino-tartranilic acid, in each case coupled to horse globulin. The subsequent injection of the corresponding active groupings coupled to chicken globulin induced anaphylactic shock. The shock could be specifically inhibited by a preliminary injection of a compound of resorcinol with the active grouping concerned. Similar results were obtained with guinea-pigs passively sensitized to *p*-amino-arsanilic acid, the active substance being coupled to tyrosine for the inhibition tests.

Specific Desensitization.—The specific desensitization of sensitized animals was recorded by many of the earlier investigators (Otto 1905, Rosenau and Anderson 1906, 1907, 1908, Besredka and Steinhardt 1907, Gay and Southard 1908). Desensitization—as then observed, and in the sense in which this term is now commonly employed—differs from the inhibitory effect induced by simple haptens in that the desensitizing agent is the complete antigen, which is capable, when administered intravenously in adequate doses, of inducing typical shock. The desensitizing effect is obtained, in place of acute shock, if a very minute dose of antigen is given and repeated, or if rather larger doses are given by a route, such as the subcutaneous, which ensures slow absorption. The essential conditions for success appear to be the administration of an amount of antigen that is adequate in total by some method that prevents any rapid accumulation in the circulating blood.

The Mechanism of Anaphylactic Shock.

It is clear that acute anaphylactic shock, as induced by any of the procedures that have been described above, belongs to the category of the specific antigen-antibody reactions. The only question at issue is the mechanism by

which this primary reaction gives rise to the series of events that characterize acute shock in any given animal species.

Two opposing views have been put forward. According to one the reaction between antigen and antibody, occurring in the circulating blood or in the tissue fluids, gives rise to a toxic product, anaphylatoxin, which, acting on susceptible cells, gives rise to the characteristic syndrome. According to the other the primary reaction occurs not in the blood or tissue fluids but on or in the tissue cells; and the reactions that characterize acute shock are due not to any toxicity of the antigen-antibody compound itself, or of any derivation from it, but to cellular disturbances initiated by the antigen-antibody reaction. As we shall see, the picture that has been pieced together during the last ten years derives, in all its essential outlines, from the cellular school, though it contains details reminiscent of the anaphylatoxin hypothesis.

The Humoral or Anaphylatoxin Hypothesis.—The chief protagonist of this conception of the mechanism of acute anaphylactic shock was Friedberger (1910, 1912, 1913), who had at an earlier period been one of the sponsors of the cellular hypothesis. Certain difficulties in the latter theory, not easily explicable in the light of the knowledge then available, had caused him to modify his views; but his complete *volte-face* was largely determined by the observation of Friedemann (1909) that a guinea-pig could be poisoned with the characteristic symptoms of acute shock by injecting a mixture of an antigen and its homologous antibody after a brief period of incubation *in vitro*.

The humoral theory in its original form postulated a union of antigen with antibody in the circulating blood, followed by a cleavage of the resulting complex, presumably of the hydrolytic type, and the setting free of toxic degradation products—a conception which seemed to accord well with the findings of Vaughan and Wheeler (1907) on the toxicity of the intermediate products of proteolytic degradation in general. Friedemann's observations, which have been repeatedly confirmed, seemed at first to afford a direct demonstration of the production of anaphylatoxin from antigen and antibody *in vitro*; but it soon became clear that sera could be rendered toxic, in this sense, by a variety of procedures in which antigen and antibody played no part. Thus anaphylatoxin can be produced by incubating normal serum with kaolin (Ritz and Sachs 1911, Keysser and Wassermann 1911, Mutermilch 1913), barium sulphate (Mutermilch), talc (Mutermilch), starch (Ritz and Sachs 1918), inulin (Nathan 1914), or agar (Bordet and Zuntz 1914).

The humoral theory in its earlier form was often described in terms of Ehrlich's hypothesis—the linkage of complement to antigen by an intermediary amboceptor followed by a proteolysis in which complement acted as an enzyme—and a certain air of probability was lent to this view by the observation that the free complement in the circulating blood may be greatly diminished during anaphylactic shock. But there is no evidence that complement can play such an enzymic rôle; and the *in vitro* toxification of serum by non-specific adsorbents is clearly not explicable in such terms as these. As an alternative hypothesis it was suggested that the formation of an antigen-antibody compound, or the addition to serum of kaolin, starch or similar reagents, might remove some stabilizing constituent, perhaps antitrypsin, and so initiate a process of auto-digestion—a kind of serum autolysis. The work of Jobling and Petersen (1914) and of Bronfenbrenner (1915), among others, is in accord with this possibility; but there are almost insuperable objections to its acceptance as an explanation of the *in vitro* toxification of serum, and still more to the view that it forms the essential mechanism of anaphylactic shock *in vivo*. Thus, there has been an almost universal failure to show any relation between the increasing

toxicity of serum under the influence of a toxifying material and chemical changes indicative of proteolysis (de Kruif and German 1917, Dale 1920b, Doerr 1922); while the rapidity of toxification—under favourable conditions a serum may be rendered highly toxic within a few minutes—is hardly compatible with the known behaviour of proteolytic enzyme reactions. Still more is this the case in acute anaphylactic shock. As early as 1908 Wells pointed out the extreme improbability of the view that the minute amount of egg albumin that suffices to produce almost immediate shock in the sensitized guinea-pig could, in the short interval available, undergo proteolysis with the liberation of a toxic dose of degradation products. The substitution of the serum proteins for the antigen as the supposed substrate removes the quantitative difficulty, but greatly increases that introduced by the time factor. We cannot believe in the formation of an antigen-antibody compound, the adsorption by it of a stabilizing serum constituent, and a consequent digestion of the serum proteins, all occurring within a very few minutes. Moreover, substances that produce *in vitro* toxification of serum may fail to produce anaphylactic symptoms when injected intravenously into animals (Dale 1920b, Doerr 1922).

The exact nature of the process which underlies the *in vitro* toxification remains obscure; but it appears to be related in some way to the redistribution of the plasma constituents that occurs during clotting (Novy, de Kruif and Novy 1917a, b, Novy and de Kruif 1917, de Kruif 1917, de Kruif and German 1917).

Anaphylactoid Shock.—Apart altogether from the difficulties referred to above, it is very doubtful how far the acute and fatal reaction that follows the intravenous injection of a toxified serum can be identified with true anaphylactic shock. The resemblances are certainly striking, and it seems certain that common factors are involved; but there are certain differences. At necropsy the emphysema of the lungs may be rather less striking, while oedema and hæmorrhage into the pulmonary tissue are more frequent. For this reason there is general agreement that "anaphylactic" should be replaced by the ambiguous but convenient "anaphylactoid" as a descriptive term for this type of shock, and that "anaphylatoxin" should be replaced by "serotoxin" as a designation for the active agent in toxified serum.

There are, in truth, many agents other than toxified sera that may produce a fatal anaphylactoid reaction when injected intravenously into the guinea-pig. A list of such substances given by Hanzlik and Karsner (1920, 1924) includes a wide variety of organic colloids and of salts of heavy metals. The syndrome produced differs a little from one anaphylactoid agent to another; and with none does it resemble completely and in detail that of true anaphylactic shock. The spasm of the bronchioles—the most dramatic of the anaphylactic reactions in the guinea-pig—is in general a prominent feature, though it may vary in degree. There is, however, a high frequency, with these anaphylactoid reagents, of capillary thromboses and embolism in the lungs.

Among such anaphylactoid reagents—and this has been a source of error in several experiments supposed to support the humoral view—must be included all antisera that contain a high concentration of Forssman antibody (see Doerr and Pick 1913, Taniguchi 1922, Scott 1931). The toxic effect is here due, in part at least, to a direct action of the heterophile antibody on the heterogenetic antigen contained in the guinea-pig's tissues. When the serum is injected by the ordinary intravenous route the first stress of this reaction falls on the endothelium of the lung capillaries, resulting in lesions which appear to be identical with those produced by the serotoxins.

Peptone Shock.—Another anaphylactoid reagent is ordinary peptone (de Waele 1907, Biedl and Kraus 1909, Hirschfelder 1910, Doerr 1914, 1922, Dale 1920a, 1929, Manwaring, Clark and Chilcote 1923). The resemblance of peptone shock to true anaphylactic shock is very striking. In the guinea-pig there is the typical contraction of the bronchioles, in the dog the fall in blood pressure, and the congestion of the liver. In both there may be found the loss of coagulability of the blood and the multiple small subserous hæmorrhages that are characteristic features of acute anaphylaxis in general. It is probable that peptone, like the serotoxins, acts as a direct endothelial poison (see Dale 1929). In the case of the dog there appears to be an additional action on the liver cells.

Histamine Shock.—Histamine is an amine derived by decarboxylation from histidine. It was isolated from ergot by Barger and Dale (1911) and its pharmacological action has been studied in considerable detail by Dale and his colleagues, and by others (Dale 1913, 1920a, b, 1929, Dale and Laidlaw 1919, Maunter and Pick 1915, Longcope 1922, Voegtlin and Dyer 1924).

The toxic syndrome produced by histamine reproduces very faithfully several of the characteristic features of acute anaphylaxis. In the guinea-pig the spasm of the bronchioles is constantly present in its most typical form. In the dog (Maunter and Pick 1915, Dale 1929) the muscular walls of the efferent hepatic veins are very sensitive to histamine; in response to traces of this substance they constrict and dam back the blood in the capillary spaces of the liver. But there are also quite definite points of difference. The loss of the coagulability of the blood and the capillary hæmorrhages that are associated with anaphylactic shock do not occur in histamine poisoning; and the distension of the dog's liver with blood and lymph in anaphylaxis is far more severe and persistent than that produced by histamine.

The importance of these observations lies in the fact that histamine is a relatively simple substance of known chemical constitution, the pharmacological action of which has been studied in considerable detail. It can hardly be a mere coincidence that on intravenous injection it mimics so closely the syndrome produced by the injection of a non-toxic antigen into a sensitized animal.

Summarizing the evidence up to this point we may say that sera may be treated in various ways that render them toxic, in the sense that they produce when injected intravenously into the guinea-pig a syndrome that closely resembles, but is not identical with, that of acute anaphylactic shock. There is, however, no evidence that an "anaphylatoxin" is produced as the result of an antigen-antibody reaction occurring in the blood stream, nor has any mechanism for the production of such an anaphylatoxin been yet suggested which is compatible with all the known facts. Moreover, many reagents other than toxified sera will produce similar anaphylactoid reactions; and one reagent of known chemical composition will give rise to some of the most characteristic features of acute shock. It seems clear that the apparent uniformity of the major symptoms of these different *in vivo* reactions is misleading, if we regard it as denoting an identity of underlying mechanism, though we can hardly be mistaken for supposing that some common factor runs

through them all. It will be convenient to consider the cellular hypothesis of anaphylactic shock before discussing what this factor is likely to be.

The Cellular Hypothesis.—The essence of this hypothesis is that, for the production of anaphylactic shock, the primary antigen-antibody reaction must occur, not in the blood stream or tissue fluids, but in or on the tissue cells. Why this cellular reaction should give rise to the striking syndrome that characterizes acute shock is a separate problem that we may consider later.

The earliest experiments made it clear that those procedures that led to a high concentration of antibodies in the circulating blood were not those that produced the anaphylactic state. Repeated injections of antigen into a normal animal lead to a high antibody content but not to hypersensitiveness. In passive sensitization the highest concentration of antibody in the blood occurs immediately after the sensitizing injection of antiserum; but it is only after the lapse of a latent period, during which most of the antibody has disappeared from the blood stream (Fenyvessy and Freund, see Doerr 1922, Weil 1913), that hypersensitiveness is established.

Such facts as these clearly suggested that "fixed," not "circulating" antibody was concerned in the reaction. But far more decisive support for the cellular hypothesis was derived from experiments carried out with isolated tissues from actively and passively sensitized animals.

Schultz (1910) excised portions of intestine from sensitized guinea-pigs, suspended them in a bath of Ringer's solution, and studied their response to the addition of the specific antigen. He found that such isolated muscle contracted far more vigorously, when the antigen was added to the bath, than did similar preparations from normal animals when submitted to the action of similar amounts of the same foreign protein. These observations were confirmed and greatly extended by Dale (1913), using an improved technique in which one horn of the guinea-pig's uterus was substituted for the intestinal preparation, and this technique has since been used by Dale and his colleagues (Dale 1920*a, b*, 1929, Dale and Hartley 1916, Dale and Kellaway 1922), and by Weil (1913-17) in attempts to determine the underlying mechanism of the acute anaphylactic reaction.

The reaction of the sensitized uterine horn is extraordinarily delicate; a typical contraction may occur after the addition of as little as 1 c.c. of a 1:10,000,000 dilution of horse serum to a bath containing 150 c.c. of Locke's solution. It is also sharply specific; not only does the uterus removed from a guinea-pig sensitized against horse serum fail to respond to the serum of cat, rabbit, dog or man, but it is possible to sensitize separately against the euglobulin, pseudoglobulin and albumin fractions of horse serum (Dale and Hartley 1916).

All the essential features of anaphylaxis can, indeed, be demonstrated *in vitro*. Uterine horns removed from guinea-pigs sensitized either actively or passively respond specifically to the addition of the specific antigen to the bath of Ringer or Locke solution in which they are suspended. Perfusion of the uterus with warm Ringer solution before removal from the body does not

abolish its sensitiveness. After a single maximal response to the addition of a relatively large amount of antigen, followed by a change to fresh Ringer's solution, the uterus no longer responds to the addition of fresh antigen: it has been specifically desensitized. If such a desensitized uterus is again washed, soaked for $2\frac{1}{2}$ hours in a 10 per cent. dilution of the specific antiserum, washed in numerous changes of Ringer's solution, and again tested by the addition of antigen, it will give a typical contraction, showing that it has been passively sensitized *in vitro*. The uteri of normal guinea-pigs may be rendered hypersensitive by prolonged perfusion with diluted serum from anaphylactic or immunized guinea-pigs. This hypersensitiveness is hardly perceptible after 1 hour's perfusion, but it is easily perceptible after 5 hours' perfusion, thus affording a striking analogy to the latent period in passive sensitization in the living animal.

A fact of great significance was established by Dale while investigating the response of the uteri from immunized guinea-pigs. These animals had received repeated injections of horse serum increasing in amount. Some of them were injected intraperitoneally with 5 c.c. of horse serum but showed no sign of shock. Nevertheless, the uterine horns, removed from other animals of the same series, reacted with a typical contraction on the addition of horse serum to the bath, thus showing that fixed antibody is present in the immune as well as in the hypersensitive animal, and lending strong support to the view that the immune guinea-pig is protected by the excess of antibody in its circulating blood.

The protective function of circulating antibody is very clearly shown in some of Weil's experiments. A series of guinea-pigs were passively sensitized by the intraperitoneal injection of 0.1 c.c. of rabbit-*v*-horse serum, i.e. the serum from a rabbit immunized by the repeated injections of horse serum. Three days later one animal received 2 c.c. of this antiserum (20 sensitizing doses) and immediately thereafter 0.01 c.c. of horse serum (1.5 anaphylactic doses). This guinea-pig showed slight symptoms only. It was killed; its uterus was tested by Dale's technique, and its serum was injected into another normal guinea-pig which was tested 24 hours later by the injection of horse serum. The uterus of the first animal gave a typical reaction, showing that it had anchored the antibody injected 3 days previously, and that this fixed antibody had not combined to any appreciable extent with the 0.01 c.c. of antigen subsequently injected under cover of a protective dose of antibody. The second guinea-pig developed typical shock, showing that a considerable amount of the antibody injected into the first guinea-pig immediately prior to the injection of the antigen had remained unneutralized in the circulation. Another sensitized guinea-pig of the same series received 2.5 c.c. of the rabbit-*v*-horse serum (25 sensitizing doses) and immediately thereafter 0.4 c.c. of horse serum (52 anaphylactic doses). It died in acute shock, showing that the amount of antibody present was not sufficient to protect the cells containing the fixed antibody from this excessive dose of antigen. The uterus, removed immediately after death, showed itself to be desensitized. A normal guinea-pig was injected with the serum of this animal and 24 hours later received an injection of horse serum. It developed moderate symptoms only, showing

that the greater part of the circulating antibody in the animal that died of acute shock had been neutralized by the large dose of antigen. These results may be made clearer by reference to Fig. 33.

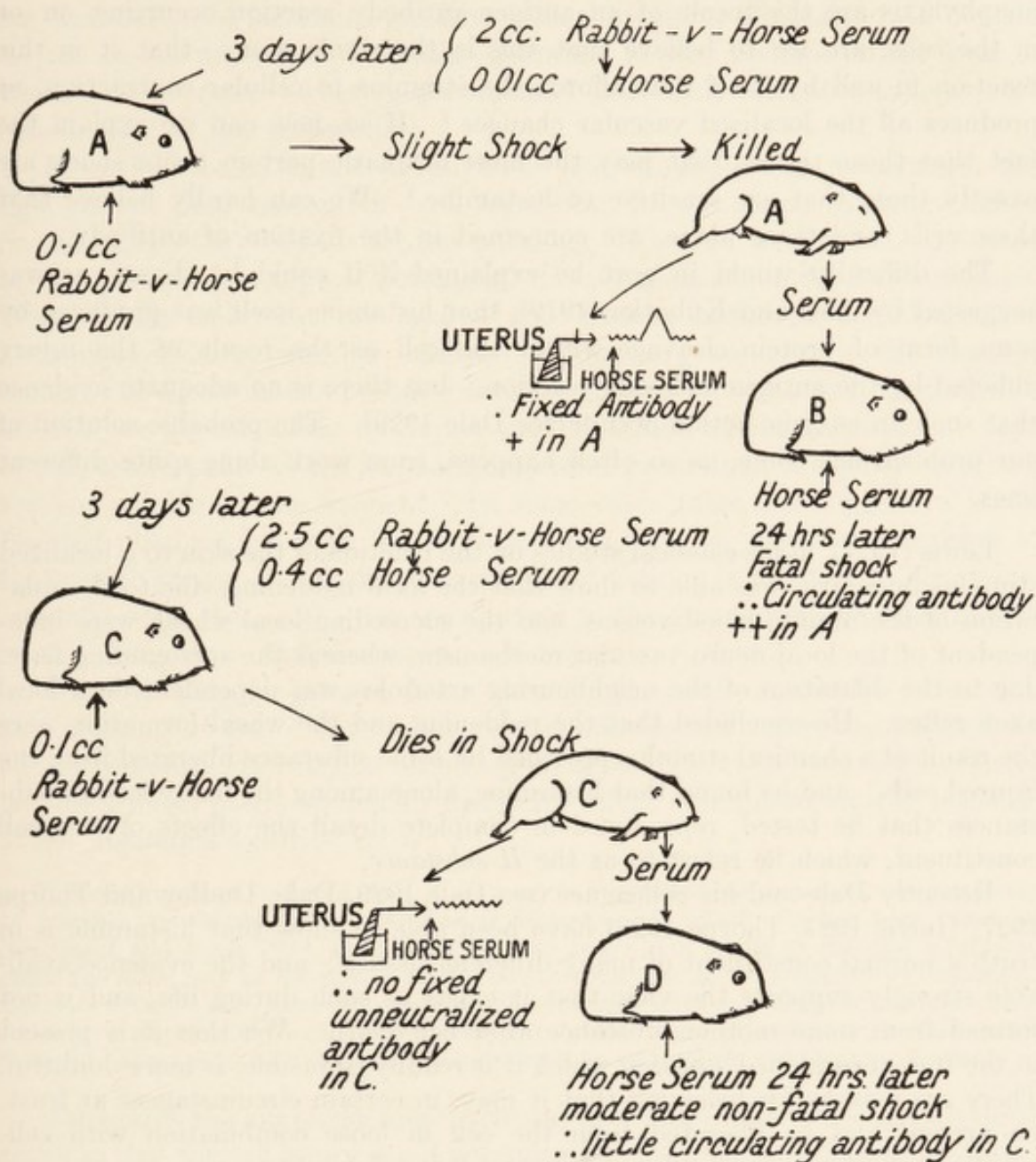


FIG. 33.

We may state with some confidence that, so far as the guinea-pig is concerned, the different behaviour of the hypersensitive and "immune" animal may be explained by postulating a difference in the relative concentration of fixed and circulating antibody.

If, however, we turn to other animals the evidence is more confused (see Dale 1920*b*, Scott 1931); and the observations of Manwaring and his colleagues (1923-28) on acute anaphylaxis in the dog are particularly difficult to fit into the relatively simple picture that we have drawn above. It seems, however, altogether unlikely that the mechanism of acute anaphylaxis differs fundamentally from one animal to another; and it is probable

that apparent discrepancies will in time resolve themselves into the expression of secondary divergences in a reaction of one essential type.

If we accept the view that the cellular responses that characterize acute anaphylaxis are the result of an antigen-antibody reaction occurring on or in the cells, are we to believe that this is the whole story—that it is this reaction in and by itself that affords the stimulus to cellular contraction, or produces all the localized vascular changes? If so, how can we explain the fact that those tissues that play the most dramatic part in acute shock are exactly those that are sensitive to histamine? We can hardly believe that these cells, and these alone, are concerned in the fixation of antibody.

The difficulty would in part be explained if it could be shown, as was suggested by Abel and Kubotka (1919), that histamine itself was produced by some form of protein cleavage within the cell as the result of the injury inflicted by the antigen-antibody reaction; but there is no adequate evidence that such an enzyme action occurs (see Dale 1929). The probable solution of our problem has come, as so often happens, from work along quite different lines.

Lewis (1927), in his classical studies on the reaction of the skin to a localized stimulus or injury, was able to show that the local reddening, due to the dilatation of the minute blood vessels, and the succeeding local wheal, were independent of the local neuro-vascular mechanism, whereas the surrounding flare, due to the dilatation of the neighbouring arterioles, was dependent on a local axon reflex. He concluded that the reddening and the wheal formation were the result of a chemical stimulus provided by some substance liberated from the injured cells; and he found that histamine, alone among the many known substances that he tested, reproduced in complete detail the effects of this cell constituent, which he refers to as the *H-substance*.

Recently Dale and his colleagues (see Dale 1929, Dale, Dudley and Thorpe 1927, Harris 1927, Thorpe 1928) have been able to show that histamine is in truth a normal constituent of many different tissues; and the evidence available strongly supports the view that it exists as such during life, and is not formed from some mother-substance after cell-death. Whether it is present in the free uncombined state, in which it is readily diffusible, is more doubtful. There are reasons for believing that it may, in certain circumstances at least, be present and be liberated from the cell in loose combination with cell-constituents of high molecular weight and low diffusibility.

The relative histamine content of different tissues is interesting and suggestive. Using a physiological method of assay (see Thorpe 1928, Harris 1927) the lungs have been found to contain between 35 and 75 mgm. of histamine per kilo, the liver between 2.5 and 6.6 mgm., the skin as a whole about 6 mgm. (24 mgm. in the epidermis and 4 mgm. in the dermis). Other tissues contain much less. The voluntary muscles contain about 1 mgm. per kilo, the testis about 1.8 mgm., the submaxillary gland about 0.5 mgm. and the thyroid about the same.

There is thus strong presumptive evidence that cellular injury, however it is produced, is liable to liberate histamine into the immediately surrounding

tissue fluids. The amount liberated will depend on the extent of the injury, and on the type of tissue injured. The extent of the resulting histamine effect—its localization to the site of injury or its generalization with the resulting stimulation of histamine-sensitive cells throughout the body—will depend in part on the amount of histamine liberated, in part perhaps on the form in which it is liberated—whether uncombined and highly diffusible or in a loose combination of low diffusibility.

If we look back on the anaphylactic and anaphylactoid reactions in the light of this dual conception—cell injury on the one hand, histamine effect on the other—they become far less puzzling. We need not, indeed we cannot at the moment, attempt a histological disentanglement of the two reactions. Whether the cells that are injured and liberate histamine are themselves histamine-sensitive, whether minimal injury may so disturb the internal economy of a cell that it becomes reactive to its own contained histamine, or whether we should locate the injury and liberation of histamine in one type of cell, the response to the liberated histamine in another, are problems which are not soluble at the moment. In some cases there are suggestive hints. Isolated plain muscle from the sensitized dog gives no significant response to the specific antigen. Yet the plain muscle of the dog is sensitive to histamine. It would seem that in the dog, as opposed to the guinea-pig, the sensitization to the antigen is limited mainly to the liver.

We may then, in general terms, describe the acute anaphylactic reaction as consisting of two different but related effects—cell injury determined by an antigen-antibody reaction occurring on or in those cells by which antibody has been fixed, and a secondary histamine effect determined by the response of the histamine-sensitive cells.

It should perhaps be noted that, if we want to generalize our statement as completely as possible, we should write *histamine or histamine-like* effect. It has been observed (see Grant and Jones 1929, Dale 1929) that the frog's skin reacts to minimal injuries in much the same way as the skin of man; but this reaction is not produced by histamine. Extracts of frog's skin, however, reproduce this response as closely as histamine reproduces the injury response in man. The active principle of the frog's skin is not histamine, though it appears to be a base that resembles it in some of its chemical properties.

As regards the anaphylactoid reactions, they differ from acute anaphylactic shock in that the cell injury is not determined by an antigen-antibody reaction; they resemble it in that the secondary histamine effect is essentially the same. It should be noted that most of the anaphylactoid reagents, such as toxified sera, do not stimulate contraction of the isolated guinea-pig uterus. This histamine-liberating injury presumably occurs elsewhere when these agents are injected *in vivo*. Peptone, on the other hand, has a direct stimulating effect on plain muscle, which suggests that it may contain histamine.

The complete picture of an anaphylactic or anaphylactoid reaction will thus consist of a description of cellular injury, differing from case to case according to the reagent employed and the susceptibilities of the experimental animal, and a histamine effect, differing from one animal species to another

according to the distribution of the histamine-sensitive cells, but common to all reactions in one animal species, by whatever reagents they are caused.

LOCAL ANAPHYLACTIC REACTIONS

Within a year of the original observations of Portier and Richet, and before the phenomenon of acute anaphylactic shock had been submitted to any detailed study, Arthus (1903) described a curious reaction in the rabbit, which was soon recognized as belonging to the same order of exaggerated response to a foreign antigen. Inoculating rabbits subcutaneously with repeated doses of horse serum, he noted that the initial injections were without any detectable effect, but that, as one injection succeeded another with suitable intervals between them, the local reactions became more intense and more persistent. After a few injections the site of inoculation became swollen and oedematous. These local swellings were at first transient, but with each successive injection they lasted longer, with more definite induration, until finally the response took the form of a firm indurated swelling which progressed to a localized necrosis. This altered response was not confined to the tissues in the immediate neighbourhood of the earlier injections. The rabbit, as a whole, had become so sensitized that it reacted in this characteristic and abnormal way to the injection of the specific antigen at any site (see also Seegal, Seegal and Jost 1932, Davidoff, Seegal and Seegal 1932). It may be noted that this reaction, in its typical form, appears to be peculiar to the rabbit. Nicolle (1907) failed to reproduce it in the guinea-pig, and Opie (1924) failed to elicit it in either the guinea-pig or the dog.

HYPERSENSITIVENESS IN MAN

Clinical observations have long been on record containing descriptions of reactions in human subjects that present obvious analogies to the anaphylactic reaction in experimental animals.

SERUM SICKNESS.—It has been known for many years that a proportion of those persons who receive injections of antitoxic or antibacterial serum for prophylactic or therapeutic purposes develop a curious and characteristic condition which has been given the name of "serum sickness." An excellent description of the disease and a detailed discussion of its significance are contained in the monograph of von Pirquet and Schick (1905). For our immediate purpose we need refer only to the following points.

The reaction occurs after primary as well as after subsequent injections. It is in no way related to the antibody content of the injected serum, but is a response to the foreign protein as such, which is usually horse serum. There is, in the great majority of cases, an incubation period of 8 to 12 days between the injection of the serum and the onset of the symptoms. The illness itself is characterized by rashes, especially of an urticarial type and often commencing at the site of the inoculation, by fever, joint pains, slight oedema without albuminuria (or with a mere trace of albumin in the

urine), and by a varying degree of glandular swelling usually confined to the regional glands that receive the lymphatics from the site of inoculation. The illness is variable in duration, protean in its symptoms, and seldom of a serious nature, though it may be associated with severe discomfort. It is probably never fatal.

In a tiny minority of cases an entirely different reaction follows the injection of serum, the patient dying within a few minutes with symptoms resembling those of acute anaphylactic shock. Park (1908) records two such cases among 50,000 persons who received serum injections in New York.

THE IDIOSYNCRASIES.—Another group of reactions, which clearly come into the general category of hypersensitiveness, are the so-called idiosyncrasies, in which certain persons develop a characteristic symptom-complex as the result of natural contact with some particular substance, or range of substances. The substances that may evoke this response are of the most varied nature: in hay fever, pollens of various species; in asthma, pollens, horse dander or the cutaneous débris from other animals; in the food idiosyncrasies, a wide range of different materials; in the drug idiosyncrasies, a whole series of chemical compounds which seem to have no characteristic or common structure.

One of the most striking features of the idiosyncrasies as a group is that—as in the anaphylactic reaction—the symptom-complex bears no relation to the particular type of substance by which it is evoked. In spite of the varied nature of the exciting materials concerned, the syndrome induced shows an essential uniformity; though there are minor differences which seem to depend in the main on the particular route by which the reacting substance gains access to the tissues. It is particularly striking that, in the case of drugs, the response exhibited by the hypersensitive subject—using hypersensitive in this particular sense—bears no relation to the pharmacological action of the drug in question.

The reactions induced may be either local or general. Among the former are the local reaction to the intracutaneous injection of horse serum or to the application to the skin of such a drug as iodoform, the coryza of hay fever and perhaps the associated asthma, and the acute vomiting and gastro-intestinal disturbance that follow the ingestion of a particular food stuff. Among the latter are the generalized urticarial eruptions or oedema that may follow the administration by any route of a substance to which a person is hypersensitive, the occasional pyrexia, and the asthmatic symptoms that sometimes follow the entry of the exciting substance by a route other than the respiratory tract.

There are obvious points of similarity between this group of reactions on the one hand and anaphylactic hypersensitiveness on the other, but there are also obvious divergences.

Among the points of similarity we may include the following (see Coca 1920*a*):—

In both cases the reaction is specific. Although the naturally hypersensitive person may react to several different substances the range is usually

limited, and may be confined to a single chemical substance. In both cases the type of response is essentially the same for a single animal species irrespective of the nature of the exciting substance.

Among the features that have been held to differentiate between the two conditions are the following. The exciting agent in anaphylaxis is always an antigenic substance; the exciting agent in human hypersensitiveness is often non-antigenic. Anaphylactic hypersensitiveness is induced by the injection of a sensitizing dose of antigen; hypersensitiveness in man is often exhibited upon first contact with the exciting agent. The anaphylactic state, apart from congenital passive anaphylaxis, is not a heritable condition; natural human hypersensitiveness shows a definite tendency to run in families, though the hypersensitiveness is often exhibited towards different substances in parents and offspring. Specific desensitization is far more easily demonstrated in anaphylaxis than in hypersensitiveness.

Taking these points of difference *seriatim* we may attempt to assess their significance in the light of the evidence now available. Coca and his colleagues, who would regard the differences between the two conditions as sufficient to justify a formal separation into different categories, employ the term *atopy* to denote the natural hypersensitive state, and the term *atopen* to denote the exciting agent (see Coca and Cooke 1923). We may define our first problem as the determination of the differences between atopens and antigens.

Atopens and Antigens.—It is certain that many substances are at the same time antigenic and atopic. Horse serum forms an excellent example, though we should note that Coca and Cooke separate serum sickness from the true atopies. If, however, we confine ourselves to the more typical atopens we still find that a single substance commonly displays both types of activity.

Alexander (1924) found that guinea-pigs could be rendered anaphylactic to rye pollen though he could not induce the formation of precipitating or complement-fixing antibodies in detectable amount. Parker (1924) reports the production of precipitins for ragweed pollen in rabbits and guinea-pigs, and Ramsden (1926) obtained similar results. Walzer and Grove (1925) showed that the pollens of timothy-grass and ragweed have definite antigenic properties, since both induce the anaphylactic state in guinea-pigs, demonstrable either by intravenous inoculation or by Dale's technique. They note, however, that sensitization with pollens is far more difficult and uncertain than with such an antigen as horse serum, and they failed to demonstrate precipitins in the serum of the sensitized animals. Caulefield and his colleagues (1926) prepared various fractions from ragweed, timothy-grass and birch pollens, and obtained with them active and, in some cases, passive sensitization. Ramsden (1926) demonstrated both active and passive sensitization of guinea-pigs to pollen. Longcope and others (1925, 1927) have demonstrated the active sensitization of guinea-pigs to extracts of horse dander, and have succeeded in specifically desensitizing such animals by the usual procedures. There seems little doubt that many of the reagents that have been classed as typical atopens have a definite antigenic action.

There is, of course, no doubt at all that many of the substances that function as atopens—for example many drugs—are non-protein in nature and are therefore not antigenic in the full sense. Moreover, it has been found (Grove and Coca 1925) that the removal of all proteins and other detectable nitrogenous substances from extracts of pollen, or of house dust, does not cause any appreciable loss of atopic activity, as tested by intracutaneous inoculation in sus-

ceptible persons. On the other hand, the tryptic digestion of the proteins of horse dander, or of the green pea, caused a loss of atopic activity corresponding with the loss of protein.

The fact that non-protein substances can excite a specific response in the hypersensitive person cannot, however, be taken as a proof that antigens and atopens differ in any essential respect. We have seen that many haptens, when injected intravenously into actively or passively sensitized guinea-pigs, will induce acute anaphylactic shock, so that it would be in no way surprising if a fundamentally similar reaction were produced in naturally sensitive persons by contact with antigenic components of a similar kind.

There remain instances of drug idiosyncrasies that are not at present readily explicable along these lines, since the chemical structure of the exciting agent is not of the relatively complex kind which characterizes those haptens that are capable of inducing acute shock in a sensitized animal. But recent observations suggest that animals may under suitable conditions be sensitized to relatively simple chemical compounds (see Klopstock and Selter 1929, Mayer and Sulzberger 1931, Sulzberger and Mayer 1931), and, taking the evidence as a whole, there seem no sufficient grounds for believing that antigens and atopens must be placed in different categories.

Active and Passive Sensitization in Man.—The second criterion that has been accorded differential significance is the mode of sensitization. There is no question that human hypersensitiveness occurs naturally and apart from any known previous contact with the particular antigen to which the sensitive subject reacts, just as the "natural" antibodies may be found in persons who, so far as we can tell, have never been infected with or immunized against the particular bacterium on which their sera react.

There are, however, good reasons for believing that active sensitization plays some part in the distribution of hypersensitiveness to any particular antigen among any random sample of the normal population.

Hooker (1924) tested a large number of persons by the intracutaneous injection of horse serum before and after they had been immunized by the injection of toxin-antitoxin mixtures. Of 367 persons tested before immunization 308 failed to react to horse serum. Of these, 100 were found to be Shick-positive and were immunized by toxin-antitoxin injections. On retesting these 100 persons at a later date, 66 gave positive reactions to horse serum. Park (1924) carried out a similar series of observations, in which he tested the sensitiveness to horse serum of immunized and non-immunized groups of children. He found that 84 of 116 children who had received toxin-antitoxin injections reacted positively to horse serum, while of 90 children who had not been immunized 45 were sensitive, giving a difference of 22.5 per cent. between the two groups. Other observers (Cowie 1914, Longcope and Rackeman 1918, Gordon and Creswell 1924, Mackenzie 1924) have obtained results that point in the same direction. It would appear that the injection of horse serum significantly increases the percentage of sensitive persons in a sample of the normal population, so that the relation between natural and artificial hypersensitiveness differs little from that between natural and artificial immunity, using the term immunity to denote the presence in the blood of specific antibodies.

The most striking evidence in favour of the view that experimental anaphylaxis and natural hypersensitiveness depend on essentially similar mechanisms has, perhaps, been provided by the demonstration that hypersensitiveness

to a particular atopen or antigen can be passively transferred from one person to another, since this clearly places human hypersensitiveness with anaphylaxis in the category of the antigen-antibody reactions.

Ramirez (1920) reported the case of a man who was sensitive to horse dander. He was employed as the donor in the transfusion of an anæmic patient who received 600 c.c. of his blood. The recipient, who gave no history of such an idiosyncrasy, thereafter developed marked hypersensitiveness to horse dander. The evidential value of this case is somewhat lessened by the fact that the same donor was used in another transfusion without rendering the recipient hypersensitive.

An observation that has provided a basis for the experimental study of passive sensitization in man was recorded by Prausnitz and Kustner in 1921. Kustner himself was markedly sensitive to the cooked flesh of certain fish. Using an extract of cooked fish as antigen these observers failed to demonstrate the presence of precipitins in Kustner's serum, nor could they produce passive sensitization of guinea-pigs. If, however, a little of Kustner's serum was injected into the skin of a normal person and the antigen was injected into the same site 24 hours later, a marked local reaction was produced. This reaction did not occur if the antigen and the serum were injected simultaneously, a finding strikingly analogous to the latent period in the passive anaphylactic sensitization of the guinea-pig.

The detailed study of this passive atopic sensitization has been taken up by Coca and his colleagues and by other investigators. Coca and Grove (1925) gave the name *atopic reagins* to the antibodies present in the serum of naturally hypersensitive persons, through the agency of which the specific hypersensitiveness can be passively transferred to the skin of normal non-sensitive persons. They record that these reagins have been demonstrated in the serum of all those subjects of hay fever or of asthma in whom the reaction to the intradermal injection of the specific atopen is positive. They find, however, that not all normal skins are receptive, about 11 per cent. failing to react at all, and some 5 per cent. giving slight or doubtful reactions. The remaining 84 per cent. are fully receptive, and the local passive sensitization lasts for at least 4 weeks once it has been established. They find that the reagin may be neutralized by the specific atopen in the test-tube, but that it gives no precipitin reaction *in vitro*, nor will it induce passive anaphylaxis in the guinea-pig. In a control series of experiments they noted that the normal human skin could not be passively sensitized to egg white or to ragweed-pollen with rabbit antisera, although these contained specific precipitins. Exactly similar phenomena have, however, been noted in the case of passive anaphylaxis—neither horse antisera nor chicken antisera are effective in the passive anaphylactic sensitization of the guinea-pig, though antisera prepared in the rabbit are highly efficient from this point of view.

Clarke and Gallagher (1926) give a very clear-cut demonstration of the latent period in passive atopic sensitization and of the *in vivo* neutralization of reagin by atopen without the production of any tissue reaction. They injected 0.05 c.c. of atopen into one area of skin, and 5 minutes later they injected 0.05 c.c. of reagin-containing serum into the same site and 0.05 c.c. of the same serum into a second site some distance from the first. No reaction occurred after these injections, showing that the union in the tissues of atopen and reagin under conditions that give no time for the preliminary fixation of the reagin does not produce the typical response. Next day each site was reinjected with the same quantity of atopen. At the second site, where no atopen had previously been injected, a typical reaction occurred. At the first site no obvious response was elicited, showing that the reagin had been neutralized by the atopen that was present when the sensitizing injection was given.

Levine and Coca (1927) found that there was a proportional relationship between the degree of sensitivity of the skin and the reagin content of the serum in 33 cases of hay fever.

Reagins and Antibodies.—The observations quoted above do not suggest that any clear separation can be made between these two types of antibody. It is true that reagins may be demonstrated in sera that give no precipitation with the specific antigen; but, as we have seen, passive anaphylaxis may be induced with sera in which no precipitins can be detected. It is by no means clear, therefore, that reagins and precipitins are different antibodies; the apparent qualitative differences may in reality be quantitative—as they appear to be in anaphylaxis—depending on a difference in delicacy between the *in vivo* and *in vitro* tests. De Besche (1918), for instance, has reported the presence of precipitins in the serum of a considerable proportion of a small group of asthmatics who were sensitive to the proteins of the horse, cat, dog or cow. He notes that patients who showed the presence of precipitins at one time might fail to do so at another. The antibodies appeared to fluctuate above and below the level at which they were demonstrable *in vitro*.

Otto and Adlesberger (1931) have recently recorded a careful series of observations on the relation between the atopic reagins and the anaphylactic antibodies. They note that the typical reagin, as exemplified in the sera of persons sensitive to horse dander, is extremely effective in the local transference of specific sensitivity to the normal human skin. It is less effective in sensitizing the skin of the guinea-pig, and quite ineffective in sensitizing that animal to acute anaphylactic shock. The sera of persons suffering from serum sickness, or of those who have been injected with horse serum without suffering from the clinical disease, are effective—though somewhat less effective—in the transference of sensitivity to the normal human skin. They are far more effective in sensitizing the guinea-pig's skin; and are not infrequently effective in inducing generalized anaphylactic sensitization. Anti-horse sera, prepared in rabbits or guinea-pigs, are relatively—but not entirely—ineffective in the transference of sensitivity to the human skin, but are uniformly effective in inducing passive anaphylaxis in the guinea-pig.

The evidence as a whole suggests minor differences in the antibodies with which these various workers have been concerned—differences perhaps connected with the proteins in antisera obtained from different animal species rather than with the active grouping of the antibody; but there seems no adequate reason for regarding reagins and anaphylactic antibodies as belonging to essentially different types. *Atopens* and *reagins* might well give place to *antigens* and *antibodies* in immunological nomenclature, except where some convenient term is required to denote the way in which an antigen or antibody is functioning at a particular time.

The Genetic Factor in Anaphylaxis and Hypersensitiveness.—The third character that has been held to differentiate human hypersensitiveness from anaphylaxis is concerned with the part played by inheritance in the two conditions. There is evidence (Cooke and Veer 1916, Coca 1920a, Spain and Cooke 1924, Clarke, Donnally and Coca 1928) that human hypersensitiveness tends to run in families. There is at present no evidence that the anaphylactic state, as induced in the sensitized guinea-pig, occurs naturally or is determined by genetic factors. Does this indicate any essential difference in the nature of the two phenomena?

Even if we accepted the premises as warranting such a conclusion we should require much more evidence than we have before we accepted the second premise as valid. Anaphylaxis in its usual experimental form is, of course, never inherited; since one of the necessary conditions for its study is that the injection of the antigen into the unsensitized animal shall not produce shock, *i.e.*, the experimental animal selected must not be naturally sensitive to the antigen under test. Whether there are any cases in which the injection of an antigen into an animal that has not been artificially sensitized produces typical anaphylactic shock, demonstrably due to an antigen-antibody reaction, we do not know, because no systematic search has ever been made. In the absence of such knowledge we cannot tell what part inheritance plays in the natural anaphylactic state, if that state occurs. As Doerr (1922) points out, we have for obvious reasons studied natural hypersensitiveness in man and anaphylaxis in experimental animals. It is at least possible that the differences noted are referable in large part to the different universes selected for study.

With regard to the inheritance of human hypersensitiveness we have already noted (see Cooke and Veer 1916, Spain and Cooke 1924) that the genetic factor appears to be concerned with a tendency to develop atopic hypersensitiveness in general, rather than a sensitiveness to any one atopen in particular. It appears to be unusual to find that parents and offspring, or several members of the same family, are all hypersensitive to the same substance; one may respond to ragweed pollen, another to horse dander, another to cat hairs, and so on. It has also been noted (Clarke, Donnally and Coca 1928) that the type of reaction to atopic substances in general appears to be in some degree inherited; the tendency to develop asthmatic symptoms, for instance, being particularly frequent in certain families.

There seem insufficient grounds for postulating an essential difference between anaphylaxis and hypersensitiveness on the premises of our present knowledge of the influence of genetic factors in the two conditions.

Desensitization in Hypersensitiveness and Idiosyncrasy.

Fourthly, some observers, and particularly Coca (1920*b*), would lay great stress on the difficulty of desensitization in the sensitive human subject as compared with its relative ease and certainty in the anaphylactic animal. While admitting that a degree of lessened sensitivity to natural contact with the specific atopen may be attained in a hypersensitive person by suitably graded injections, Coca points out that the further administration of the atopen by subcutaneous or intracutaneous inoculation will always demonstrate the persistence of the hypersensitive state.

It seems doubtful, however, whether this criterion has any greater differential value as between the atopic and anaphylactic states than those we have discussed above. It is true that desensitization may readily be demonstrated in the anaphylactic guinea-pig; but even here it depends on a strict observance of the necessary quantitative and time relationships, as may be seen in many of Weil's experiments. In other species the problem is far less simple; it is for instance notoriously difficult to desensitize the rabbit.

Apart from these theoretical considerations, the possibility of effective general desensitization in human hypersensitiveness is obviously one of practical importance, not only as a measure of affording relief in such conditions as hay fever or recurrent asthma but as a precaution against anaphylactic accidents in the administration of therapeutic sera. In the latter connection, Besredka (1907) regards the results established in the guinea-pig as directly applicable to man; but clinical experience has not supported his view,

and it is extremely doubtful whether any of the methods of desensitization in common use, such as the subcutaneous injection of a small amount of serum shortly before the administration of the main dose, have any effect in lessening the risk of serum disease, or the far more remote risk of acute shock. Even if the conditions in the sensitive human subject were strictly analogous to those in the actively or passively sensitized guinea-pig, which they certainly are not, there would be no justification for believing that one or more small subcutaneous injections of serum would infallibly protect against the intravenous or intramuscular injection of 10-20 c.c. some hours later. Even in the guinea-pig recovery from severe anaphylactic symptoms following the injection of a relatively small dose of antigen does not necessarily avert the occurrence of fatal shock when a much larger dose is given on the following day. On the principle of neglecting no precautions, it is wise to employ one of the recognized methods of desensitization before administering a large dose of serum to a patient suspected of hypersensitiveness; but the clinician relies in the main on the statistical evidence, which shows quite clearly that fatal shock following the injection of therapeutic sera is an extremely rare event.

Bacteria and Bacterial Products in Relation to Anaphylaxis and Hypersensitiveness.

We have already noted that active anaphylactic sensitization can be induced by the inoculation of bacterial cells, and passive sensitization by the injection of a suitable antibacterial serum; and we have seen that, in the sensitized animal, acute shock can be produced by the intravenous injection of certain separated bacterial haptens.

No useful purpose would be served by discussing in any detail the extensive literature dealing with bacterial anaphylaxis in general. The available data may be summarized by stating that active sensitization has been induced with various bacterial antigens and passive sensitization with the corresponding antibodies as demonstrated by the occurrence of acute shock (Rosenau and Anderson 1907, Kraus and Doerr 1908, Holobut 1909, Kraus and Admiridzibi 1910, Zinsser and Parker 1917, Tomcsik and Kurotchkin 1928, Lancefield 1928, Enders 1929, Avery and Tillet 1929), or by Dale's technique with the isolated uterus (Zinsser and Parker 1917, Zinsser and Mallory 1924, Tomcsik and Kurotchkin 1928). The reactions are specific and desensitization can be demonstrated. Sensitization to bacterial antigens cannot, however, be produced with the same ease as can sensitization to such antigens as horse serum or egg white. A single injection of bacteria frequently fails to produce any demonstrable sensitization, and the most successful results have been obtained by giving repeated small injections of antigen, followed by an interval of three weeks or so before the intravenous injection of the test dose, which should be relatively large. A technical difficulty in work of this kind is introduced by the fact that many of the bacterial suspensions employed are themselves toxic when administered in any considerable dose, and the symptoms that develop in normal animals after the injection of massive doses of bacteria or bacterial products may be very similar to anaphylactic shock. The difference in the response of normal and of sensitized animals may, therefore, be quantitative rather than qualitative.

ALLERGIC REACTIONS IN BACTERIAL INFECTIONS

The current terminology in this field of immunity is as chaotic as elsewhere, and it is impossible to define with any exactness the content of the group of conditions covered by the term *allergy*. From its derivation—signify-

ing simply an altered or abnormal reaction—it is logical to use it as it is used by Doerr (1922) to cover any departure from the normal response of the tissues to contact with a foreign substance. Here we are using it in a widely accepted sense, as a loose and general term to cover a group of reactions characterized by a heightened or accelerated response to a particular type of antigen, irrespective of the balance of harm or benefit that the altered response confers on the allergic host.

The case of serum sickness affords an instance of a reaction of this type, in which bacterial infection plays no part. Although this syndrome may develop after a first injection of horse serum, it is quite clear that the response may be significantly modified as the result of a previous injection. Von Pirquet and Schick (1905) have described these secondary reactions in considerable detail. The most significant feature is the shortening of the incubation period, followed by the development in unusually rapid succession of the various signs and symptoms that together make up the complete syndrome. This is referred to as an accelerated response. In some cases this acceleration is so pronounced that œdema at the site of inoculation, associated with generalized urticaria, fever and all the symptoms of severe serum sickness, may develop within 24 hours.

Turning to those instances in which specific sensitization occurs during the course of a bacterial infection—the type of hypersensitiveness with which we are most closely concerned—the classical example of an allergic response to a non-living bacterial antigen is afforded by the tuberculin reaction in its various forms.

This experimental allergic reaction is readily demonstrated in the guinea-pig. The injection into a normal guinea-pig of 2 c.c. of Koch's old tuberculin—a culture of tubercle bacilli in 5 per cent. glycerine broth, grown for 6–8 weeks at 38° C., concentrated to 1/10th volume at 100° C., and freed from bacilli by filtration through a clay or kieselguhr candle—has little effect. But the injection of 0.01 c.c. into a guinea-pig in the 8th or 10th week of tuberculous infection may kill it within a few hours. At necropsy, supposing the injection to have been given subcutaneously, the site of inoculation is found to be very congested, dark red or almost violet in colour. The focal glands are swollen and congested, the liver and spleen show on their surface numerous dark red spots, particularly in the neighbourhood of the multiple tuberculous lesions which are commonly present. Microscopically these red spots are seen to consist of enormously dilated capillaries. Thus the tuberculin produces a local reaction at the site of inoculation, numerous scattered focal reactions in the immediate neighbourhood of pre-existing tuberculous foci, and a rapidly fatal toxæmic effect, probably secondary to the widespread focal reactions.

This fatal generalized tuberculin reaction in the guinea-pig exemplifies the allergic response in its severest form. The development of hypersensitiveness in an infected animal may, however, be demonstrated quite as clearly by means of the localized reaction that follows the intracutaneous injection of 0.2 c.c. of a high dilution (1 : 800 to 1 : 4,000) of tuberculin (see Römer and

Joseph 1909-10, Lewis and Aronson 1923, Eagleton and Baxter 1923). Both types of response—the generalized in a mitigated form as evidenced by a febrile reaction, and the localized as demonstrated by the scarification method of von Pirquet or by some alternative procedure—are employed as diagnostic tests in human and veterinary medicine (see Chapter XVIII).

We have seen that a polysaccharide hapten has been isolated from tubercle bacilli, but it would seem that this is not the antigenic component concerned in the allergic response as commonly demonstrated (see Laidlaw and Dudley 1925, Mueller 1926). There is, however, evidence that an allergic response to bacterial haptens of this type may develop as the result of a natural infection. Tillett and Francis (1929) have described a characteristic local response to the intracutaneous injection of the type-specific pneumococcal polysaccharides in patients convalescent from lobar pneumonia; and similar observations have been recorded by Finland and Sutliff (1931).

We have so far been concerned with the allergic response to a non-living antigenic material; but there is abundant evidence that the sensitized tissues respond differently from the non-sensitized to contact with living bacteria. Here again, the classical example is afforded by Koch's observations on experimental tuberculosis (Koch 1891).

When a normal guinea-pig is injected subcutaneously with living tubercle bacilli, a localized nodule appears at the site of inoculation after 10-14 days. This nodule increases in size, involves the skin, and undergoes necrosis, leaving an indurated ulcer that persists until the animal's death. Meanwhile there occurs the characteristic lymphatic spread of infection, involving first the regional lymphatic glands and later the more remote tissues and organs. But if living tubercle bacilli are injected into a guinea-pig that is already infected with tuberculosis of some weeks' duration the result is quite different. On the 1st or 2nd day following the reinoculation the site of injection shows an area of acute superficial inflammation 0.5-1 c.m. in diameter, without the formation of any nodule in the underlying subcutaneous tissue. Next day the affected area of skin is found to be necrotic and finally sloughs, leaving a flat, shallow ulcer which undergoes rapid healing. The regional lymph glands are not affected; and there is no evidence of the spread of infection from the localized focus caused by the second inoculation. This rapid and energetic response does not, in this case, confer any permanent benefit on the guinea-pig, for it is destined to die from the primary infection; but it affords a striking example of a reaction that betrays at one and the same time the character of hypersensitiveness and of immunity.

THE RELATION OF HYPERSENSITIVENESS TO INFECTION AND IMMUNITY

The terms *anaphylaxis* and *hypersensitiveness*, and the connotation of the term *idiosyncrasy* as used in this restricted sense, suggest an alliance with susceptibility rather than immunity—an altered response that weighs the scales against the infected host rather than in his favour. They yield indeed an obvious and attractive suggestion that the phenomena of hypersensitiveness

may play an essential rôle in the whole sequence of events that characterize an attack of an infective disease.

The latent period in sensitization offers an analogy to the incubation period in an infective fever; and the hypothesis that the fully developed disease is an expression of an anaphylactic response would at least afford a tentative explanation of the pathogenic action of those bacteria that are not known to produce specific toxins, and would suggest a reason for the similarity of the constitutional symptoms in a diversity of bacterial infections. Such a conception has not, however, received any adequate experimental support. It has passed through an unfortunate and troubled infancy, the result of its early adoption by the protagonists of the anaphylatoxin school. This phase was of course inevitable. Had the anaphylatoxin hypothesis in its simplest form established its validity, some of the general febrile and constitutional reactions in an acute infective fever might have been described in very simple terms. During the incubation stage the bacteria would be multiplying in the body and increasing the supply of bacterial antigen. During the same stage the tissues would respond by a gradual production of antibody. For a time antigen and antibody would co-exist in the blood and tissue fluids, the latter in relatively small amount. These conditions would be those required for the formation of anaphylatoxin from the antigen-antibody complex; and this period would correspond to that of the clinical disease. Gradually the concentration of antibody would increase and the antigen would be eliminated. This would correspond to the period of convalescence and recovery.

Even if the anaphylatoxin theory had not been discarded, such a conception as that outlined above could never have explained more than a small part of the phenomena of infection. As has been pointed out elsewhere, it is quite certain that many of the phenomena that characterize infective diseases are referable to the inherent characters of the infecting organisms as living things; while the anaphylactic, or allergic, reaction appears to be independent of the nature of the agent that evokes it. It does, however, seem quite possible that anaphylactic or allergic reactions may play a significant part in the pathogenesis of many bacterial infections, and this problem awaits re-investigation in the light of our present knowledge.

It is, however, with immunity rather than with susceptibility that the allergic response shows the clearest affinities; and the all-inclusive classification of Doerr, who would place acute anaphylaxis, natural hypersensitiveness and immunity in a single generic group, with subgroups clearly marked by certain features but shading into each other when the focus of attention is shifted from the obvious manifestation to the underlying mechanism, offers the most reasonable as well as the most coherent picture.

So far as our present knowledge goes it seems very probable that all the phenomena of anaphylaxis, of hypersensitiveness, and of immunity of the type dependent on the development of specific antibodies, result from the working of a single underlying mechanism, the character of the total reaction being determined in each particular case by a variety of secondary factors. Anaphylactic shock, for instance, is an experimental curiosity of high informative value to the student of immunity but not of any obvious significance in determining the survival or extinction of a species during the course of its evolution. The sudden introduction of a foreign antigenic substance into the circulating

blood is not an event that is likely to menace any living thing, unless it is subjected to intentional human interference. It is neither paradoxical nor surprising that a mechanism, which in natural circumstances tends directly towards immunity, should react to the disadvantage of its possessor when exposed to a stimulus that formed no part of the environment in which it was evolved.

The idiosyncrasies, it is true, seem to confer nothing but disadvantage under natural conditions; but this may well be because a mechanism that, in its modal application against an infecting parasite, has a high survival value is here brought into play, perhaps in an exaggerated form, against agents that in themselves are harmless.

Infection-Immunity.

This is as convenient a place as any in which to mention a conception that is often found in current bacteriological literature—the conception that there is a kind of immunity that is associated with existing, but not with past infection. In its extreme form this conception suggests that so long as an infection with a particular bacterial parasite exists, even though that infection is latent, the host will be immune to reinfection with that particular bacterium, but that once the host's tissues are freed altogether from the infecting parasite susceptibility will return.

We have already noted Koch's observation that a guinea-pig behaves differently towards a primary and secondary infection with living tubercle bacilli. The studies of many subsequent workers have confirmed and greatly extended his findings, and have shown quite clearly that a subacute or chronic tuberculous infection is associated with a considerable resistance to reinfection.

Significant evidence bearing on the problem of infection-immunity has been obtained during the study of experimental syphilis in the rabbit. In this infection it is possible, by administering an adequate dose of neosalvarsan, or of one of the related arsenical drugs, to cure the initial infection and observe the effect of so doing on the resistance of the rabbit to reinfection.

A syphilitic keratitis may be induced in the rabbit by the injection of *Trep. pallidum* into the anterior chamber of the eye (see Bertarelli 1906, 1907, 1908), a chancre may be produced by injection into the skin of the scrotum (Tomaszewski 1910), and a characteristic lesion follows injection into the testicle (Parodi 1907).

When an infected rabbit is reinoculated with the same strain of *Trep. pallidum* after a relatively short interval a local chancre does not develop (Truffi 1910); but reinfection with a different strain may induce a typical local lesion (Kolle and Schloszberger 1926). Brown and Pearce (1921) infected a number of rabbits and treated some of them with arsphenamine shortly after the development of the primary chancre. Five days later these rabbits were injected with the same strain of *Trep. pallidum*. Most of the treated animals developed chancres. The untreated animals did not.

Kolle (1922, 1924) treated rabbits with neosalvarsan at various stages of infection. He found that rabbits treated within about 6 weeks after the initial infection usually responded to a subsequent injection by developing a new chancre. When treatment was delayed beyond this point, reinfection seldom gave rise to a chancre. When treatment was delayed for about 3 months a new chancre could very rarely be produced. Rabbits that were treated and reinfected at long intervals after the primary infection (75–250 days) were not, however, completely resistant to reinfection (see Kolle and Prigge 1927, 1929). Tissue extracts from treated rabbits that had not been reinfected were non-

infective for normal rabbits, but similar extracts from treated and reinfected rabbits gave rise to characteristic lesions, showing that the spirochaetes had gained access to the tissues and were presumably multiplying in them, at least to a limited extent. On these grounds Kolle and his colleagues maintain that the immunity to reinfection displayed by rabbits that have been treated in this way is only a "chancre immunity." The exact significance of this term is a little dubious. We have noted many examples of a partial antibacterial immunity in which bacteria may persist for long periods in the tissues without giving rise to any of the lesions that they produce in non-immunized animals. Moreover, the evidence suggests that a more complete immunity against *Trep. pallidum* may be induced under optimal conditions. Uhlenhuth and Groszmann (1928) treated syphilitic rabbits with neosalvarsan 107 to 506 days after the primary infection. Attempted reinfection 3 to 10 months after treatment gave rise to no obvious lesions; and in only 4 of 11 rabbits could spirochaetes be demonstrated in the tissues (see also Voegtlin and Dyer 1925, Chesney 1927, Chesney and Kemp 1924, 1925*a*, *b*, 1926, Breinl and Wagner 1929).

In summary, we may say that immunity to reinfection appears to be established within a short time after the appearance of the initial lesion. At this stage the maintenance of immunity depends on the maintenance of infection; complete chemotherapeutic cure is followed by a return of susceptibility. But after a longer period of infection a partial immunity is established that persists after complete chemotherapeutic cure; and, after still longer periods of infection, a complete immunity may be established that is independent of the persistence of infection.

Whether it is necessary to assume the existence of some mechanism other than those we have considered in this and in preceding chapters, in order to account for the immunity to reinfection—or, as it is more appropriately termed, to "superinfection"—during the early stages of the initial infection, is a question that cannot yet be answered. We may, at least, note that this resistance to superinfection appears to be type-specific. As regards the resistance developed during the later stages of infection, it seems highly probable that it can be accounted for by a slow acquirement of an immunity of the usual antibacterial type.

SUMMARY

(1) Acute anaphylactic shock may be produced by inoculating into an animal any antigen to which it has previously been sensitized. The symptoms and lesions associated with shock in any particular case are determined by the anatomical and functional peculiarities of the species of animal employed, not by the character of the antigenic material injected.

(2) Sensitization is specific. It may be induced either actively or passively, and hence must depend, at least in part, on some form of antigen-antibody reaction.

(3) Active sensitization is induced—in the case of such an animal as the guinea-pig and such an antigen as horse serum—by a single minimal injection of antigen. After this injection there follows a latent period during which hypersensitiveness develops, and during this period no further injections of antigen must be given. Once the anaphylactic state has been actively induced it lasts for months or years.

(4) Passive sensitization is induced by the injection into a normal animal of the serum from a sensitized or from an immunized animal—that is by any serum containing the specific antibody. There is a correlation between the antibody content of a serum and its effectiveness as a sensitizing agent; and for this reason the serum of an immune animal is more effective (*i.e.*, sensitizes in smaller doses) than the serum of a hypersensitive animal. The anaphylactic state that is induced by the injection of an antibody-containing serum takes some hours to develop. To ensure typical shock the assaulting dose of antigen should not be injected until 8 to 24 hours have elapsed after the injection of the antiserum. During this latent period the antibodies have, for the most part, disappeared from the blood and become fixed on or in the tissue cells. The anaphylactic state when passively induced passes off in a few days or weeks.

(5) To produce typical shock in either an actively or passively sensitized animal, the assaulting dose of antigen must be introduced into the blood stream rapidly and in adequate, though not in large amount. Intravenous injection gives the most consistent results, and in many cases is the only route by which typical shock can be induced.

(6) The injection of haptens, or partial antigens, fails to induce active sensitization, just as it fails to induce active immunity. Complex haptens, such as the bacterial polysaccharides, will induce acute shock when injected into an animal actively sensitized by the injection of the corresponding full antigen, or passively sensitized by the injection of a specific antiserum. Less complex haptens will neither induce active sensitization nor induce shock in a sensitized animal; but when injected into such an animal they will inhibit the induction of shock by the corresponding antigen. In a sensitized animal antigens and haptens behave *in vivo* as they behave in the precipitin reaction *in vitro*.

(7) The sensitized animal may be desensitized by the injection of the specific antigen in amounts too small to induce acute shock or by a route that ensures slow absorption so that there is no rapid transport to the circulating blood.

(8) The anaphylactic state can be demonstrated in an isolated tissue or organ as well as in the animal as a whole; thus, the uterus of a sensitized guinea-pig reacts *in vitro* to contact with the antigen employed in sensitization.

(9) The balance of experimental evidence is strongly in favour of the view that the anaphylactic response is the result of an antigen-antibody reaction occurring on or in the cells that have removed the antibody from the blood and in some way fixed it themselves. The anaphylactic state is associated with the presence of fixed antibody and the absence of circulating antibody. The immune state is associated with the presence of circulating antibody in a concentration sufficient to protect the fixed antibody that is also present.

The difference between the anaphylactic and immune state is quantitative rather than qualitative, depending upon the balance between circulating and fixed antibody.

(10) Many of the most dramatic features of the syndrome of anaphylactic shock are the result not of the antigen-antibody reaction itself but of the liberation of histamine by the injured cells, and the secondary response of the histamine-sensitive cells throughout the body.

(11) A great variety of substances, when injected intravenously, may give rise to a syndrome resembling, in many but not in all particulars, that of anaphylactic shock. These reactions are spoken of as anaphylactoid reactions. The resemblance is not due to any similarity in the underlying mechanism, but to the fact that, in each case, cellular injury is followed by the liberation of histamine, and this by the characteristic syndrome of histamine shock.

(12) Apart from acute anaphylactic shock there are other examples of hypersensitiveness, among them the natural idiosyncrasies that have been observed in man to contact with particular materials, such as pollens, horse serum, horse dander, and certain drugs.

(13) In spite of obvious superficial differences between this condition and experimental anaphylaxis in the guinea-pig, the knowledge that has been gained in recent years—and particularly the demonstration that skin sensitivity to these atopic substances can be passively transferred—has lent support to the view that the underlying mechanism is essentially the same.

(14) Specific hypersensitiveness may also develop in the course of natural or experimental bacterial infection. This type of hypersensitiveness—which is sometimes referred to as *allergy*—is characterized by an accelerated and exaggerated reaction to contact with the infecting bacterium or its products. It partakes of the character of hypersensitiveness in these respects. It partakes of the character of immunity in that the allergic reaction is often associated with the localization of infection. This type of reaction may sometimes be demonstrated in animals that have been immunized by the injection of bacteria or bacterial products, as well as in animals that are suffering from an active or quiescent infection. It is probably an important factor in the development of natural or artificial immunity.

(15) Acute anaphylaxis, natural hypersensitiveness, the allergic response and specific antibacterial immunity would appear to depend on the same fundamental mechanism—the union of an antigen with its specific antibody. The differences between them depend on the nature of the antigen concerned (living parasite, toxic substance or intrinsically inert material), the nature of the animal into whose tissues it penetrates, the rate and route of penetration, the distribution of the antibody in the animal's tissues and body fluids, the secondary effects of the primary cellular reactions, and a variety of other factors.

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CHAPTER XIII

CERTAIN NON-SPECIFIC MECHANISMS IN GENERAL IMMUNITY

THE integrated protective mechanisms which we have described in the preceding chapters are specific in their action. When fully established, by nature or by art, they increase the resistance of the body against some particular bacterial infection, not against bacterial infections in general.

It would, however, be foolish to suppose that the picture we have so far pieced together tells the whole story; it is quite certain that it does not. In this chapter we shall consider certain non-specific antibacterial mechanisms, and the part they play in resistance to infection.

β Lysins, Leukins and Plakins.—Among the earliest records of experimental immunity are a series of observations on anthrax infection, which establish clearly the presence in the serum of certain animal species of bactericidal substances different in kind from the bactericidal complex formed of specific sensitizers and complement. Thus the serum of the rat is actively bactericidal for the anthrax bacillus, and this activity remains after heating the serum at a temperature of 56°–60° C. for 30–40 minutes (von Behring 1889, 1892, Malvoz 1902, Pirenne 1904). The name *β lysins* has since been applied to these relatively thermostable bactericidal serum constituents.

Another series of observations, dating from the same early period, have shown that similar thermostable bactericidal substances can be extracted from suspensions of polymorphonuclear leucocytes. Like the *β lysins* these *leukins* are restricted in their range of activity, some bacterial species, in particular the spore-bearing aerobes such as *B. anthracis* and *B. subtilis*, being highly sensitive to their action, other species being relatively or entirely resistant. There is some divergence of evidence with regard to the susceptibility or insusceptibility of different bacteria, and it seems possible that the leukins derived from one animal species may differ in this respect from those derived from another. Nor can it be said that there is yet any certainty as to whether the *β lysins* and *leukins* are relatively simple substances, or complex systems like serum complement, with its end-piece, mid-piece and third and fourth components.

(See Hahn 1895, Schattenfroh 1897, 1899, Pettersson 1906, 1908 *a, b*, Hiss 1908, Hiss and Zinsser 1908, Zinsser and Hiss 1908, Schneider 1909, Zinsser 1910, Kling 1910, Weil 1911, Blum 1922, Haussmann 1925, Mackie and Finkelstein 1932, Mackie *et al.* 1932.)

There are observations which suggest that substances with a general similarity to leukins may be extracted from blood platelets (see Pettersson,

above, Gruber and Futaki 1907, Barreau 1909), and these have been called *plakins*. It is not certain what relation the β lysins bear to the leukins or to the plakins. There are apparent differences in thermostability—the β lysins are inactivated at temperatures between 65° and 70° C., the leukins not below 75° C. But these differences have hardly been established with sufficient sharpness, or with sufficient regard to possible effects of differences in the containing medium, to be accepted as a clear differential criterion. It seems certain, however, that many animal species may contain no demonstrable β lysins in their serum, although leukins may readily be derived from their leucocytes.

Our present knowledge may be summarized by the statement that there exist in the normal sera of certain animal species thermostable bactericidal substances which are non-specific in their action, in the sense in which immunological specificity is generally understood—though their range of activity is limited by the varying sensitiveness of different bacterial species—and which are not increased in amount as the result of immunization. Bactericidal substances having very similar properties may be extracted from leucocytes and perhaps from blood platelets.

The relation of these substances to effective immunity is by no means clear. To take the case of anthrax, the relatively resistant rat has β lysins in its serum, so has the susceptible rabbit, while the relatively resistant dog has none (see Ledingham 1922).

The significance of the leukins and plakins is still more difficult to assess. That the leukins—using that term for the moment as a generic name for the antibacterial substances present in or derived from leucocytes—play some part in resistance to infection, seems a highly probable assumption; but we do not know whether their rôle is primary or secondary, nor how it is related to the defence mechanisms that we have considered in more detail above. We may note, in this connection, an observation by Buxton and Torrey (1906) which suggests that the polymorphonuclear cells play a significant rôle as bactericidal agents, even when they are not concerned in the primary phagocytic reaction. The injection of typhoid bacilli into the peritoneal cavity of the rabbit is followed by the rapid deposition of a large proportion of the injected bacteria on the surface of the omentum. Here they are in part destroyed by extracellular lysis, in part ingested by the omental histiocytes. During the first 4–6 hours the polymorphonuclear cells play little if any part in the events on the omental surface, but after that interval there is a marked and increasing polymorphonuclear reaction which varies in intensity in different animals, and the fate of the rabbit appears to depend in part on the successful mobilization of the polymorphonuclear cells. When they collect rapidly and in large numbers the clearance of typhoid bacilli is complete, and the animal recovers. When they are scanty or slow in arrival secondary centres of bacterial multiplication appear, and the animal dies with a generalized bacteræmic infection. These observations have not been repeated in detail by later observers, and are not therefore established on a sufficiently broad basis to allow of any general induction; but they are clearly suggestive.

Variations in the Phagocytic Power of the Leucocytes.—We have seen that one animal differs from another in the phagocytic power of its whole blood and that this difference is determined, at least in part, by the presence or relative concentration of sensitizing antibodies, the opsonins or bacteriotropins, whose action is increased in effectiveness by the normal serum complement. This opsonizing action is specific, so far as the sensitizing antibody is concerned, and may be increased or induced *de novo* as the result of specific immunization. But the phagocytic activity of the leucocytes, as was first shown clearly by Shattock and Dudgeon (1908), is itself variable. Leucocytes from different persons, and particularly from persons suffering from various infections, show a significant difference in their ability to ingest not only various bacteria, but also inert particles, such as melanin. The latter observation is of importance, since it indicates that such variations in phagocytic activity are independent of the nature of the phagocytizable object (see also Glynn and Cox 1909-10, Boughton 1910, Rosenow 1910, Hektoen 1911, Wright 1931).

Lysozyme.—In Chapter IV we referred to the bactericidal substance described by Fleming under the name of lysozyme and noted that, in high concentration, it has some bactericidal effect on pathogenic bacteria as well as on those non-pathogenic organisms that are particularly susceptible to its action. What part this substance plays in the defence mechanisms of the tissues it is, at the moment, impossible to say.

Induced Variations in the Non-specific Bactericidal Power of the Blood.—This leads us to a consideration, necessarily brief, of the possibility of inducing an effective immunity by increasing the non-specific bactericidal mechanisms of the cells and fluids of the body. Within recent years Wright and his collaborators (see Wright, Colebrook and Storer 1923, Wright 1931) have been active in exploring this field. Using specially devised *in vitro* methods of testing the bactericidal power of the whole blood, they have found that the addition of living or killed bacterial suspensions to blood in the test-tube, or the injection of similar suspensions into the living animal, results in a very rapid increase in bactericidal power. This increase they attribute, in greater part, to the liberation by the leucocytes of directly bactericidal substances, in lesser part to increased opsonic power. Although this response is not specific—in the sense that there need be no antigenic relationship between the bacterium employed to induce the response and the bacterium against which the bactericidal effect is exerted—some bacterial suspensions have been found to provide a more effective stimulus than others.

This increased bactericidal power is developed with great rapidity—within a few minutes or hours—and is relatively transient, lasting at most for a period of days. It may be induced by a wide variety of stimuli.

Thus Prausnitz and Meissner (1925) record an increase in the bactericidal power of the whole blood after the injection of yatren and aolan. Fleming (1926) records a characteristic response after the injection of salt solutions into rabbits, or after the injection of nuclein (1928). Colebrook, Eidenow and Hill

(1924) have recorded a similar response after exposure to ultra-violet light to dark heat, or to the application of a mustard plaster.

In part, at least, these non-specific effects seem to be due to a temporary increase in the normal opsonins (see Ledingham and Bulloch 1906, Bedson 1915). Thus, Bedson found that an injection of nuclein produced an increase in the opsonic power of the serum that was detectable within 20 to 30 minutes, was still obvious after 24 hours, but then rapidly faded. The opsonic power of the serum, at any period of the reaction, was destroyed by heating for 30 minutes at 56° C., or by dilution with normal saline, indicating that there had been no significant increase in the thermostable opsonins.

It is possible that some of the instances in which an increase in resistance has been noted within so short an interval after an immunizing injection as almost to exclude the elaboration of specific antibodies, have been due to factors of this kind. Thus H. D. Wright (1927) noted that rabbits might show an increased resistance to the intravenous injection of living virulent pneumococci within 5 hours of the injection of a killed suspension of the same organisms. The injection of killed typhoid bacilli failed in most cases to produce a similar result, but there was in two instances some evidence of an improved clearing power of the blood. Teague and McWilliams (1917) report that the intravenous injection of killed typhoid bacilli into rabbits induces, within 24 hours, a condition of increased resistance that enables a proportion of the treated animals to resist a dose of living typhoid bacilli fatal to controls.

A recent series of experiments by Nukada and Arifuku (1931) are of interest, in that they show the combination of non-specificity, in the antigenic sense, with unequal effectiveness of different inocula. They injected guinea-pigs subcutaneously with killed suspensions of various bacteria—three doses given at 5-day intervals—and then determined resistance to the intraperitoneal injection of doses of living typhoid bacilli large enough to kill untreated controls in 12–24 hours. The results recorded (the numbers of animals in each group were small) indicate that the injection of pneumococci or of plague bacilli induces an increased resistance to the typhoid bacillus, evidenced in a few cases by survival beyond the 7th day but more usually by death delayed beyond that of the controls. Paratyphoid bacilli (A and B), dysentery bacilli and cholera vibrios produced a slight increase in resistance evidenced by a relatively delayed death; the gonococcus, the whooping-cough bacillus, the influenza bacillus, *Ps. pyocyanea*, *Proteus vulgaris*, a streptococcus, a staphylococcus and the meningococcus produced no increase in resistance. This effect is reported to have been most marked 5–10 days after the last injection, and to have decreased considerably by the 15th day. (It will be noted in connection with the phenomena which are considered in the following chapter that in these experiments the immunizing inoculum was administered by one route, the infecting inoculum by another.)

We may recall the effect of non-specific immunization in protecting mice against natural infection with *Bact. aertrycke* (see Table XV, p. 131). The limited mean survival time of control unvaccinated mice, in an infected herd, was 26.26 ± 0.641 days, that of mice vaccinated with *Staphylococcus aureus* (an entirely unrelated organism) was 29.86 ± 0.714 days. Mice immunized with a specific vaccine lived on the average 35.31 ± 0.884 days. The difference between the non-specifically immunized and the control mice was trivial, barely attaining statistical significance, but two other non-specific vaccines gave a closely similar result, while a third, not included in Table XV, gave no protection at all. It would seem that the increase in resistance afforded by non-specific stimulants of this class is too slight, and altogether too transient, to have any significant prophylactic value. Whether they can be usefully exploited therapeutically is a different problem.

SUMMARY

(1) In addition to the specific factors that determine antibacterial immunity, there are certain non-specific factors which appear to play some part in the reaction of the tissues to invading bacteria. The exact significance of these factors, and their relation to the general defence mechanism, is at the moment doubtful.

(2) Among these factors are certain thermostable bacteriolysins—the β lysins—which are present in the sera of certain animal species. Their bactericidal action is limited to particular bacterial species, and it seems doubtful whether they confer an effective immunity even against these. They are not increased in amount as the result of immunization.

(3) Bactericidal substances may be extracted from leucocytes (the leukins) and perhaps from blood platelets (the plakins). These substances bear many resemblances to the β lysins, and appear to be even more thermostable. They probably play some part in antibacterial immunity, but what that part is remains, at the moment, an unsolved problem.

(4) Leucocytes vary in their phagocytic activity, and it seems likely that such variations may be induced in response to infection, and perhaps to immunization.

(5) Various non-specific stimuli are capable of inducing a rapid but transient increase in the bactericidal power of the blood, probably determined by some of the factors referred to above. The resulting increase in resistance is too slight, and too temporary, to be of practical prophylactic value. Its possible therapeutic value will be discussed later.

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CHAPTER XIV

LOCAL IMMUNITY

THE defence mechanisms with which we have hitherto been concerned have all exerted a generalized effect. By whatever route bacteria or their products gain access to the tissues these mechanisms will tend to protect the body as a whole.

Another type of immunity may be conceived, confined to one particular area of the body, and to one particular kind of tissue. Such a heightened local resistance may or may not result in, or be associated with, a significant increase in the resistance of the body as a whole. There has been a tendency to confuse the issue by an inadequate definition of the problem to be solved. The concept of a local immunity as such has been imperfectly separated from hypotheses regarding the mechanisms on which it depends. In some cases the term has been used as though it implied a tissue or cellular immunity, not conditioned by humoral factors. In others there has been a failure to distinguish clearly between specific and non-specific effects. It will simplify discussion if we consider the available evidence from each point of view in turn.

What evidence is there for the existence of a local as opposed to a general immunity?

As long ago as 1887 Meierowitsch noted that rabbits which had recovered from an experimental attack of erysipelas were resistant to subsequent inoculations of the causative streptococcus during periods up to 2 months. This observation was confirmed by many later workers, and Gromakowsky (1895) noted that animals which had become resistant to a local cutaneous inoculation did not always withstand an intraperitoneal inoculation.

This phenomenon was studied in much greater detail by Cobbett and Mel-some (1895, 1898). They concluded, on the basis of a considerable series of experiments, that an attack of erysipelas in the rabbit completely protects the area of skin affected against a subsequent inoculation of the specific streptococcus; while on the rest of the body it confers an immunity which is usually less marked and is sometimes entirely absent. They noted also that, when streptococci were inoculated into the skin of a rabbit's ear after the subsidence in that ear of an attack of erysipelas, an inflammatory reaction quickly appeared but subsided before the typical erysipelatos lesion had made any appreciable progress in a control rabbit. This rapid but transitory inflammatory reaction was most marked in ears that had been locally immunized, less marked in ears that shared in a general protection. Gay and Rhodes (1922) have recorded experiments bearing on the same problem.

They conclude that a rabbit which has recovered from an attack of erysipelas is completely protected against subsequent intracutaneous injections of the causative streptococcus, irrespective of its exact locality, for a period of at least 3 months. They were unable to obtain a similar immunity by the intracutaneous injection of cultures killed by heat or by alcohol. Immunity against intracutaneous inoculation was not associated with immunity against intravenous inoculation; while immunization by the intravenous route was found to afford more protection against subsequent intravenous inoculation than against intracutaneous inoculation. Rivers (1925) carried out a series of experiments in which rabbits were given repeated intracutaneous injections of living hæmolytic streptococci derived from a case of erysipelas. He notes that the reactions following successive injections showed a progressive decrease in severity. After several such injections the sera of the rabbits were tested for the presence of protective antibodies. The serum to be tested was mixed with a suspension of living streptococci and injected into the skin of a normal rabbit; at another site in the same animal another injection was made consisting of a mixture of the same streptococcal suspension and normal serum. The sera from the immunized rabbits had a marked protective action, normal serum had none. The studies of Amoss and Bliss (1927) bring out further points of interest. Like other observers they found that an area of skin which had been involved in an erysipelatos reaction was definitely more resistant to a subsequent injection of the causative streptococcus, and that if the injections were repeated the degree of resistance progressively increased. They noted that the primary lesion in the skin of the rabbit's flank tended to spread ventrally from the site of inoculation in the direction of the lymph flow; and they found that when the area involved in the primary reaction and the skin in its immediate neighbourhood were tested by subsequent intradermal inoculations the area of increased resistance extended ventrally beyond the area of primary reaction, but not anteriorly, posteriorly or dorsally. At this period no increased resistance could be demonstrated in the skin of the opposite flank. After prolonged immunization the skin at a distance from the site of inoculation might show increased resistance, but this generalized skin immunity was associated with a humoral immunity, evidenced by the appearance of specific antibodies in the blood.

Taking the evidence as a whole it would appear that a local immunity may exist in the absence of a general immunity, and that, when repeated local injections lead to a general immunizing response, the level of immunity may, for a time at least, be higher in the immediate neighbourhood of the site of inoculation than in the tissues generally. It may be noted that the severe inflammatory reaction associated with an attack of erysipelas is more effective in inducing a local immunity than is the relatively trivial reaction that follows the injection of a killed suspension of hæmolytic streptococci.

Is the distribution of a local immunity confined to a particular type of cell or tissue, affecting that type of cell or tissue wherever it occurs in the body, or is this distribution determined by the site of inoculation, affecting all cells or tissues in the immediate neighbourhood of that site?

We may particularize our question in regard to the phenomena considered in the preceding section by inquiring whether it is the skin, as such and apart from other tissues, that is rendered resistant by the intracutaneous injection of living hæmolytic streptococci, or whether the effect is limited to the area of the initial erysipelatous reaction, affecting in that area all the cells or tissues involved in the inflammatory process.

The evidence is somewhat conflicting. The observations of Gay and Rhodes appear to favour the view that the skin as such irrespective of its nearness or remoteness to the site of the primary inflammatory reaction is rendered resistant by a localized experimental erysipelas. The findings of most other workers, and particularly those of Amoss and Bliss, suggest that the distribution of resistance is, in the first instance, confined to the area affected by the erysipelatous reaction, though other areas of skin share in the more general immunity that may be induced by repeated injections of streptococci.

The question posed leads naturally to the one that follows it and data relevant to its solution will be considered under this and other heads.

Does the local immunity as such, and apart from an associated humoral immunity, confer an increased resistance on the body as a whole?

It will be convenient to commence our discussion of this question by setting out, in their baldest terms, the views that have been put forward by Besredka (1919, 1920, 1921).

He notes that many, if not all, pathogenic bacteria show a marked tendency to specific localization in particular tissues of the body. Thus the typhoid, paratyphoid and dysentery bacilli cause intestinal infection, while the anthrax bacillus commonly causes infection of the skin. He would, however, carry this argument much further than those who regard such frequency distributions as the expression of a modal type of infection, determined by a variety of interacting factors and often departed from in particular instances. He believes, for instance, that the localization is in no way dependent on the route by which the bacteria enter the body. By whatever path typhoid, paratyphoid or dysentery bacilli gain access to the tissues they pass to the intestines, and there produce their distinctive lesions. Similarly, for a fatal anthrax infection to result, the anthrax bacillus however introduced must reach the skin.

As a logical corollary, a naturally immune or an actively immunized animal owes its increased resistance to the local immunity possessed by the particular tissues which are, in susceptible animals, specifically liable to attack. If these susceptible tissues are rendered immune then the whole animal is immune, because the specific localization which is an essential feature of bacterial infection can no longer occur. Thus, in the last analysis, general immunity is simply the expression of effective local immunity. The various antigen-antibody reactions that have been observed are, in reality, of altogether minor importance.

These conclusions are clearly revolutionary and provocative; and like all provocative conclusions based on experimental study they have acted as a stimulus to further research. Taken as they stand, it seems hardly necessary to do more than confront them with the evidence set out in the preceding chapters in order to demonstrate that they cannot be accepted as an adequate picture of the facts. Whether a localized immunity in Besredka's sense plays any part in resistance to bacterial infection is another matter.

Taking the case of experimental typhoid, paratyphoid or dysentery infection in rabbits, Besredka lays emphasis on the point that these animals can be effectively immunized by feeding with dead or living cultures. When dead bacterial cells are used

for immunization, it is sometimes necessary to administer in addition a small dose of bile, which causes minimal lesions of the mucous membrane. The fact that an increased resistance can be induced in this way does not, however, afford any evidence in favour of the conception of a specific induced immunity of the intestinal mucosa. We have seen (p. 178) that the administration of dead or living bacteria by the mouth is often followed by the passage of bacterial antigens from the intestines to the tissues and this, in its turn, by the usual antibody-forming response. The argument on which Besredka lays most stress in this particular instance is the following. He states that when dysentery bacilli are administered to rabbits by the mouth agglutinins may appear in the blood after the first administration, but they soon disappear, and do not reappear after any subsequent administration. He records analogous observations in the case of rabbits immunized by the oral administration of typhoid bacilli after preliminary treatment with bile; and, in both cases, he interprets the early disappearance of agglutinins, their failure to reappear after subsequent administration *per os*, and the increasing resistance of the rabbit to infection by any route, to the establishment of a local immunity of the intestinal mucosa, which presents an impenetrable barrier to the passage of bacteria from the intestines to the tissues, and at the same time prevents the essential selective localization of the bacilli should they gain access to the tissues by some other route. The few experiments recorded by Besredka seem quite inadequate to form a basis for such an important and far-reaching conclusion; and, as Otten and Kirschner (1927) have pointed out, the crucial observation of the disappearance of antibodies from the blood during a series of repeated administrations of vaccine *per os* finds no adequate confirmation in the results obtained by other workers.

Turning to the case of anthrax, controversy has centred round Besredka's contention that the skin alone is susceptible to this infection. Balteano (1922) states that he has confirmed Besredka's findings. Plotz (1924) carried out a series of experiments in which cultures of *B. anthracis* were enclosed in thin-walled glass bottles and introduced into the subcutaneous tissue of rabbits. The wounds were allowed to heal and at various periods thereafter the bulbs were broken *in situ* and the contained culture was allowed to escape into the surrounding tissues. The majority of such animals survived, and Plotz concludes that the rabbit can resist a large subcutaneous inoculation of *B. anthracis*, provided that the bacilli do not come into contact with any wound of the skin.

Other investigators have, however, recorded quite different results. Sobernheim and Murata (1924) estimated the minimal lethal dose of a culture of *B. anthracis* when administered by different routes. They found that about 1/10,000 of a loopful would produce death when introduced intraperitoneally or intravenously, about 1/100,000 of a loopful when introduced subcutaneously or intracutaneously and about 1/1,000,000 of a loopful when injected intramuscularly.

It is obviously difficult to avoid infecting the skin when making injections into any subjacent tissue, and this objection has been urged against those who have recorded successful injection by some route other than the intracutaneous; but Besredka's assumption can hardly be reconciled with the observation that a *smaller* dose of bacilli is required to produce a fatal infection when the inoculation is made into the muscles than when it is made directly into the skin. Similarly, Basset (1925) was unable to demonstrate any superiority of the intracutaneous as compared with the subcutaneous or intramuscular route. Müller (1925) has recorded results which are directly opposed to those obtained by Plotz. He states that, several years before his paper was published, he had inoculated a pigeon's egg with anthrax bacilli, sealed the opening, and introduced the egg into the peritoneal cavity of a rabbit. Five weeks later this animal died from anthrax. Unable to reconcile this result with Besredka's views he carried out further experiments of the same type, but with a slightly different technique. Finding that anthrax bacilli would grow slowly through the pores of an L₁ candle, he placed cultures of the organism in such candles, sealed them carefully, and introduced them into the peritoneal cavities of nine rabbits. Seven of these animals died after intervals varying from 6 to 51 days.

This long incubation period would appear to preclude an infection of the skin occurring at the time of the introduction of the candles. Burke and Barnes (1931) have utilized the bactericidal action of gentian violet in an attempt to produce an initial subcutaneous infection with *B. anthracis* unassociated with any primary infection of the skin. A guinea-pig was injected with a young culture of *B. anthracis* by introducing the needle through the skin of the abdominal wall in one flank, and then passing it subcutaneously across the abdomen to the other flank, where the inoculum was deposited. The point of the needle was then withdrawn to just beneath the skin puncture, the syringe barrel was detached, another barrel containing a 1 per cent. solution of gentian violet was attached, and the dye was introduced into the puncture as the needle was withdrawn. The guinea-pig died in 56 to 72 hours. The characteristic gelatinous infiltration occurred at the point where the inoculum had been deposited, not at the point where the needle had been inserted. Cultures from the site of the skin puncture and from the dye-stained area remained sterile.

It seems fair to conclude that Besredka's contention with regard to the unique susceptibility of the skin to anthrax has not been proved. This does not, of course, mean that all tissues are alike in their reaction to this or any other organism. It is quite certain that they differ, and that such differences are important in determining the outcome of infection in particular instances; but it by no means follows that the prevention, or significant reduction, of selective localization is followed by effective general immunity.

Thus, Hach, Borodaj and Melynk (1928) state that staphylococci, after intravenous inoculation into rabbits, tend first (3-3½ hours) to accumulate in the spleen, few remaining in the blood and few being present in the skin. As the infection progresses towards its rapidly fatal issue the distribution changes; the number of cocci in the spleen rapidly diminish, the number present in the skin show an enormous increase. Multiple intradermal inoculations of a staphylococcal filtrate, preceding the intravenous injection, prevented this rapid accumulation of staphylococci in the skin (Hach and Melynk 1928); but there was no effective immunity, the rabbits dying of typical acute staphylococcal infection.

Returning to the type of infection that we considered in the earlier sections of this chapter—a localized infection of the skin—we may inquire a little more closely into the mechanism on which resistance to reinfection depends.

Here again we may usefully take as a text certain observations recorded by Besredka and his colleagues (Besredka 1924*a, b*, Besredka and Urbain 1924). They applied killed broth cultures of staphylococci or streptococci, or filtrates from such cultures, to the shaved skin of rabbits, and tested the resistance of these areas of skin to subsequent infection with the homologous organisms. From their results they conclude that this procedure confers a high degree of local immunity. In later reports they, and others, have recorded valuable curative properties in these filtered cultures—the so-called “antivirus” preparations—but these latter observations do not bear directly on the problem at issue, and need not be further considered.

The discussion of these observations, and of those recorded by others who have studied the same problem from other angles, raises a question of primary importance.

Is local immunity specific or non-specific?

Cobbett and Melsome (1898) found that a local non-specific inflammatory reaction, such as that following the injection of mustard oil, would effectively

protect the skin of a rabbit's ear against subsequent attempts to produce experimental erysipelas.

Amoss and Bliss (1927) noted that the localized resistance following an erysipelatous lesion was not sharply specific. It was effective against strains of hæmolytic streptococci antigenically dissimilar from that producing the initial lesion, and to a less extent against *Staphylococcus aureus*. Rivers and Tillett (1925), studying the properties of the serum derived from the rabbits in which they had produced a localized antistreptococcal immunity, found that such immune serum injected into the skin 24 to 48 hours before the injection of living streptococci into the same site rendered this site relatively refractory to infection. Similar results were, however, obtained by the injection of normal serum, or of ordinary nutrient broth, though the protection in this case was less effective and quite non-specific. Mallory and Marble (1925) prepared filtrates from cultures of staphylococci and streptococci. With these they infiltrated localized areas in the skin of a rabbit, and found that such treatment conferred a strictly localized but non-specific immunity lasting for 5-6 weeks. This increased resistance was, however, little if any greater than that conferred by the infiltration of the skin with sterile broth. Analogous results have been recorded by Miller (1927), Freedlander and Toomey (1928), Hanger (1930) and Toomey and Freedlander (1931).

Freedlander and Toomey carried out a detailed histological study of the reactions induced in the cutaneous tissues by the application of compresses of sterile broth, and of the reactions that followed the subsequent injection of *Staphylococcus aureus* into these treated areas. They found that the application of the broth compresses to the abdominal wall of the guinea-pig, for a period of 48 hours, produced a localized œdema, a marked proliferation of histiocytes and a moderate exudation of polymorphonuclear and small mononuclear cells. The cellular response to the subsequent injection of *Staphylococcus aureus* differed widely in treated and untreated animals. In the broth-prepared animals there was an increase in the histiocytes and fibroblasts with a dense exudation of polymorphonuclear cells, which showed little tendency to degenerate. The histiocytes phagocytosed the staphylococci at an early stage of the reaction, while the proliferating fibroblasts tended to wall off the lesion. In the control animals the œdema following the bacterial infection was more marked, there was little proliferation of histiocytes or fibroblasts, and the polymorphonuclear cells, which collected in considerable numbers during the earlier stages of the reaction, showed a marked tendency to undergo degeneration at a later period. The reaction was in general more severe and destructive and far less localized.

A localized non-specific immunity of this type is by no means confined to the skin.

Pfeiffer and Isaëff (1894) showed that the injection of such materials as broth, peptone solutions or urine into the peritoneum of the guinea-pig would so alter the local conditions that the animal became resistant to the intraperitoneal injection of living cholera vibrios in doses fatal to untreated control animals. It is not justifiable to regard this particular non-specific immunizing reaction as local, in the same sense as the cutaneous reactions considered above, since material injected into the peritoneum is rapidly transferred to the blood stream, and a generalized non-specific response of the type discussed in Chapter XIII may well be concerned, in addition to any possible local effect.

Gay and Morrison (1923) injected various substances intraperitoneally into rabbits, and injected living streptococci 24 hours later. They found that certain substances, such as meat-extract broth, afforded marked protection, while others, such as aleuronat, did not. They noted that the substances which conferred protection were those that produced exudates in which macrophages were particularly numerous, while those that produced exudates containing numerous polymorphonuclear cells but few macrophages were far less effective.

These observations, in that they relate the degree of resistance produced to the character of the local cellular response, are more nearly comparable to findings recorded in experiments on non-specific immunization of the skin.

Is local immunity necessarily associated with a cellular or tissue immunity in the strict sense?

There is, as has been pointed out above, no necessary correlation between local immunity and cellular immunity in the sense here implied—an immunity which belongs to the cell as such apart from its immediate environment and, in particular, apart from any antibodies which may be present in the tissue fluids in its immediate neighbourhood. A cellular immunity might well be widespread in its effects: a local immunity might be due to a variety of factors other than an acquired resistance of particular cells. The existence of a cellular type of immunity will be discussed again in relation to virus infections. In the meantime it will suffice to note that none of the experimental data so far recorded in regard to localized immunity require us to assume that a strictly cellular immunity is involved.

We may note also that an immunity depending on specific antibodies may quite well have an effect on local cellular reactions. We have seen, in discussing the phenomena of anaphylaxis, that antibody is in some way taken up by many of the body cells, and that these cells then react specifically to contact with the corresponding antigen. We do not know what part this fixed antibody plays in the defence mechanism of the tissues; but our ignorance is no reason for assuming that it plays no part at all.

SUMMARY

The evidence available appears to justify the following conclusions.

(1) By the injection of various materials into the tissues, and by certain analogous procedures, it is possible to induce an immunity which is confined to the neighbourhood of the treated area, and is not shared by the body as a whole.

(2) This immunity appears to be distributed in accordance with the area involved in the original inflammatory reaction, not in accordance with the distribution throughout the affected part or throughout the body generally of one particular type of cell. There is good evidence of a localized immunity

affecting an *area* of skin, or a particular serous sac. There is no adequate evidence of an immunity affecting the skin as a whole, or the intestinal mucosa as a whole, or serous surfaces as a whole.

(3) It follows that we cannot accept the conclusion that a localized immunity of the latter type is an essential factor in a general immunity. Even had an induced skin immunity, or mucous membrane immunity, been shown to exist, our knowledge of the general defence mechanisms of the body would prevent us from regarding such a local increase in resistance as the essential factor in antibacterial immunity.

(4) The problem of local immunity is quite distinct from that of cellular, as opposed to humoral, immunity. There is nothing in the recorded data with regard to local immunity that necessitates the assumption of an immunity of the purely cellular type.

(5) All the evidence suggests that non-specific factors are of primary importance in those instances of local immunity that have been submitted to experimental study. It would appear to be the initial inflammatory reaction as such, and the cellular changes which persist for some time after it has subsided, that determine the relative resistance of the treated area to subsequent experimental infections. In particular it would seem that any treatment which induces a local mobilization and concentration of histiocytes will confer on the treated area an increased resistance, which will last as long as the local cellular changes persist.

(6) When a localized area is repeatedly treated with a particular bacterium, a general immunity is often superimposed on the local immunity. There is nothing in the evidence at present available to suggest that such general immunity, in so far as it is specific, differs essentially from the specific antibacterial immunity that we have considered in earlier chapters.

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CHAPTER XV

THE INFLUENCE OF DIET, FATIGUE, CHANGES IN TEMPERATURE AND HUMIDITY, CHEMICAL AGENTS AND OTHER FACTORS ON GENERAL OR LOCAL IMMUNITY

THE INFLUENCE OF DIET ON IMMUNITY.

It will be convenient to discuss first the effects produced by deficiency or excess of those accessory food factors that have come to be grouped together under the name of *vitamins*.

Variations in the Intake of Vitamin A.—In 1909 Knapp described a curious condition of the eyes developing in rats fed on a deficient diet. This condition (xerophthalmia) is characterized by a thickening and drying of the superficial epithelium covering of the eye, patchy in distribution, and associated in its later stages with a subacute bacterial infection of the underlying tissues. Knapp did not ascribe these lesions to any specific dietary deficiency; but we know now that the essential factor in its causation is lack of the fat-soluble Vitamin A, and Knapp's protocols show that the diet he employed was, in fact, deficient in this constituent. Similar lesions have since been described by many other observers, not only in the rat but in other animals, such as the rabbit (Nelson and Lamb 1920, Boock and Trevan 1922) and the dog (Steenbock, Nelson and Hart 1921). Particularly interesting is the account of Bloch (1921, 1928) of the occurrence of numerous cases of xerophthalmia in Danish children during the Great War, when the supply of animal fats to the general population, and in particular to the dwellers in large towns, was greatly restricted. The increased susceptibility to bacterial infection that results from Vitamin A deficiency is, however, by no means limited to the superficial tissues of the eye. In describing the condition of the children in Copenhagen Bloch remarks: "It is absolutely characteristic of these dystrophic children how little they are able to withstand infections, and how quickly they die of serious intercurrent fever." The most important killing disease that developed among them was broncho-pneumonia. Blegvad (1923) records the following incidence of serious complicating infections among the cases of xerophthalmia—broncho-pneumonia 63, bronchitis 45, pyuria 42, otitis media 30. These clinical observations are in entire accord with the results obtained in dietary experiments on animals.

The liability of rats fed on a diet deficient in Vitamin A to develop intercurrent infections, such as glandular abscesses, suppurative arthritis, suppurative otitis, pyelonephritis and broncho-pneumonia, has been noted by many

observers (Drummond 1919, Hess *et al.* 1921, Cramer 1923, 1924, 1927, Gross 1924, Wolbach and Howe 1925, Green and Mellanby 1928, 1930, Gudjónsson 1930). The more precise knowledge that has accrued within recent years in regard to the chemical nature of certain of the vitamins has made it possible to demonstrate clearly that the fat-soluble vitamin concerned is A, not D (see Goldblatt and Benischek 1927, Green and Mellanby 1928); and to establish, in this respect as in others, the functional similarity of Vitamin A and carotene (Green and Mellanby 1930).

These observations on the increased frequency of spontaneous infections have been confirmed and extended by testing the resistance of animals kept on an A-deficient diet to experimental infection (Werkman 1923*b*, Hotta 1928, Reiter 1929, Lassen 1930, 1931, 1932, McClung and Winters 1932, Greene 1933). Lassen's studies are of particular interest, since the course of infection in the avitaminotic and control animals was followed in considerable detail. Rats were reared on a diet lacking Vitamin A, and were then infected, together with control rats of the same age fed on a complete diet, by administering living cultures of *Bact. aertrycke* by the mouth, or by subcutaneous or intravenous injections. The avitaminotic rats showed a significantly lower resistance than the normal controls. In the experiments in which the bacilli were administered by the mouth, the course of the infection was studied by the technique employed by Ørskov, Jensen and Kobayashi (see p. 122). The rats were killed at various intervals after infection and cultures taken from the intestines, Peyer's patches, mesenteric glands, liver, spleen, lungs and heart's blood. In the normal rats the infection tended to be limited to the Peyer's patches and mesenteric glands, with some extension to the liver and spleen. In the avitaminotic rats it tended to become generalized, and to produce a fatal bacteræmic infection. In the few experiments in which the bacilli were injected intravenously, the avitaminotic rats showed the same initial clearing of the blood stream as did the normal controls, but in the former this initial clearance was followed by a secondary bacteræmia and a fatal generalization of infection.

By adding irradiated ergosterol to the Vitamin A-deficient diet and noting that this procedure did not prevent the fall in resistance to experimental infection, Lassen (1931) was able to show—as Green and Mellanby had done in the case of spontaneous infections—that the effect of removing all fat-soluble vitamins from the diet was due to the absence of Vitamin A.

It may be noted, and this is a point of some importance, that Lassen found it necessary to induce a pronounced degree of avitaminosis before he could demonstrate a well-marked break in resistance. He states that a slight decrease in resistance occurs after 2 to 3½ weeks on a Vitamin A-free diet, at a time when the animal shows no clinical evidence of avitaminosis, but that the clear and significant fall in resistance sets in suddenly at a later stage, coincidentally with the appearance of xerophthalmia.

The mechanism that underlies the decreased resistance of animals deprived of Vitamin A is as yet far from clear. Cramer (1923, 1924) has described atrophic changes in the intestinal mucosa of avitaminotic rats, and believes that the lesions facilitate the passage of bacteria from the intestines to the tissues. Wolbach and Howe (1925) record striking changes in the surface epithelium in certain situations though they locate these lesions in the lining membranes of the nares, trachea, bronchi and genito-urinary tract, rather

than in the intestine. These observations would suggest that the increased susceptibility to infection associated with A-avitaminosis may be due, in part at least, to a failure of the first line defence mechanisms discussed in Chapter IV; but such a view is difficult to reconcile with other evidence on record. Thus Lassen (1931) found no difference in the rate at which bacteria passed from the intestine to the mesenteric glands *via* the Peyer's patches and lymph follicles, as between normal and avitaminotic rats. It was in the subsequent course of events—the fatal generalization of infection as opposed to its successful localization—that the avitaminotic animals displayed their lack of effective resistance. Moreover (Hotta 1928, Reiter 1929, Lassen 1930, 1931, 1932) such animals are more susceptible than normal controls to the subcutaneous, intraperitoneal and intravenous injection of bacteria—in which the first line of defence is artificially ruptured—as well as to the administration of pathogenic bacteria by the mouth.

It would seem, therefore, that there must be some defect in the defence mechanisms of the tissues—in the efficient functioning of the reticulo-endothelial system itself, or in the conditioning or accessory action of the humoral antibodies—but of the nature of that defect we have as yet no knowledge. It will be recalled in this connection that most workers have failed to find any satisfactory evidence that deprivation of Vitamin A—or indeed of any other vitamin—reduces the efficiency of antibody production in response to a specific stimulus (see Zilva 1919, Werkman 1923*a, b*, Werkman *et al.* 1924, Cramer and Kingsbury 1924), though Greene (1933) records an apparently significant decrease in the ability of A-avitaminotic rabbits to produce haemolysins for sheep's red cells. Lassen (1931, 1932) notes that the sharp difference in resistance between normal and avitaminotic rats cannot be demonstrated when both groups have been previously immunized by the injection of a heat-killed vaccine, which indicates that the antibody-producing mechanism is still functioning with effect.

It may be noted, as further evidence that some deep-seated break in resistance is involved, that observations have been recorded suggesting that the deprivation of Vitamin A renders animals more susceptible to the injection of bacterial toxins, as well as to the invasive action of living bacterial cells (Werkman, Baldwin and Nelson 1924, Schubert 1928).

We have been concerned so far with a comparison between animals fed on a normal diet and those deprived of Vitamin A. The former have clearly an advantage; but it is by no means a necessary corollary that an excess of Vitamin A will raise the resistance above normal level.

There are as yet no adequate experimental or field data on which a sound opinion on this further problem can be based. It is intrinsically a difficult one, since it really resolves itself into comparisons of resistance over a wide range of different levels of Vitamin A intake. The amount that is required for normal growth may fall short—even if not far short—of the amount required for optimal resistance. But no one has yet defined the optimal level of intake, as determined by the effect of minor or transient deficiencies.

Among other experiments that have a bearing on this point are those of Webster and Pritchett (1924) on the influence of diet on susceptibility to experimental infection with *Bact. aertrycke*. They compared the resistance of mice fed on the ordinary diet of the Rockefeller Institute (a daily ration of bread, soaked in fresh, pasteurized, Grade B milk, supplemented by two weekly feedings of dog-biscuit) with that of mice fed on a modified McCollum diet (67.5 per cent. milk powder, 5 per cent. butter fat, 1 per cent. sodium chloride and 1.5 per cent. calcium carbonate). Mice kept on these diets were tested, in groups of 10–36 animals, by the injection through a stomach tube of 2–5,000,000 *Bact. aertrycke*. The results indicated that the mice fed on the McCollum diet were signific-

antly more resistant than those fed on the ordinary laboratory diet. Pritchett (1927) records a more numerous series of experiments along similar lines, in which an attempt was made to assess the influence of the different constituents of the McCollum diet. The increased resistance of the mice fed on this diet was in general confirmed; but the effects produced by varying its constitution were not easy to interpret. For instance, the entire withdrawal of butter fat—the only source of Vitamin A—left the mice more resistant than the mice on the ordinary laboratory diet, though less resistant than those on the full McCollum diet. On the other hand the addition of butter fat to the normal laboratory diet was followed, in four of seven trials, by a lowering of mortality as compared with the control group; and a similar result was obtained by the addition of cod-liver oil. In a later series of experiments on the effect of diet on the epidemic spread of mouse typhoid Webster (1930) records results from which he concludes that the substitution of the McCollum diet for the ordinary laboratory diet increases the resistance of mice to contact infection.

In another series of observations along similar lines (Topley, Greenwood and Wilson 1931) different results were obtained. Mice fed on a basal diet of whole-wheat flour, casein, butter and a salt mixture showed no advantage over mice fed on a diet of whole oats, milk and water as regards their ability to resist infection during an experimental epidemic of *Bact. aertrycke* infection. The addition of an excess of fat, butter or lard, or of a Vitamin A concentrate to this diet appeared to react unfavourably on the mice at risk. The unfavourable action of these artificial diets was not manifest when the mice were infected by intraperitoneal inoculation. Under these conditions there was a suggestion that the mice fed on the usual laboratory diet of oats, milk and water were slightly less resistant than the other groups, but the differences observed were quite insignificant. In order to avoid the possible complication introduced by the excess of fat in the vitamin-rich diets, other experimental epidemics were initiated in which the vitamin content of the normal laboratory diet was increased by the addition of a ration of carrot or of cabbage. The mice receiving carrots appeared to be at a disadvantage as compared with those on the normal diet; the addition of cabbage produced no significant change in mortality. It may be noted that Lassen (1931) records that a considerable excess of shark-liver oil in the diet caused a slight decrease in the resistance of rats to experimental paratyphoid infection.

These experiments are considered here because some of them at least have a bearing on the effect of an excess of Vitamin A over the amount required to maintain growth and apparent well-being; but it would in truth be unwise to lay much stress on their significance, since it is clear that the diets employed varied in many ways besides their differences in Vitamin A content. It does, however, seem fair to conclude that no adequate experimental evidence has yet been produced that an excess of Vitamin A over the level required to prevent all overt signs of avitaminosis induces an increased resistance to bacterial infection.

We have been concerned above solely with resistance to the inception of spontaneous or experimental infections—with the prophylactic aspect of variations in a particular dietetic factor. If it could be shown that an excess of Vitamin A significantly increased resistance in this sense, it would be arguable that its administration might produce a therapeutic effect in already established infections.

Mellanby and Green (1929) have recorded a few observations from which they conclude that such an effect is in fact exerted in the particular case of puerperal fever. Confirmatory evidence is at present lacking. Thomas (1931), in a detailed study of puerperal sepsis in Glasgow, records that 86 patients were treated by the administration of a Vitamin A concentrate. Neither in

the milder nor in the frankly septicæmic cases was any beneficial effect noted.

Orenstein (1932) has failed to find any evidence that the administration of a highly potent preparation of Vitamin A has a beneficial effect on cases of pneumonia occurring among the native workers on the Rand mines. Over a 1-year period alternate cases were treated with this preparation. Of 375 treated cases 10·13 per cent. died; of 389 untreated cases 10·28 per cent. died.

Variations in the Intake of Vitamin B.—It would appear both from clinical records and from experimental studies that a deficiency in the intake of Vitamin B—the anti-neuritic vitamin—has no effect on resistance to infection at all comparable to that produced by a lack of Vitamin A. There have been suggestions that B-avitaminosis is associated with a minor increase in susceptibility to infection, but the evidence is of very doubtful significance (see Werkman 1923, Werkman, Baldwin and Nelson 1924, Lassen 1929, 1931, 1932).

Variations in the Intake of Vitamin C.—The evidence with regard to the anti-scorbutic vitamin on resistance is peculiarly confusing and discrepant.

On the clinical side Hess (1920) stresses the frequency of infective complications in scurvy, while Bloch (1928) states that scorbutic children show no tendency to infection in any way comparable to that displayed by children suffering from xerophthalmia.

As regards spontaneous infections in guinea-pigs suffering from experimental scurvy, Hamburger and Goldschmidt (1923), Grant (1926–30) and Schmidt-Weyland and Költzsch (1927) state that they are very common. On the other hand, Holst and Frolich (1912) state that they are uncommon. The observations of Glenny and Allen (1921) on the occurrence of epidemic infection among a stock of guinea-pigs on a vitamin-deficient diet, and its suppression by the addition of a ration of green stuff, may perhaps be taken as relevant evidence, though we clearly cannot assume that C-avitaminosis was the determining factor.

As regards the resistance of scorbutic guinea-pigs to experimental infection, Findlay (1923), Werkman, Nelson and Fulmer (1924), Grant (1926) and Schmidt-Weyland and Költzsch (1927) record a slight increase in susceptibility. Findlay would ascribe this to the degenerative changes and feeble leucoblastic reaction seen in the bone-marrow of guinea-pigs suffering from chronic scurvy. Werkman, Nelson and Fulmer stress the association between lowered resistance and lowered body temperature.

The observations of Zinsser and Castaneda (1931) on the increased susceptibility of guinea-pigs and rats on a vitamin-deficient diet to experimental typhus infection may be referred to here. It is probable that the guinea-pigs were suffering from some degree of C-avitaminosis; but the fact that rats, which are generally regarded as being relatively insensitive to the deprivation of this vitamin, showed the same decrease in resistance makes it difficult to assess the significance of these results.

Variations in the Intake of Vitamin D.—The similar behaviour of Vitamin A and the anti-rachitic vitamin as regards their fat-solubility make many of the earlier observations on the effect of a Vitamin D-deficient diet of doubtful significance. Certain of the experimental studies that we have summarized above show that the addition of Vitamin D to a diet from which the fat-soluble vitamins have been removed does not prevent the lowering of resistance: indeed there is a suggestion in some of them (see Lassen 1931) that the addition of irradiated ergosterol increases the susceptibility of the animals receiving it. In other experiments Lassen found that rats on a rachitogenic diet not entirely lacking in Vitamin A showed no appreciable lowering of their resistance to experimental infection *per os*, even when the macroscopic and histological evidence of rickets was clear and unmistakable; and Freund (1932) was unable to demonstrate any significant difference in the resistance of rats and mice maintained on normal, Vitamin D-free or Vitamin D-excess diets.

If we attempt to assess the significance of the data at present available with regard to the influence of variations in vitamin intake on resistance to infection, we shall probably conclude (1) that there is good evidence that gross deficiency of Vitamin A produces a serious lowering of resistance; (2) that it is doubtful whether the addition of any extra source of Vitamin A to an ordinary unrestricted human or animal diet is likely to lead to any significant increase in resistance, but that this question must await the answer of properly controlled trials carried out on an adequate scale; (3) that the evidence with regard to the effect of Vitamin C deficiency is so divergent that no sound conclusion is possible at the moment, though there are definite suggestions that animals such as the guinea-pig, that are peculiarly susceptible to the deprivation of this vitamin, are rendered more susceptible to infection when it is withheld; and (4) that there is at present no satisfactory evidence that either Vitamin B or Vitamin D are in any way associated with the factors that determine resistance or susceptibility.

The Influence of Other Dietary Factors on Resistance.

Without making any attempt to review in detail the scattered, but not inconsiderable literature dealing with the possible or probable effects of dietetic variations of a broad and as yet unanalysable kind on resistance to infection, a few recent observations may be briefly summarized, as pointers to the lines along which inquiry is proceeding.

First, perhaps, may be mentioned studies of a quite general kind, in which an attempt has been made to establish preliminary correlations that will serve to define problems rather than to answer them. In this category fall the studies of Orr and Gilks (1931) on the health of two African tribes—the Masai and the Kikuyu—and correlated studies (Orr, MacLeod and Mackie 1931) on the effect of nutritional variations on the resistance of sheep. The Masai, particularly the males, live mainly on a diet of milk, meat and raw blood. The Kikuyu live almost entirely on cereals, roots and fruits. Apart from a marked difference in physique—the adult male Masai is 5 inches taller, 23 lb. heavier and 50 per cent. stronger than the Kikuyu—there appears to be a significant difference in the frequency of certain types of infective disease; though the facilities for obtaining statistical data among native tribes are not of the kind that allow the collection of entirely convincing evidence. With this reservation, it would seem that pulmonary infections (bronchitis, pneumonia and phthisis) and tropical ulcer are far more frequent among the Kikuyu than among the Masai, while the latter are more prone to arthritis, presumably of an infective type. This difference in susceptibility to particular kinds of infection is associated with a low calcium content of the blood in the Kikuyu (9.4 mg. per 100 c.c. as against 11.4 mg. among a small sample of Europeans in Kenya and 10–10.5 mg. among Europeans in general). Samples of Masai blood could not be obtained on account of a tribal tabu. In the experiments on sheep, which were carried out in Scotland, animals were allowed to graze on different types of pasture, or fed on different diets in pens, and studies were made of the blood calcium, blood phosphorus, and (as an index of immunity *faute de mieux*) of certain of the natural agglutinating or lytic antibodies. The results, as one would expect, are by no means easy to interpret; but there appears to be a definite correlation between ample pasturage, a high calcium and low phosphorus content of the blood, and general well-being, and there is a suggestion that these conditions are associated with active lytic properties of the serum, while the agglutinins in certain experiments appear to have varied inversely as the lysins. In later experiments (Mackie *et al.* 1932) a slight indication was obtained that the animals on the poor pasture were more susceptible than controls to the intradermal injection of the toxin of the lamb dysentery bacillus.

The possibility that variations in the intake of mineral salts may have an influence on resistance has been explored by various experimental workers (Zilva 1919, Lange 1925, 1927, Pritchett 1927, Hotta 1928, Klieger and Geiger 1928, Mayer and Sulzberger 1931, Sulzberger and Mayer 1931) but the evidence is highly confusing. We can, at the moment, do little more than suspend judgment, and note that a question has been asked, but not answered.

We may pass to the effect of a general deficiency of food, apart from qualitative changes. We are here in little better case, so far as experimental evidence is concerned. Hotta (1928) records a definite decrease in the resistance of mice kept on a starvation diet, while Lassen (1931) finds only a trivial decrease in resistance of rats suffering from a relative but well-marked inanition for a period of 17 weeks (see also Müller and Simons 1919, Bieling 1925, Bickert 1931).

Apart altogether from their effect on the defence mechanisms of the tissues, there can be no question that variations in diet alter the local conditions in the gastro-intestinal tract. We know (see Rettger and Cheplin 1921, Dudgeon 1926) that the ingestion of certain foodstuffs produces characteristic changes in the gastro-intestinal flora; and Arnold (1926, 1927*a*, *b*, 1929) has stressed the importance of various food materials in deranging or maintaining the efficacy of the gastro-duodenal bactericidal mechanism, particularly in infants. He suggests that such results as those recorded by Webster and Pritchett (1924) and Pritchett (1927) may be due in part to effects of this kind.

THE INFLUENCE OF FATIGUE ON RESISTANCE

In spite of the abundant clinical experience that establishes beyond question the conclusion that bodily rest is an important factor in the treatment of any infective disease, the experimental evidence with regard to the effect of fatigue on resistance to infection is more than a little puzzling.

Spaeth and his colleagues (Oppenheimer and Spaeth 1922, Nicholls and Spaeth 1922, Spaeth 1925) have published a series of observations carried out on rats and guinea-pigs, from which they conclude that the resistance of these animals to tetanus toxin, and to experimental infection with the pneumococcus, is increased as the result of the fatigue induced by making the animals run for a long period in a revolving cage. Friedberger and others (1931) have injected rats and guinea-pigs intraperitoneally with *El Tor* vibrios and have compared the fate of resting animals with that of others fatigued before, or after, or both before and after, the inoculation. There was no significant difference in mortality among the different groups. In the same series of experiments these authors studied the effect of fatigue on antibody production. The results were entirely negative. Such observations must clearly be interpreted conservatively, and without uncritical generalization. They emphasize the stability of the defence mechanisms of the tissues under a variety of different stresses; but they do nothing to lessen the significance of our common experience that fatigue is harmful in established infections, and it would be very unwise to conclude from them that fatigue has no influence on the liability to contract an infection under natural conditions.

More illuminating are the experiments recorded by Boycott and Price-Jones (1926). It may be noted that the infection they studied—that produced by *Bact. enteritidis* in the rat—is a natural disease of that animal, and therefore far more analogous in its pathogenesis to natural human infections than are the experimental conditions selected for study by the workers referred to above. When rats were infected with *Bact. enteritidis* by subcutaneous or intraperitoneal inoculation, fatigue induced before the inoculation, or during the first few days after it, appeared to have very little effect on the subsequent mortality rate. But when the rats were infected by feeding and then fatigued on several occasions during the subsequent few days the results were quite different. Twenty-seven rats were treated in this way and of these 13 died. Twenty control rats were fed on the same cultures, but were not subsequently fatigued; of these none died. The animals that survived the whole period of observation were submitted to necropsy, and it was found, in conformity with the findings recorded by many other observers, that the

majority of them were still harbouring *Bact. enteritidis* in the spleen. There is an obvious suggestion that fatigue has a significant effect in activating a latent infection and favouring its fatal generalization.

THE EFFECT OF VARIATIONS IN TEMPERATURE, HUMIDITY AND OTHER PHYSICAL CONDITIONS OF THE ENVIRONMENT

The evidence available under this head is concerned mainly with the effect of these environmental factors on the first-line defence mechanisms of the kind considered in Chapter IV. Arnold and his colleagues (Arnold 1927*b*, 1928, 1929, Arnold and Brody 1927) have studied in considerable detail the effect of high temperature, especially when associated with high humidity, on the gastro-duodenal bactericidal mechanism of the dog. They find that such conditions are associated with a decrease in gastric secretion, a consequent fall in the acidity of the gastric and duodenal contents, and an increased passage of bacteria from the stomach or duodenum to the cæcum.

There are in the literature many other records of experiments on the effect of temperature and humidity on resistance to infection (for recent papers see McDowell 1923, Kligler and Olitzki 1931, Robertson and Weld 1932), but the numbers of animals in the comparable groups are so small, and the effects of these two factors seem so difficult to disentangle, that the significance of the results reported cannot be assessed.

Hill and Muecke (1913) have shown that the mucosa of the human nose becomes turgid with blood and covered with a thick, mucous secretion when subjects are exposed to a warm, moist atmosphere, and that when such subjects pass immediately to a cold atmosphere the mucosa pales quickly as the result of a constriction of the vessels, but remains swollen and œdematous for a considerable period. Mudd and his colleagues (1921) have found that chilling of the body surface is associated with a marked fall of temperature in the nasal and post-nasal cavities, and that the return to the normal temperature in these situations is at first rapid, but then very slow, when the body surface is again warmed. There is, then, good evidence of significant changes in the physiological conditions of the nose and nasopharynx in response to changes in temperature and humidity, and it may well be that some of these changes are associated with a lowering of the local resistance; but such a correlation will clearly be very difficult to establish by any statistical survey of naturally occurring infections, and we have as yet no experimental data bearing directly on this point.

Among other physical agencies mention may be made of the possible effect of ultra-violet irradiation of the body surface since this procedure has been advocated as a means of increasing resistance to infection. We have noted (p. 227) that Colebrook, Eidenow and Hill (1924) have demonstrated a temporary increase in the bactericidal power of the blood as the result of exposure to ultra-violet rays, and a similar effect after exposure to dark heat, or to the blistering of the skin with a mustard plaster. The general experience of the beneficial effect of sunlight as enjoyed under optimal natural conditions, combined with such observations as the above, made it natural to inquire whether any isolated constituent of the solar radiation, and particularly the rays of short-wave length, would produce a significant increase in resistance to infection.

Some favourable views have been recorded (Maughan and Smiley 1928, 1929, Hill and Laurie 1931); but the balance of evidence appears to be very definitely against the view that exposure to such rays has any significant effect. Barenberg and Lewis (1928) report entirely negative results in a small group of infants. Colebrook, D. (1929), in a very careful and detailed study carried out on school children of 5-7 years of age, compared the frequency of infection in a group of 101 children irradiated 3 times a week with light from a carbon arc lamp with a high output of ultra-violet rays; in a second group of 94 children irradiated under the same conditions except that light of wave-length shorter than 3,200 A.U. was cut off by a glass screen; and in a third group of 92 children who received no irradiation. Over the period of observation—August 1927 to

March 1928—the number of colds per 100 child-weeks' exposure to risk was 20.2 in the group irradiated with the unscreened lamp, 18.7 in the group irradiated with the screened lamp, and 18.6 in the unirradiated group. The average duration of each cold was 7.5, 7.5 and 7.1 days respectively. The incidence of measles among those not previously attacked—a small group—showed no significant difference between the treated and untreated: the attack rates were 80.0 per cent. in those irradiated without screening, 70.6 per cent. in those irradiated but screened, and 68.2 per cent. in the unirradiated controls. In the case of whooping cough the corresponding attack rates were 8.8 per cent., 7.7 per cent. and 8.6 per cent. In no case did the irradiated group show any benefit. A later report by Doull, Hardy, Clark and Herman (1931) is entirely confirmatory. It deals with a careful study of the frequency of colds among irradiated and unirradiated groups of volunteer students, or members of the staff, at Johns Hopkins University. Every statistical precaution was taken in planning the trial and in assessing its results. These were unequivocal. Among 169 persons in the irradiated group, the attack rate during the period September 29, 1929 to May 31, 1930 was 78.6 per 1,000 student-weeks, among 184 persons in the unirradiated control group it was 71.9. Whatever test was employed—duration of colds, the presence of fever, the presence of cough or sputum—or whatever allowance was made for previous history or differences in physical conditions, the result was the same, the irradiated group showed no advantage.

The experimental data available are, so far as they go, in accord with field observations. Chapman and Hardy (1931) were unable to obtain any evidence that irradiation increases the resistance of rabbits to the subcutaneous injection of *Pasteurella lepi-septica*. Hill, Greenwood and Topley (1930) found that irradiation conferred no benefit on mice submitted to contact infection with *Pasteurella muriseptica*.

SEASONAL VARIATIONS IN RESISTANCE

Seasonal variations in the frequency of particular infective diseases are a commonplace of epidemiology. How far such variations are dependent on fluctuations in the average resistance of the host species, how far on environmental factors that increase the opportunities for the spread of infection, and how far on factors the nature of which we cannot even guess, are problems to which we can as yet give no answer.

Observations are on record from which the conclusion has been drawn that seasonal changes in the efficacy of the normal defence mechanisms do in fact occur (see Pritchett 1925, 1926, Glenny and Waddington 1928, Blake and Okell 1929, Mayer and Sulzberger 1931, Sulzberger and Mayer 1931, Orr, MacLeod and Mackie 1931). Wilson (1930) has noted synchronous fluctuations in the mortalities following the injection of three different strains of *Bact. aertrycke* into mice during a sixteen months' period, each strain being tested on 15 occasions. It appeared impossible to relate these synchronous changes in mortality to any common factor acting on the three bacterial strains, and it seemed difficult to escape from the view that they must have been due to synchronous transient fluctuations in the resistance of the mice, but these fluctuations showed no definite seasonal distribution.

Even if the occurrence of seasonal variations in resistance were clearly established, it would be an exceedingly difficult task to disentangle the various factors involved; and without such disentanglement there could be little hope of intelligent interference. The vitamin content of foodstuffs, and many other dietetic variables, are subject to seasonal influences; the condition of the nasal mucosa and the efficiency of the gastro-duodenal bactericidal mechanism are, as we have seen, affected by changes in temperature and in humidity; and there is little doubt that many other types of physiological response, of which as yet we know little or nothing from the immunological viewpoint, may be subject to influences of a similar kind. Until our knowledge of the relative significance of these factors, working in isolation, is much more accurate and detailed than it is at present we can hardly hope to construct a useful working chart of the total effect of the environmental changes associated with the changing seasons.

THE EFFECT OF CERTAIN CHEMICAL SUBSTANCES IN FAVOURING BACTERIAL INFECTION

We may close this chapter with a brief survey of a series of observations that have, during recent years, focussed attention on certain secondary factors that play a significant part in the local pathogenesis of infective lesions. It is becoming increasingly clear that some of these factors exert an important influence on the frequency and severity of naturally occurring infections, particularly in industrialized communities.

As a first example we may cite the observations of Gye (Bullock) and Cramer (1919) on the effect of calcium salts in certain anærobic infections, and the more recent studies of Fildes (Fildes 1927, 1929*a, b*, Knight and Fildes 1930, Campbell and Fildes 1931). Gye and Cramer found that ionizable salts of calcium, inoculated together with the washed spores of *Cl. welchii*, of *Cl. tetani* or of certain other anærobic bacteria, led to the development of the corresponding infections in their most typical and fatal forms, while the washed spores alone showed no tendency to develop in the tissues after inoculation. The chlorides of sodium, potassium, ammonium, strontium or manganese had no such effect. They were unable to determine the exact mechanism by which this effect, to which they gave the name *kataphylaxis*, was brought about; though they were able to show that it was not due to an absence of leucocytes from the lesions, nor to a lack of phagocytic activity on the part of the cells that collected at the site of inoculation. Fildes (1927), attacking the particular problem of experimental tetanus in the guinea-pig, studied the vegetation of spores in the normal testicle, after ligation of the blood vessels. He found that the injection of tetanus spores into the ligated testicle was followed by a rapidly fatal toxæmia, indicating rapid vegetation in the tissues. Comparison of sections from ligated and unligated testicles, at various intervals after the injection of the spores, showed that active vegetation had occurred in the ligated testicle at a period when emigration of leucocytes was at its earliest stage in the unligated testicle, and therefore before any significant degree of phagocytosis could in any case have occurred. He concluded that there must be some definite stimulus to vegetation in tissues cut off from their blood supply, either by ligation of vessels or by the vascular injury following the injection of calcium salts, and suggested that this stimulus was probably the result of the diminished oxygen tension, although Gye and Cramer had tentatively rejected this view. Later (Fildes 1929*b*) he carried out a detailed study of the oxidation-reduction potential of the tissue fluids under different conditions using suitable indicator dyes. He found that oxidized methylene blue injected into the subcutaneous tissues of the living guinea-pig is not reduced, while reduced methylene blue is rapidly oxidized. In the dead animal the oxidized methylene blue undergoes a slight reduction, while the reduced methylene blue is not oxidized. Analogous results were obtained with thionin and with indophenol I. The general conclusion may be drawn that, in the subcutaneous tissues of the living guinea-pig, the oxidation-reduction potential (Eh) at the prevailing pH of about 7.0, is in the neighbourhood of +0.12 volts, while, as shown in a previous communication (Fildes 1929*a*), tetanus spores will not germinate readily at a poten-

tial more oxidizing than $+0.01$ volts at this pH (see also Knight and Fildes 1931). In further experiments it was shown that the injection of solutions of calcium chloride led to the production of localized areas of oxygen deficiency, with an Eh on the negative (reducing) side of that of methylene blue, thus providing conditions suitable for the germination of tetanus spores. Campbell and Fildes (1930) studied the effect of other methods of varying the oxygen tension of the tissues. Guinea-pigs were maintained in atmospheres containing different partial pressures of oxygen (60 per cent., 20 per cent. and 7 per cent.) and in air containing 0.15 per cent. of carbon monoxide. Control experiments showed the corresponding pressures of oxygen in the subcutaneous tissues (in mm. Hg) to be approximately 26, 20, 5 and 6. When animals subjected to these atmospheres were injected intramuscularly with a mixture of tetanus spores and aleuronat the animals maintained in atmospheric air (20 per cent. oxygen) showed a mortality of 45 per cent., those maintained in an atmosphere of 60 per cent. oxygen a mortality of 6 per cent., those maintained in 7 per cent. oxygen, or in air containing 0.15 per cent. CO, a mortality of 75 per cent. These results clearly support the view that the oxygen tension of the tissues controls the germination of the tetanus spores.

Gye and Cramer (1919) noted that the local defence rupture produced by the injection of calcium salts favoured infection with streptococci, as well as with anaerobic bacilli. The nature of the effect has been studied in more detail by Kettle (1927). Here again there is no evidence of any diminished emigration of leucocytes, or lessened phagocytic activity, and the main effect of the calcium, as revealed by histological examination of the lesions, is a striking increase in the rate of bacterial multiplication. As Kettle points out, the streptococcal-calcium lesions in the mouse bear a close resemblance to the rapidly spreading streptococcal cellulitis that occurs in man. It would appear that the characteristic exudative lesion that is caused by the calcium salt provides peculiarly favourable cultural conditions for streptococci, as it does for anaerobic bacilli; though it does not, of course, follow that the two effects are determined by identical chemical or physical factors.

Calcium is not the only substance which produces defence rupture. That silica has an injurious effect on the lungs and that there is an association between silicosis and tuberculosis has been known for some 30 years, and Gardner (1920) has shown experimentally that a dust containing a high percentage of silica has a significant effect on pulmonary tuberculosis in the guinea-pig.

The factors concerned in this enhancement of tuberculous infections by silica have been studied in detail by Kettle (see Gye and Kettle 1922, Kettle 1924, 1926, 1927, 1930, 1932). The majority of these experiments have been carried out on mice. These animals are normally far more resistant to tuberculosis than is the guinea-pig; and the selective action of silica can be readily demonstrated by subcutaneous injection of the material under test followed by an intravenous injection of living tubercle bacilli. Silica, or silica-containing material, is injected at one spot, some other substance to act as a control at another. If the control substance is an irritant, such as calcium chloride or turpentine, the bacilli that are injected intravenously appear in both the silica

and control lesions, but it is in the silica lesion that they show the most active proliferation. The specific action of the silica is clearly evidenced by the fact that the more extensive calcium lesion provides far less favourable conditions for the growth of the tubercle bacillus. It is further evidenced by the results of careful comparative tests on a large number of industrial dusts (Kettle 1932), which show that it is the presence of silica, or of a silicate, that determines the characteristic effect. Crystalline silica, unlike colloidal or amorphous silica, is very slowly dissolved in the tissues, and only when dissolved does it exert its injurious action. Silica crystals coated with oxide of iron are not dissolved in the tissues, and they remain inert and harmless.

Experiments have been carried out in guinea-pigs and rabbits in which dusts have been brought into direct contact with the lung tissue, either by injection into the trachea (Kettle 1930) or by repeated or prolonged exposure to the breathing of an active dust.

An illuminating experiment is recorded by Gardner (1930). Guinea-pigs were infected, by inhalation, with a strain of tubercle bacillus of unusually low virulence, which normally produces in these animals a retrogressive type of tuberculosis confined to the lungs and bronchial glands. The infection is practically never fatal. When animals so infected were exposed for 8 hours a day to quartz dust an entirely different picture was produced. After 3 to 5 months the bacilli in the lungs began to proliferate actively. The infection spread to all parts of the lungs and to the abdominal viscera, producing large and progressive lesions. The bacilli had not become more virulent, since cultures isolated from the dusted animals produced the usual type of retrogressive tuberculosis when administered to normal undusted guinea-pigs. This particular instance of the selective action of a simple chemical substance in promoting the growth of a particular type of bacterium within the tissues is of course of great practical importance, since it is the cause of a serious form of industrial sickness.

Experimental studies of the kind outlined above open up wide fields for further exploration. We may reasonably hope to obtain significant information on many problems by studying the effects of local changes in the chemical constitution of the tissue fluids. Two recent contributions along these lines may be noted.

Findlay (1928) has attempted to test the tentative hypothesis that the localization of pathogenic bacteria, or of filtrable viruses, at the site of localized tissue injury might be the result of the liberation of histamine, or of histamine-like substances, from the injured tissues, leading to a localized dilatation of the capillaries and an increased permeability of the capillary endothelium. Animals of a susceptible species were inoculated intravenously with an infective agent—the virus of fowl-pox, or of vaccinia, or of the Rous sarcoma, *Staph. aureus*, *Strep. haemolyticus*, or the pneumococcus—and histamine acid phosphate in a 0.5 per cent. solution was then injected into a site suitable for the observation of any lesion, the same amount of phosphate-buffer solution being inoculated into a corresponding site on the opposite side of the body to serve as a control. In each of the experiments the lesions appearing at the site of the histamine injections were significantly more frequent and more severe than those appearing at the site of injection of the phosphate-buffer solution. The latter were indeed infrequent in occurrence and minimal in degree.

Rosher (1931) has studied the effect of the growth-promoting factors (X and V) on

experimental infection with *H. influenzae* in mice. When a suspension of influenza bacilli in ordinary nutrient broth was used for intraperitoneal inoculation, 26 per cent. of 45 strains killed one or more of five mice. When 0.05 c.c. of Fildes' solution (containing both growth factors) was added to the inoculum, 71 per cent. of these strains killed one or more mice.

In more recent (unpublished) studies, Rosher has found that the X and V factors, acting together, enhance the toxic action of dead influenza bacilli; so that we are not, in this case, dealing with a pure growth-promotion effect.

SUMMARY

(1) With regard to the effect on resistance of variations in diet, the one fact that stands out clearly is that a gross deficiency in the intake of Vitamin A, especially during the period of rapid bodily growth, is associated with a marked increase in susceptibility to infection. There are suggestions that C-avitaminosis, in animals peculiarly liable to this condition, may be associated with a similar break in resistance; but the evidence is highly confusing. There is no evidence that a deficiency of Vitamin B or of Vitamin D has any significant effect on resistance. There is little if any evidence that an excess of Vitamin A, above the limit required to prevent the occurrence of detectable avitaminosis, has any prophylactic or therapeutic effect in infective disease.

(2) Our knowledge of the influence of other dietetic defects on resistance in general is as yet too scanty to justify any definite conclusions. There is, however, evidence which suggests that the effect of ingested foodstuffs on the local bactericidal mechanisms of the intestinal tract may play a part in the frequency of natural infections by this route.

(3) The experimental evidence with regard to the influence of fatigue on resistance suggests that it is more important as a factor leading to the activation of a latent infection than as predisposing to infection *ab initio*.

(4) The data with regard to the effect of variations in the physical environment are scanty. It is certain that fluctuations in temperature and humidity affect the local conditions in the upper part of the respiratory tract, and it seems possible that the changes produced may in some cases lower the local resistance. There is evidence that similar environmental changes may derange the gastro-duodenal bactericidal mechanism. There is no satisfactory evidence that irradiation with ultra-violet rays produces any significant increase in resistance to natural or experimental infection.

(5) There are suggestions that a seasonal variation may occur in the general resistance to infection. If this be the case, such fluctuations are probably dependent on variations considered in the previous sections; but which factors are concerned or what their relative importance may be we cannot tell.

(6) Evidence is accumulating with regard to the importance of certain chemical substances as agents favouring local infective processes. Thus ionizable calcium salts favour the germination of tetanus spores in the tissues, and this fact is of importance in aiding the natural pathogenesis of tetanus in wounds grossly contaminated with soil. Silica favours the local proliferation

of the tubercle bacillus in the tissues, and is an important predisposing cause to this infection in certain industrial populations. The mechanisms upon which these kataphylactic effects depend have been subjected to detailed study with results of considerable interest and importance.

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CHAPTER XVI

IMMUNITY IN VIRUS DISEASES

WITHOUT making any attempt to review the voluminous literature dealing with virus diseases in general, it is necessary to refer briefly to our present conceptions—based largely on studies carried out within the past few years—of the mechanisms by which these diseases are resisted. There was a tendency among earlier workers to differentiate somewhat sharply between immunity to virus diseases on the one hand and to bacterial infections on the other; and this differentiation involved, mainly by assumption, the mechanisms brought into play as well as the quality and duration of the immunity induced. We may, for our present purpose, ignore most of the earlier literature and much of the more recent, confining our attention to those records that show us where the observed reactions of antiviral immunity fit our picture of immunity to bacterial infections, or where they suggest ways in which that picture must be modified.

Up to the present time our study of virus infections has been beset with technical difficulties. We cannot as yet cultivate any filtrable virus—with the exception of the curious organism of pleuropneumonia which some would exclude from the filtrable-virus class—in a medium completely freed from living tissue cells. Almost all experiments on the transmission of infection, and on immunity reactions *in vivo* or *in vitro*, have of necessity been carried out with tissue extracts or with other crude material containing host products as well as virus. Under these conditions the qualitative or quantitative analysis of such reactions is rendered very difficult, and for this reason the study of antiviral immunity has tended to lag behind that of antibacterial immunity at just those points in which the latter has become most precise. We have, however, learned enough of the mechanisms concerned to construct a useful working outline which can be filled in rapidly when our technical difficulties have been overcome, and which has already passed the sound empirical test of enabling us to interfere with considerable success in the natural propagation of infection.

Antiviral Immunity as Observed under Natural Conditions.

The first fact that forces itself on our attention when we examine the natural history of virus diseases is the frequency with which an effective and lasting immunity is afforded by a single attack (see Andrewes 1931). Smallpox, chicken-pox, measles, mumps, cattle plague, swine fever, and dog distemper—

all these are common virus diseases, and all confer an immunity that, in the great majority of cases, appears to last throughout life. There are exceptions. Herpes simplex is one; influenza and the common cold are others—if we accept the view that these are virus diseases. Not long ago foot-and-mouth disease would have been placed in this non-immunizing or poorly immunizing class; but we have had to reconsider our views in that regard, and perhaps the relations that have misled us in this instance have misled us in others also. It would, however, be unwise to stress the value of the relative effectiveness of immunity as a differential criterion between virus and bacterial diseases, if only because, as pointed out in Chapter I, we really know very little of the immunizing value of generalized bacterial infections.

There is another way in which bacterial and virus diseases have been thought to differ in their natural behaviour. We shall note in Chapter XVII the frequency of latent or atypical bacterial infections, emphasizing the fact that a clinical survey of diphtheria or scarlet fever or enteric fever gives a quite inadequate picture of the frequency of infection with the bacteria concerned. The current clinical conception of many of the commoner virus diseases would seem to minimize the significance of latent, even of atypical, infections, suggesting by implication that every infected person provides a frank case of disease, or at least a case that is clinically diagnosable if suspicion is aroused. Those who hold this view would base it in part on the high attack rate among the previously-not-attacked during an epidemic prevalence of such a disease as measles, in part on their belief that it is unnecessary to assume the existence of healthy carriers of infection, or of individuals who have undergone a process of latent immunization, in order to explain observed epidemic happenings. But the attack rate among the previously-not-attacked exposed to risk, even in so highly contagious a disease as measles, is certainly not 100 per cent.; and there are good reasons for a belief in the existence and importance of latent immunization.

Stocks (1928, 1930*a, b*), in a series of careful analysis of the relevant data, has shown that the morbidity figures for measles, for chicken-pox and for German measles are inexplicable except on the assumption of immunization in the absence of diagnosed disease, or of an effective inherited immunity. That the former factor is involved can be demonstrated by comparing, during any epidemic prevalence, the attack rate among children who have previously been intimately exposed to risk without contracting the disease with that among children who have previously been less intimately exposed. The natural history of these widely prevalent virus diseases, as developed by Stocks, presents striking analogies to the natural history of diphtheria or of scarlet fever.

In other virus diseases the intervention of atypical cases and of the healthy carrier has obtained general recognition. In poliomyelitis the specific virus has been demonstrated in the nasal secretions obtained from healthy carriers, from persons long convalescent from an attack of the disease, and from atypical cases (Kling, Wernstedt and Pettersson 1912, Flexner, Clark and Fraser 1913, Lucas and Osgood 1913, Kling and Pet-

tersson 1914, Taylor and Amoss 1917, Flexner and Amoss 1919, Paul and Trask 1932).

There is still a divergence of views with regard to the frequency of healthy carriers of poliomyelitis virus, though there can now be none in regard to the frequency of atypical cases (see pp. 372-374). The epidemiological evidence, especially when viewed from a statistical angle, is in entire accord with the suggestions offered by the bacteriological findings. Wickman (1907) pointed out that it was impossible to account for the epidemiological behaviour of the disease without assuming the existence of latent and atypical infections; and Stocks (1932), examining the available data of recent outbreaks, calculates that the ratio of those developing latent immunizing infections to those developing clinical attacks of the disease is probably of the order of 100 to 1 or more. In the case of another virus disease, encephalitis lethargica, he would put this ratio in the neighbourhood of 800 to 1.

In this connection it may be noted that protective antibodies acting on the virus of poliomyelitis are widely distributed among the population at large.

Aycock and Kramer (1930*a, b, c*), using the highly susceptible monkey as a test animal, determined the protective power of samples of serum obtained from convalescent cases and from normal persons. Some 90 per cent. of the former and just over 50 per cent. of the latter showed protective antibodies in the serum. Similar tests were later carried out on normal persons, giving no history of poliomyelitis, living in another area of the United States. Of 21 adults, 18 showed neutralizing antibodies in their blood. Brodie (1932) has recently recorded similar findings in Montreal.

If we accept the view that the presence of such antibodies indicates previous contact with the virus of poliomyelitis we can hardly escape the corollary that mild atypical attacks, or a purely immunizing carrier infection, must be an exceedingly common event in this particular disease.

A similar method has recently been employed in mapping out endemic centres of yellow-fever infection in West Africa. Using protection tests in the monkey (Beeuwkes, Bauer and Mahaffy 1930), or in the mouse (Russell 1932, see also Theiler 1930, 1931, Sawyer and Lloyd 1931), it has been found that in areas in which clinical cases of yellow fever are infrequent and sporadic a high proportion of the children show neutralizing antibodies in their blood, indicating that the overt cases of disease give a quite inadequate picture of the real frequency of infection.

Recent observations by Duran-Reynals (1931) provide an interesting laboratory analogy to the field observations summarized above. In the course of certain experiments on vaccinia infection he found that some of his control rabbits showed the presence of neutralizing antibodies in their blood. Of 21 controls, 8 were shown to be immune. These controls had been injected with material that did not contain vaccinia virus, but they were housed in the same room as other rabbits that had active vaccinia lesions. Fourteen normal rabbits, housed in another room, were treated in the same way as these 21 controls; none developed any immunity to vaccinia. The conclusion that this spontaneous immunity had developed in the infected room as the result of latent or unrecognized infection was confirmed by the discovery, on one of the immune controls, of three or four very small papules which were proved, by transfer to another rabbit, to be vaccinia in nature.

The Mechanism of Virus Infections.

It would appear that the general process of tissue invasion in virus diseases does not differ in any essential way from that described in the case of bacterial infections. There would seem to be the same sequence of local proliferation, blood-stream invasion and secondary foci of infection, each phase varying in prominence according to the virulence of the virus and the susceptibility or resistance of the host.

Thus, Todd and White (1914) note that, when cattle are injected subcutaneously with a small dose of the virus of rinderpest, the blood usually remains non-infective for 72 hours, becoming infective coincidentally with the onset of illness; and Andrews and others (Report 1931) note that the virus of foot-and-mouth disease does not appear in the blood of experimentally infected cattle until shortly before the commencement of the febrile reaction, though it may be present in high concentration some hours before the temperature begins to rise, or before vesicles appear. The virus then tends to persist in the blood throughout the febrile period, though the degree and persistence of blood-stream infection varies widely in different animals (see also Waldmann, Trautwein and Pyl 1931). In the case of yellow fever, Hudson and Philip (1929) record that monkeys bitten by infected mosquitoes show the presence of virus in their blood 1 to 2 days after infection, and at about the same interval before the onset of fever.

Vaccinia, as regards its obvious lesions, is commonly limited to the site of inoculation into the skin; though the virus may be recovered from the blood after intradermal inoculation in the rabbit (Otawara 1922, Rivers and Tillett 1923, Gildermeister and Heuer 1927), and Gins and his colleagues (1929) have reported its presence in the nasal secretion of children after ordinary cutaneous vaccination. With highly potent strains of virus a generalized vaccinia may occur after dermal inoculation in the rabbit, with a widespread eruption, pocks on the lips and tongue, and lesions in the lungs, liver and other organs (Douglas, Smith and Price 1929). When such a virus is injected intravenously this severe, and sometimes fatal, generalization occurs with considerable frequency, and there is a rough proportionality between the dose of the virus administered and the degree of resultant generalization. When less potent viruses are injected intravenously there is frequently some degree of generalization, evidenced by an eruption of pocks on previously shaved areas of skin or on the mucosa (Calmette and Guérin 1901, Camus 1917-18, Levaditi and Nicolau 1923).

In certain other respects the pathogenesis of virus diseases appears to differ more or less sharply from bacterial infections. The tendency to selective localization appears to be more definite; and it has become customary to use such terms as *dermotropic* or *neurotropic* to define the special, but by no means exclusive, affinities of a given species or strain. This tropism is very probably related to another peculiarity of the viruses—their tendency to produce characteristic inclusion bodies in certain cells. (For a good and full description of these bodies and a discussion of their probable nature, see Ludford 1928, Findlay and Ludford 1926). It seems very possible that this habit of functioning as intracellular parasites has an important bearing on antiviral immunity.

The histopathology of the virus diseases, while it differs in certain aspects from that of most bacterial infections, does not differ so sharply and fundamentally as to require a separate generic description. Such would, indeed,

be impossible; for one virus infection differs from another in the type and distribution of the cellular reactions produced. We have referred above to the formation of characteristic intracellular inclusion bodies. In general, however, the tissue lesions that are associated with virus infections—inflammatory, necrotic, degenerative, granulomatous and proliferative—are of the same kind as those that follow infection with one or other of the pathogenic bacteria; and the stress of the reparative reaction appears to fall on the same cells of the reticulo-endothelial system (see Ledingham 1924, 1926*a*, *b*, 1927, Ledingham and Barratt 1929).

Of the ultimate mechanisms by which the filtrable viruses produce their harmful effects we as yet know little or nothing. We do not, for instance, know whether any of them produce soluble toxins with a characteristic pharmacological action, such as are produced by certain bacteria. Until our technique has developed to the point at which we can secure mass cultivation *in vitro*, in a cell-free medium, this problem is likely to remain unanswered. We can at the moment go no further than the statement that no instance has as yet obtruded itself upon our notice in which it seems necessary to assume the presence of a soluble toxin acting at a distance from any focus of living virus.

Antiviral Immunity.

Before discussing the mechanism of antiviral immunity it will be convenient to consider briefly the ways in which such immunity may be induced; since it is held by many that, in this respect, there is a significant difference between the behaviour of the filtrable viruses and bacterial cells.

Active Antiviral Immunity.—As regards active immunity, we have seen that this is conferred under natural conditions by a typical attack of the disease, and probably by atypical attacks of varying severity or by a carrier infection. Artificially it may be induced by the injection of a sublethal dose of living virulent virus (a dangerous and uncertain method, even when injections are made by an unusual route), by the injection of a virus that has changed its disease-producing characters, but not its antigenic structure, during adaptation to a different host-species—the classical example is the use of vaccinia to protect against variola—or by an initial injection of a fully attenuated virus followed by injections of less and less attenuated samples until the fully virulent virus is resisted—the classical example is the Pasteurian method of protection against rabies by the injection of rabbit-cord virus attenuated by drying. The question at issue is whether we can induce an effective antiviral immunity by the injection of killed virus alone, in the way that we can produce an antibacterial immunity by the injection of killed bacterial cells. The question really reduces itself into two others. Can we produce an effective resistance by the injection of virus-containing material so completely inactivated that it produces no detectable infection when injected in large doses into a fully susceptible animal? If this is possible, may we assume that the inactivated virus is actually dead?

We may answer the first question with a qualified affirmative.

Semple (1912) has shown that the classical Pasteurian methods of rabies prophylaxis may be replaced by injections of rabbit-brain virus inactivated by treatment with 1 per cent. phenol. Boynton (1918, 1928) records successful immunization against cattle plague with a phenolized vaccine, Curasson and Delpy (1926) and Daubney (1928) with a vaccine inactivated with formol. Ducloux and Cordier (1926) report successful immunization against sheep-pox with vaccines inactivated by formol or by ethanol. Bedson, Maitland and Burbury (1927) record the partial immunization of guinea-pigs against foot-and-mouth virus by the injection of formolized virus, and the complete immunization of a proportion of animals after prolonged treatment. Todd (1928*b*), having failed to immunize fowls by the injection of heat-killed or formolized fowl plague virus, succeeded in producing an effective immunity by the injection of three doses of a phenolized vaccine. Bedson (1931) produced a high degree of immunity in guinea-pigs against herpes by the injection of a formolized virus and records similar results with inactivated psittacosis virus (see Discussion 1932).

There are, however, a large number of observations that suggest—although the evidence is not always entirely convincing—that the resistance induced by such inactivated vaccines is in many cases trivial and transitory, and that to secure an effective and lasting immunity it is necessary to supplement the initial injection of inactivated vaccine by a later injection of active virus, in a dose that the partially protected animal will be able to resist. A particularly well-documented account of this method will be found in the studies of Laidlaw and Dunkin (1928*a, b*) on dog distemper.

It would seem a fair conclusion that the injection of inactivated virus material almost always confers some degree of specific resistance, provided that a suitable method of inactivation is employed; but that the resistance so conferred varies widely in effectiveness in different virus diseases. In some it is of a high order. In others it must be reinforced by injections of active virus; and it is probable that such reinforcement always increases the solidity and duration of the resistant state.

When we inquire whether an inactivated virus may be assumed to be a dead virus, we are on very difficult ground. The immediate and logical answer is quite clearly in the negative—the assumption is certainly unjustified on the evidence at present available. Some at least of that evidence tells against the view that “inactivated” and “killed” are even approximately equivalent terms. There is general agreement that the application of violent, and therefore certain, methods of killing—such as heating to high temperatures for long periods—destroys the antigenic value of virus-containing material. Indeed, as we have seen above, inactivation by such a chemical agent as formol may remove the antigenic value of one virus but not of another. Such facts are, perhaps, in favour of the view that attenuation rather than death marks the limit beyond which we cannot pass if an effective antigenic stimulus is to be obtained; but they are also explicable on the assumption that the lack of immunizing effect is due to some alteration in an important antigenic constituent rather than to the killing of the virus. If all effective vaccines were attenuated, not killed, we might expect occasional infections with our treated vaccines. In some instances at least such accidents seem never to occur. Thus (see Harvey and McKendrick 1930), the testing of 500 samples of Semple's phenolized rabies vaccine by the injection of 1 c.c. intramuscularly into rabbits did not result in a single

infection. Similarly, if a symptomless infection occurred in an animal injected with an inactivated vaccine, it might be possible by passage from that animal through others to produce characteristic symptoms or lesions. Bedson (1931) notes that his formalized herpes vaccine produced no lesions when introduced into the plantar skin of guinea-pigs, nor was it possible by passage from animal to animal by this route to restore its activity. It is unlikely that we shall solve this problem until we have at our disposal methods of *in vitro* cultivation that will allow us to deal freely with the filtrable viruses apart from cells and tissue fluids, and to apply to them the criteria of viability on which we rely in the case of bacteria.

Whatever the final verdict may be, it is the common practice at the moment to rely on the injection of living virus under the protection either of a partial active immunity, induced by the previous injection of virus inactivated in some appropriate way, or of a temporary passive protection induced by the injection of serum from an immunized animal. The latter method is very commonly employed, and its practical exploitation has led to some interesting observations. These have been admirably summarized by Andrewes (1931). Virus-serum mixtures incubated until infection can no longer be produced by any route seem to be useless as immunizing agents. To be effective they must be slightly under-neutralized. In this state they will produce immunity against some virus diseases. A more generally effective method is the so-called side-to-side inoculation—virus into one site, serum into another. Depending upon the ratio of antiserum to virus, this procedure may be followed by a mild and atypical infection or may produce no detectable general reaction. In some cases effective immunity is secured only if the serum-virus ratio is within the range at which a generalized reaction occurs; in others, such as dog distemper (Laidlaw and Dunkin 1931), an excess of antiserum sufficient to suppress such a reaction does not prevent a successful immunizing response. Side-to-side immunization has been successfully employed in cattle plague (Kolle and Turner 1898, see also Carmichael 1928), swine fever (Dorset *et al.* 1919) and dog distemper (Laidlaw and Dunkin 1931). Theiler (1907–1909) records the successful immunization of horses and mules against African horse sickness by the simultaneous intravenous injection of virus and antiserum.

Another effective method is to administer the antiserum after the virus. This involves a mild immunizing attack of the disease, and for this reason it will clearly never be a method of choice where times and conditions may be determined at will; but it has an important application when exposure to risk of infection has already occurred, or is an imminent and unavoidable danger. By the administration of antiserum in adequate amount, and at the correct interval after exposure, it is possible to avoid a severe and typical attack, replacing it by a trivial infection that will confer a lasting immunity. The modern methods of measles prophylaxis (see Chapter XIX) afford an excellent example of successful action along these lines.

Passive Antiviral Immunity.—Turning to passive immunity, enough has already been said to indicate that antiviral sera are at least as effective in affording protection against the homologous virus as is an antibacterial serum in protecting against the homologous bacterium. It may be noted that, while antibacterial sera are commonly prepared by the immunization of some conveniently large animal, usually the horse, it has become the practice to prepare antiviral sera by the immunization of an animal of the same species as those it is desired to protect. This is in part due to the fact that much of the work on virus diseases has been carried out within the province of veterinary medicine, and that the original observations on the protective power of immune sera were made with the blood obtained from convalescent animals, or from such animals after further treatment with large injections of active virus.

These hyperimmunized animals, when of a conveniently large kind, provide a supply of highly effective antiserum. There are, moreover, many observations which suggest that homologous antisera may be more effective than those prepared in animals of another species. Thus an antidistemper serum prepared in the dog will protect both dogs and ferrets against distemper virus, but protection in the ferret is much less effective and less consistent than in the dog (Laidlaw and Dunkin 1931). As we shall see, the use of convalescent human serum, or of adult serum known to contain protective antibodies, is taking its place in the prevention and treatment of virus diseases in man.

As in antibacterial immunity, the passive immunity so obtained is transient—too transient to be of any prophylactic value except under narrowly limited conditions that only occasionally occur in practice.

There are observations on record—some of them contained in the papers already referred to—indicating that antiviral sera may have a definite if limited therapeutic value.

The Mechanisms of Antiviral Immunity.

We know very little of what happens when a virus is introduced into the tissues of a resistant animal, or when a relatively avirulent strain is injected into a relatively susceptible animal. We cannot, therefore, construct any detailed pictures of the kind outlined in Chapter VIII in the case of bacterial infections.

Our ignorance on this point is due to technical difficulties rather than to lack of interest. In the case of bacterial infections we can, by staining and by cultivation, trace the distribution of the parasites in the tissues with considerable accuracy. In virus diseases neither method is available in a form suitable for this purpose, and we are thrown back on the inoculation of a susceptible animal whenever we wish to demonstrate the presence of virus in a given tissue. If we desire our results to be quantitative—as we usually do—we are in further difficulties unless the virus produces a characteristic skin reaction. If it does, we can titrate it with some approach to accuracy. If it does not we have to rely on producing infection or death in an adequate sample of animals with each dose tested, and our task becomes formidable. Nor do our troubles end here. The material we inject contains not only virus, but products derived from the host; and the possible effects of the latter cannot be neglected. The assumption that the result obtained on the injection of virus-containing material into a susceptible animal is determined by the virus content alone is, as we shall see, quite unjustified.

Such knowledge as we have has been gained mainly by experiments on normal animals. So far as it goes it suggests that the immediate clearing mechanism involved in freeing the blood stream and tissue fluids from virus is the same as that brought into play in the case of bacteria, and that, in a naturally immune animal, or in a relatively susceptible animal injected by a route that does not allow the virus to gain easy access to the tissues for which it has a special affinity, this clearing mechanism is capable of functioning very effectively.

Thus Gins and Weber (1916) found, in conformity with the results of earlier workers, that vaccinia virus of ordinary potency disappeared rapidly from the blood stream

after intravenous injection. When large amounts of virus were injected, and the organs were tested within 5 hours, virus could be recovered from the spleen, less frequently from the liver, but never from the bone-marrow. Douglas, Smith and Price—who were, it will be remembered, working with a highly potent strain of virus—record four experiments in which various organs were tested for virus within a few days after an intravenous injection. In these the virus was recovered in each case from lungs and spleen, in 3 of 4 cases from the liver, and in 3 of 4 cases from the bone-marrow; but the fact that infection was in all cases widely generalized makes the significance of these findings doubtful. Of more interest are the relative frequencies of vaccinia lesions in the different organs and tissues. Of 36 rabbits injected intravenously the numbers showing lesions at different sites were as follows: skin 31, mucosa of lips or nose 26, tongue 24, lungs 35, liver 24, spleen 24, adrenals 14, oesophagus 1, bladder 1, intestine 1, muscles 2, heart 1, pericardium 1, peritoneum 3. Lesions were also relatively frequent in the testes among the males, and in the ovaries among the females. Thus we find the highest frequencies in those tissues for which vaccinia virus is known to have a special affinity and in those organs—lungs, liver, spleen and adrenals—which are primarily concerned in freeing the blood stream from inanimate foreign particles or from bacteria.

Flexner and Amoss (1914) record that the injection of poliomyelitis virus into the veins of monkeys—a route that does not commonly lead to the production of the typical disease—is followed by the prompt deposition of the virus in the spleen and bone-marrow, but not in the kidneys. Galloway (Rep. 1931) has recorded a few experiments in which the infectivity of various organs was determined 24–48 hours after the injection of foot-and-mouth virus into the pad of the guinea-pig's foot. The concentration of virus was highest in the blood, but the spleen, liver and lungs contained virus in detectable amount, while the mesenteric glands, ovary, testis and muscles did not. In dog distemper (Laidlaw and Dunkin 1928*a, b*), organs rich in reticulo-endothelial cells, such as the spleen, show a high virus content.

A review of the available data appears to justify the following conclusions. Where the virus concerned has an affinity for some special tissue in which it causes its characteristic lesions, it will, naturally enough, be found in the greatest concentration in those lesions or in their immediate neighbourhood. Thus we find vaccinia virus in the highest concentration in the lymph of the pustules, the virus of foot-and-mouth disease in the fluid of the vesicles, the virus of rabies, or of poliomyelitis, or of Borna disease, in the central nervous system, and so on; but even in these diseases the other tissues in which the virus is most frequently present, if we except the blood, are those concerned in the normal clearing mechanism—the spleen, liver, lungs, bone-marrow or adrenals. In generalized infections, in which localization in the skin or nervous system is not a feature of the disease, the reticulo-endothelial tissues frequently show the highest virus content.

The blood we must consider in rather more detail. That the virus may often be demonstrated in blood withdrawn during the febrile stage of illness, or even at other times, we have already seen; but many of the results recorded, particularly in the later stages of experimental infections, have been curiously irregular—and it would appear that these irregularities have been due in part to the fact that viricidal antibodies may appear in the blood stream at an early stage of the disease, while active virus is still present in the circulating leucocytes.

As long ago as 1899 Kolle showed that the infective agent in the citrated blood of an animal suffering from cattle plague was readily removed by centrifugation. The supernatant plasma was non-infective; the deposit, containing the blood cells, infective to a high degree. Todd and White (1914) studied this phenomenon in greater detail and showed that the virus was mainly associated with the leucocytes, and Schein (1917) and Daubney (1928) recorded similar findings. Russ (1906) (see also Landsteiner and Russ 1906) found that the virus of fowl plague was present in greater amount in the cellular constituents of the blood than in the plasma, and Todd (1928a) has shown that the concentration is highest in the leucocytic layer, though Doerr and Gold (1932) record experiments which they interpret as indicating an adsorption of the virus by the red cells.

Smith (1929) has studied this problem in some detail in experimental vaccinia in the rabbit. Comparing the infectivity of the whole blood, the plasma and the washed deposit of cells during the early days of a generalized infection he found that the washed cells were most infective, and the plasma non-infective. By fractional centrifugation it was possible to show that the red cells played no part in fixing the virus. The results suggested strongly that the leucocytes alone were concerned, but the possible participation of the blood platelets could not be entirely excluded. At a later stage—and this is the immediately significant point—the washed cells might be infective while neither the whole blood nor the plasma produced any lesion in susceptible rabbits. After intradermal inoculation of virus the washed cells might be found infective in animals whose whole blood gave consistently negative results.

In further experiments it was found that specific antibodies made their appearance in the plasma of infected rabbits as early as the 3rd day after infection and thereafter rapidly increased in concentration. There was, therefore, a period during which the leucocytes contained active virus while the plasma contained protective antibodies. Infectivity tests carried out with the whole blood during this period gave irregular and often negative results, depending on the balance between virus and antibody. But if washed cells, freed from antibody, were used for the test inoculation, virus could regularly be demonstrated in the blood up to the 8th day after infection. Berry and Kitchen (see Russell 1932) in a case of yellow fever noted the simultaneous presence of virus and protective antibodies in the blood on the 4th and 5th days of disease. Such observations indicate clearly that caution should be exercised in assuming that any tissue extract or body fluid is necessarily free from living virus because it is non-infective.

The data that enable us to compare the fate of a virus when injected into an immunized animal with its fate when injected into a normal animal of the same species are even more scanty. There is the clear and significant fact that the characteristic signs of infection do not occur, or are very greatly modified; but beyond that fact we know relatively little.

In the particular case of foot-and-mouth disease Bedson, Maitland and Burbury (1927) have shown that three stages—not of course sharply demarcated from each other—can be recognized in the active immunization of the guinea-pig. In the first the animal resists the intramuscular injection of a dose of virus that uniformly causes a generalized infection in controls, but responds to inoculation into the pad of the foot by developing a local vesicle followed by generalized lesions. In the second the solid resistance to intramuscular injection is associated with a heightened resistance to intradermal injection, so that the local vesicle is not followed by generalization. In the third and final stage the guinea-pig is immune to the inoculation of the virus by any route. There is here a clear suggestion of an improved clearing mechanism that, in the partially immunized animal, is able to protect the sensitive tissues if these have not been directly infected at the time of inoculation. Analogous

observations have been made during the period of fading immunity in cattle and pigs (Andrews and others ; Rep. 1931). Recent experiments by Galloway (Rep. 1931) on the survival of foot-and-mouth virus after inoculation into the tissues of immunized animals are in general accord with this view. Virus injected into the mucous membrane of the tongue of immunized rabbits could not be recovered from the blood at any time from the 2nd to the 36th hour after inoculation. Similarly, virus inoculated into the tongue or pads of immunized guinea-pigs could never be recovered from the blood. It could be recovered from the local lesions from 30 minutes to 24 hours after injection, but not after 36 hours.

Of particular interest is an isolated experiment recorded by Smith (1929). Vaccinia virus was injected intravenously into an immunized rabbit, and the washed blood cells were tested for infectivity after 30 minutes, 1, 2, 4, 6 and 8 hours. The cells were infective up to and including the 4th hour, after which they were non-infective. This rapid disappearance of virus from the blood of an immunized animal contrasts sharply with the course of events in a normal rabbit.

The Nature and Reactions of the Antiviral Antibodies.

In discussing the action of the serum antibodies on bacteria and their products it was possible—and convenient—to give a fairly detailed account of their *in vitro* reactions before considering their rôle in the living animal. We have not been able to follow that plan in this chapter—we know too little as yet of ways in which viruses react in the test-tube with the corresponding antisera ; but it will help to keep our ideas clear if we review the scanty data available, comparing them with those set out in Chapter V.

In the particular case of vaccinia virus, it has been shown that the serum of an immunized rabbit reacts *in vitro* with virus-containing material from the pocks of variola or vaccinia in man, giving complement fixation and, under suitable conditions, visible precipitation (Gordon 1925, Burgess, Craigie and Tulloch 1929, Bedson and Bland 1929, Craigie and Tulloch 1931, Havens and Mayfield 1931). These reactions are specific—suspensions of material from the lesions of chicken-pox do not react with anti-vaccinial sera—and they confirm the antigenic similarity or identity of the viruses of vaccinia and variola. It will be noted that the antigenic material employed is extremely crude. Vaccine lymph contains substances derived from the tissues of the calf and a number of non-pathogenic contaminating bacteria as well as the vaccinia virus itself, and antigenic substances of similar kinds are present in material derived from human skin lesions.

It has been suggested that the reactions referred to above may be due to foreign contaminating bacterial antigens rather than to antigens derived from the virus ; but this criticism has been adequately met.

Bedson and Bland (1929) showed that antivaccinial sera did not fix complement in the presence of bacteria derived from calf-lymph, nor did antisera prepared against those bacteria fix complement in the presence of the lymph (see also Havens and Mayfield 1931). Craigie and Tulloch (1931) record the failure of antisera prepared against the secondary bacterial infectors, found in vaccinia and variola crusts, to give visible flocculation with crust extracts. They note, moreover, that antisera prepared by injecting

rabbits with extracts of the bacteriologically sterile organs of an animal suffering from generalized experimental vaccinia give good flocculation with crust extracts, and that antisera prepared against dermal vaccine lymph gives good flocculation with extracts of virus-containing organs. Similar observations have been recorded by Thompson, Hazen and Buchbinder (1932). The conclusion that these reactions depend on antigens present in or derived from the virus itself is confirmed by the observation of Gilmore (1931) that antivaccinial sera prepared in the rabbit, or human serum derived from cases of variola minor, give specific complement fixation in the presence of vaccinia virus which has been propagated through 17 to 24 successive subcultures in an appropriate tissue medium.

Recent observations by Ledingham (1931) and by others have an important bearing on this problem. The tissues of animals infected with vaccinia may be shown by appropriate staining methods to contain minute granules, the Paschen bodies, which almost certainly represent the actual virus—such bodies may be demonstrated in this and other virus diseases as optically resolved images by the beautiful methods devised by Barnard (1919, 1926, 1932, Barnard and Elford 1931-2). By differential centrifugation Ledingham has been able to prepare suspensions of these elementary bodies almost entirely freed from extraneous material, and finds that, in this state, they are specifically agglutinated by an antivaccinial serum.

Recent observations by Craigie (1932) indicate that the flocculation observed in a mixture of vaccinia virus and antivaccinial serum is not due solely or even mainly to an agglutination reaction between an antigen in solution and a corresponding antibody. Extracts of vaccinial material were filtered through a Seitz disc, and the material retained on the disc was recovered by back-washing. The filtrate was found to be flocculable, but not infective—vaccinia virus does not easily pass through the pores of a bacterial filter under these conditions—while the material recovered from the filter was infective, but only slightly flocculable. The precipitate from a mixture of the filtrate and an antivaccinial serum was found, when examined microscopically, to consist of faintly staining masses of a homogeneous material in which no elementary bodies could be detected. The material recovered from the disc consisted almost entirely of Paschen bodies, and these were specifically agglutinated by an antivaccinial serum. Such a serum, after absorption either with washed Paschen bodies or with the filtrable material, neither agglutinated the former nor gave a precipitate with the latter. Thus the antigenic component, or components, involved in the reactions appear to be identical, and we may assume that the filtrable substance is a product of the Paschen bodies, which are themselves the virus of the disease.

Smith (1932) has recorded experiments that indicate the close similarity between the filtrable substance described by Craigie and the bacterial haptens described in Chapter VI. Rabbits were injected intratesticularly with vaccinia virus, and autolysates were prepared from the infected testicular tissue. From these autolysates a soluble specific substance was obtained, which would withstand boiling, but not autoclaving. It gave a positive biuret test, positive reactions for arginine and tyrosine, negative reactions for tryptophane and cystine, and a positive Molisch test for carbohydrate. It therefore contains both protein and carbohydrate radicles. It shares with the bacterial haptens the capacity to give specific precipitation *in vitro*, and the incapacity to stimulate antibody production *in vivo*.

Douglas and Smith (1930) have studied the action of whole blood and of serum-leucocyte mixtures on vaccinia virus. They find that leucocytes take up the virus *in vitro*; that immune is significantly more viricidal than normal blood; and that normal cells in the presence of immune plasma appear to be more viricidal than immune cells in the presence of normal plasma.

It happens that the greater part of the available data with regard to the *in vitro* antigen-antibody reactions of the filtrable viruses refers to the particular case of vaccinia; but we shall probably not err in regarding this as an illustrative example of the behaviour of viruses in general. All the evidence that we have points in this direction.

Thus Bedson and Bland (1929) have demonstrated specific complement fixation with herpes virus and an antiherpes serum, Ciuca (1929) with foot-and-mouth virus and the corresponding antiserum, Frobisher (1929) with yellow-fever virus and the sera of convalescent men and monkeys, Laidlaw and Dunkin (1931) with the virus of dog distemper, and Broom and Findlay (1932) with the virus of Rift Valley fever and the sera of men, monkeys, sheep, rats and mice that have recovered from natural or experimental infection. Ledingham (1931) has demonstrated the agglutination of suspensions of the Borrel bodies of fowl-pox by the sera of fowls that have recovered from that infection, and Bedson (1932) has demonstrated agglutination and complement-fixation reactions between the elementary bodies of psittacosis virus and a specific antiserum.

It would seem, then, safe to conclude that naturally infected men and experimentally infected animals produce specific antibodies to a filtrable virus, which react with that virus *in vitro* in the same specific way as an anti-bacterial serum reacts with the homologous bacterium, or with its products.

Certain observations on the neutralization of a filtrable virus by an anti-viral serum—as studied by inoculation tests in susceptible animals—have raised doubts as to whether the antigen-antibody reaction involved is entirely similar to that which occurs between a bacterium and the corresponding sensitizing antibody.

Andrewes (1928*b*) found that mixtures of vaccinia virus with antivaccinial serum might fail to give rise to lesions when injected intradermally, indicating effective neutralization, though they proved infective when injected intratesticularly, intracerebrally or intravenously. He found also that virus could be recovered from a neutral serum-virus mixture for periods as long as 24 hours by simple dilution, and that the specific neutralizing antibodies could be recovered from the mixture by filtration through a candle that held back the virus. Todd (1928*c*) found that mixtures of fowl-plague virus and anti-fowl-plague serum that were just neutral when undiluted, regained their infectivity on tenfold dilution with normal saline. This phenomenon was observable after 4 hours' contact between virus and serum at 37° C., but not after 24 hours' contact at 28° C. The effect of dilution at the earlier period was also evidenced by the fact that a mixture that was inactive when injected intramuscularly was infective when injected intravenously. Bedson (1928) records similar experiments with herpes virus and an anti-herpes serum, and concludes that there is a slow union between the virus and the viricidal antibodies *in vitro*, the effect of dilution being limited to the period during which this union is incomplete. In later experiments (Bedson 1929) he demonstrated that collodion particles would adsorb the specific antibodies from an antiherpes serum, and that particles so treated would adsorb herpes virus more readily than untreated particles, or than particles treated with normal serum. From this he concluded that the antigen must have united with the antibody. Andrewes (1930*a*) re-examining the reaction between vaccinia virus and an

antivaccinial serum in the light of these results found that it became less and less easy to recover active virus from serum-virus mixtures as the period of contact was prolonged, but that the time required for complete irreversible inactivation of the virus was of the order of 24 to 48 hours at 37° C., or 4 to 8 days at room temperature. The observation of Smith (1930) that it is possible by the use of optimal amounts of absorbing material to absorb separately the antibodies acting on herpes and vaccinia virus from a mixture of antiherpes and antivaccinial serum indicates quite clearly that, under suitable conditions, an effective antigen-antibody union occurs.

It would seem probable that antibodies acting on the filtrable viruses form with them a union which is at first of a loose kind, easily dissociable on simple dilution, and which attains stability and firmness only after the lapse of a considerable period of time, probably as the result of some secondary change in the antigen-antibody complex. Closely analogous relations have been shown to hold between bacterial toxins and certain antitoxic sera (see p. 384).

As is the case with the antibodies acting on bacteria and their products, the antibodies in antiviral sera appear to be confined to the globulin fractions.

Hartley (1914) concluded from the results obtained in the fractional precipitation of anti-cattle-plague serum that the antibodies were contained in the euglobulin. Maitland and Burbury (1927) found that the antibodies in the serum of guinea-pigs immunized against foot-and-mouth virus were associated with the serum globulin. Weyer, Park and Banzhaf (1929) concluded that the antibody of poliomyelitis antiserum was contained in the pseudoglobulin, while Morgan and Fairbrother (1930) found the euglobulin the most potent. Henseval (1919) found the antibodies of a vaccinal antiserum to be distributed throughout the serum proteins, but describes the euglobulin fraction as especially potent. Ledingham, Morgan and Petrie (1931) found antibody in both the euglobulin and pseudoglobulin fractions of an antivaccinial horse serum; the highest concentration was in the euglobulin, but the greatest absolute amount was in the large pseudoglobulin fraction. Laidlaw and Dunkin (1931) found the antibody of distemper antiserum to be confined to the globulin fraction; further study showed that the antibody was associated with a globulin fraction that was insoluble in water, but soluble in weak acids or alkalis and in water saturated with CO₂, thus behaving like the pneumococcal antibody described by Felton (1925, 1926, 1928).

There can be no dispute that humoral factors play an important part in antiviral, as in antibacterial, immunity; but there has been a tendency to suggest that a specific cellular immunity is even more important. It may be so. It would be folly to dogmatize in our present state of ignorance. But the scanty evidence at present available hardly favours such a view.

Andrewes (1929*a*) cultivated a particular virus (known as Virus III) in tissue cultures prepared from rabbit testis, and found that the characteristic intranuclear inclusion bodies associated with this infection *in vivo* were also formed *in vitro*. Rivers, Haagen and Muckenfuss (1929*a, b*) later described the formation of characteristic inclusion bodies in cultures of vaccinia and of herpes virus, grown in association with cells of the rabbit's cornea. Andrewes (1929*b*) employed this method in an attempt to study the relative importance of cellular and humoral immunity in Virus III infection. Cultures were prepared containing the virus associated with (*a*) normal testicular cells and normal serum, (*b*) normal testicular cells and the serum from an immune animal, (*c*) testicular cells from an immune animal in association with normal serum and (*d*) immune testicular cells with immune serum. With immune testis and immune serum no inclusion bodies were formed. With immune serum and normal testis the same result was obtained, provided that the serum

was allowed to come into contact with the testicular tissue, before the virus was added. If, however, the virus was allowed to remain in contact with the tissue for 10 minutes or longer at 37° C. before the immune serum was added inclusion bodies developed, though they were always scantier than in control cultures in normal serum. When immune testis was combined with normal serum inclusion bodies usually developed; when the immune testis was well washed by a preliminary soaking in Tyrode's solution inclusion bodies were constantly obtained. Similar results were later obtained with herpes virus and an anti-herpes serum (Andrewes 1930*b*). These observations indicate very clearly that, in the case of the viruses studied and under the experimental conditions obtaining, the humoral factor is decisive, while there is little evidence of an increased resistance of the cells from the immunized animal. In the case of vaccinia virus Rivers, Haagen and Muckenfuss (1929*b*) record observations which show apparent divergences from Andrewes' findings, but these may have been due mainly to differences in the technique employed.

It is perhaps a plausible guess, though certainly no more, that the real difference between antiviral and antibacterial immunity lies in the fact that an essential element in virus infections is entry into and multiplication within particular susceptible cells, and that an adequate concentration of antibody in the neighbourhood of these cells protects them from the virus, and so presents an effective barrier to the spread of infection within the tissues.

The Antigenic Structure of the Filtrable Viruses.

We know almost nothing of the antigenic structure of the viruses. It seems a reasonable enough assumption that living cells so small must have relatively few antigenic components; and it is, perhaps, not an abuse of the argument from analogy to suppose that, as in the bacteria, the particular component that is concentrated at the surface of the virus particle will dominate the picture from the point of view of effective immunity. We have seen that, in the particular case of vaccinia virus, there is evidence of the existence of a heat-stable, soluble antigenic component, having the properties of a bacterial hapten, and it is altogether probable that we shall in time be able to separate similar haptens from other viruses and so to apply the same chemical methods of analysis that have proved so fruitful in the study of bacterial antigens.

There is one direction in which we already have evidence of antigenic differences of a kind entirely analogous to those existing within a pathogenic bacterial species. Just as there are different serological types of pneumococci, each type acting as a specific entity so far as its more important immunity reactions are concerned, so there are at least three types of the virus of foot-and-mouth disease, each type producing the same clinical syndrome in susceptible animals but producing immunity only against itself. By cross-immunity experiments Vallée and Carré (1922*a, b*) demonstrated the existence of two types of foot-and-mouth virus, to which they gave the labels A and O. Waldmann and Trautwein (1926) and Trautwein (1927) described three immunologically distinct types of virus which they called A, B and C. It has been found that the A type of Vallée and Carré is identical with the B type of Waldmann and Trautwein, and the O type of the former observers with the A type of the latter (see Rep. 1928). These immunological differences may be demon-

strated either by active or passive immunization of susceptible animals, and the antibodies may also be differentiated by complement-fixation tests *in vitro* (Ciuca 1929, Rep. 1931).

It is clear that observations of this kind should make us very cautious in accepting statements with regard to the effectiveness of the immunity that follows a single attack of a particular virus disease. One attack of foot-and-mouth disease does not always protect against a second; because foot-and-mouth disease is a clinical but not an immunological entity. One attack of O foot-and-mouth infection protects against another infection with that strain of virus, though we cannot yet assess with any accuracy the exact degree or duration of the protection afforded, but an attack of O infection does not protect against a subsequent attack of A infection. If influenza in man is a virus disease, it seems very probable that the same antigenic heterogeneity of the causative virus will be found to exist.

No other instances of clear-cut antigenic differences, such as those that exist between the different types of foot-and-mouth disease virus, have as yet been put on record. Burnet and Macnamara (1931) have reported observations that suggest, but do not prove, the existence of antigenic differences between different strains of poliomyelitis virus, and Flexner (1932) has recorded experiments that suggest an antigenic modification of this virus as the result of repeated passage in the monkey. Such evidence as exists, and it is considerable in total mass though very various in kind, suggests that most of those viruses that have been adequately studied have a relatively stable and uniform antigenic structure. This is a field that is as yet almost unexplored, but there seems no reason to suppose that the picture finally constructed will differ in kind, though it may differ in emphasis, from that which we have described in the case of bacteria.

Allergy in Virus Diseases.

The characteristic response to reinoculation with vaccinia virus affords one of the classical examples of an allergic reaction. The typical sequence of events after a successful primary inoculation commences with an incubation period lasting about 3 days, followed by the appearance of papules at the sites of inoculation on about the 4th day. These develop into compound vesicles during the next 5 days, become definitely pustular about the 10th day, and heal by the well-known scabbing process between the 14th and 21st days. If a person who has been successfully vaccinated is again injected with calf-lymph during the period of waning immunity, but before he has again become fully susceptible, the most noticeable feature in the local reaction is an acceleration of all stages. The papules appear earlier, sometimes within 24 hours; and vesiculation and pustulation, when they occur, are in evidence at a far earlier period than after primary vaccination. Very frequently, however, the reaction ceases at the papular stage, and this may be so transitory as to be missed unless daily examinations are made. In any case, the induration round the papules or vesicles is usually far less marked after a secondary than after a primary vaccination, and the constitutional symptoms are slighter. We have, in fact, the typical allergic combination of accelerated response with localization of infection. It would appear (Andervont and Rosenau 1930) that

vaccinia virus killed by heating to high temperatures is capable of producing the characteristic immediate reaction with papule formation in persons previously vaccinated; while it has no effect in unvaccinated persons, nor does it produce an active immunity. There are, of course, many analogous observations in the case of bacterial allergy and hypersensitiveness.

There is no reason to believe that other virus infections differ from vaccinia in their ability to induce the allergic state, though this aspect of antiviral immunity has not yet been studied in any detail. A few observations are on record. Thus, Andrewes (1928*a*) records an allergic reaction in rabbits infected with Virus III. Animals that had been solidly immunized showed a relatively small local lesion when reinoculated in the testes, but no nuclear inclusions were present in these lesions. Partially immunized rabbits developed more pronounced testicular lesions, as judged histologically, than did either normal or solidly immunized animals. These lesions, however, unlike those produced in normal rabbits, were usually free from nuclear inclusion bodies.

Other Factors in Virus Infections.

Our knowledge as to interplay of factors other than those we have referred to above is very scanty; but so far as it goes it suggests no fundamental differences between the mechanisms involved in antiviral and antibacterial immunity.

We have seen that the reticulo-endothelial cells are intimately concerned in the pathogenesis of the virus infections, and it seems altogether probable that they play an important rôle in the production of antiviral antibodies. The time relations of the antibody response to virus infections would seem to be of the same general type as those that occur in bacterial infections, or in active immunization against a bacterial antigen (see Ciuca 1929, Ledingham 1932).

In virus as in bacterial infections there is evidence that ancillary factors may play an important part. Duran-Reynals (1928) records the enhancing effect of extracts of embryonic or sarcoma tissue, or of suspensions of kieselguhr, on the dermal infectivity of partially inactivated vaccinia virus. He also (1929) notes a similar effect produced by extracts of testicular tissue. This latter phenomenon has been studied by McClean (1930) who finds that the active principle in testicular extract produces a local increase in the permeability of the skin, and thus increases the area of diffusion of the virus.

A series of observations by M. Maitland (see Rep. 1928) on the factors determining the localization of the virus in experimental foot-and-mouth disease in the guinea-pig are of considerable interest. Seeking an explanation of the limitation of the vesicles to the mouth and the hairless part of the feet, she transferred strips of hairy skin to the soles of guinea-pigs' feet and found that vesicles developed in the hairy skin so transferred, whether the virus was injected directly into it or introduced elsewhere in the body. She also showed that, when virus was inoculated intramuscularly, vesicles failed to appear on the sole of one hind foot, if that foot was protected by cotton-wool and a bandage. On the unprotected feet vesicles appeared as usual. Again, when the bottom of the cage was made of wire mesh, the pressure on the toes and anterior part of the feet resulted in the appearance of vesicles on these parts, though they are not the usual sites of vesicle formation.

SUMMARY

It is perhaps a fair summary of the evidence presented in this chapter to suggest that it is compatible with the view that there is no essential difference between the mechanisms involved in antiviral and antibacterial immunity. In so far as immunity to virus infections is more effective than immunity to bacterial invasion, it seems possible that the difference is due rather to the greater limitations imposed on the virus by its habit of intracellular parasitism than to any special reaction on the part of the host.

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CHAPTER XVII

HERD INFECTION AND HERD IMMUNITY

IN the preceding chapters we have taken as our host unit the individual man or animal. It is clearly possible to work with a different unit—the herd. If we wish to study the epidemic spread of infection, and the factors that favour or prevent it, then it is with this larger unit that we must deal.

The herd, like each of its members, has a characteristic structure ; and this structure, from our present point of view, includes not only the hosts belonging to the herd species, and their spatial relationship to one another, but the presence and distribution of alternative animal hosts and possible insect vectors of infection, as well as all those environmental factors that favour or inhibit the spread of infection from host to host. This herd structure, apart altogether from the susceptibility or resistance of the individual hosts, may play a decisive part in the immunity of the herd as such. A herd may be immune to a particular disease—in the logical sense that it will resist the introduction of infection from without—although each of its members is fully susceptible, and would fall an easy victim if he strayed to a herd with a structure that allowed an endemic prevalence of the disease in question. In this sense the English herd is immune to plague ; because the association of man, the rat and the flea is not now of a kind to allow spread along natural routes. It is probably immune to cholera, as the result of an adequate system of water purification. It is not—nor does it seem likely to become—immune to any of those diseases that are spread by droplet infection. It would take us altogether beyond our present scope to consider the known or problematical effects on herd resistance of such changes in environmental conditions ; but we may at least note that many of the most striking successes of preventive medicine have been attained by altering herd structure without inducing any increased resistance in its individual members. By attacking insect vectors of infection, such as the mosquito, by preventing the frequent passage of bacteria from one person's intestine to another person's mouth by way of water and food, and by a general improvement in environmental conditions, we have succeeded in eliminating, or reducing to negligible proportions, diseases that formerly took a heavy toll of lives, and still take that toll in areas where such measures are not applied.

The type of herd immunity with which we are here concerned is that in which this freedom from the spread of infection has not yet been attained, so that contact with the bacterial parasite is at least an occasional event in the normal experience of the host species. Under such conditions the course of

events in any infected herd will be determined mainly by the distribution of the parasite and the distribution of those kinds of specific immunity that we have considered in earlier chapters. Intelligent interference with the course of events is impossible without a clear idea as to what is actually happening.

We may recognize, in theory, at least six categories of hosts among any infected herd. Four of these categories include individuals who are themselves infected, (1) the typical case, (2) the atypical case, (3) the latent infection, and (4) the healthy carrier; while two categories are not themselves infected, or infective, (5) the uninfected immune and (6) the uninfected susceptible. The division between (2), (3) and (4) is formal rather than actual—these conditions shade into each other by imperceptible degrees. It is doubtful whether the division between (3) and (4) is justifiable even for convenience of description, since many, perhaps most, of the class commonly described as healthy carriers are in reality suffering from a symptomless, and often negligible infection. It may be noted that the individuals falling in categories (3) and (4) are in general, though in a very varying degree, resistant to further infection from without; so that the only fully susceptible hosts at risk are those in class 6.

Fig. 34 may help the student to visualize the kind of distribution, both of infection and of immunity, that may be met with in infected herds under different epidemic conditions. No distinction has been made in the diagram between latent infections and healthy carriers. A few arrows have been introduced to indicate the direction of effective spread—effective in the sense of producing new cases of disease or in converting susceptibles to immunes. As we shall see, an epidemic of an infective disease is usually accompanied by an epidemic of symptomless immunization.

A may be taken as an example of an epidemic phase in an endemic-epidemic prevalence, that is, as representing the state of affairs in a herd that is affected by an outbreak of an infective disease from which it never completely frees itself, epidemics of varying severity recurring at more or less frequent intervals. B might be regarded as a later stage of A, or as a small epidemic wave occurring in a herd in which susceptibles are few, while carriers are frequent. C may be taken as representing the state of affairs during a severe epidemic occurring in a herd with little initial immunity. An extreme example of this catastrophic type of prevalence has occasionally been afforded by the introduction of such a disease as measles into an isolated island community that has either never experienced the infection before, or has been free from it for many years. D may be regarded as representing a stage of relative quiescence between two outbreaks of the type depicted in A. It will be noted that the proportion of susceptibles is higher than in A or B, and with such a distribution as this a fresh outbreak of the A type is likely to occur.

As an illustration of the types of distribution depicted in A, B and D, no better example could be selected than that of diphtheria. We are here dealing with a disease that is essentially a toxæmia; and, as we have seen, an effective antitoxic immunity will in this case protect the host against clinically

detectable infection. In the Schick test we have a method which allows us to divide the members of any herd into susceptibles and immunes. Except in the case of very young children a negative reaction may be taken as an

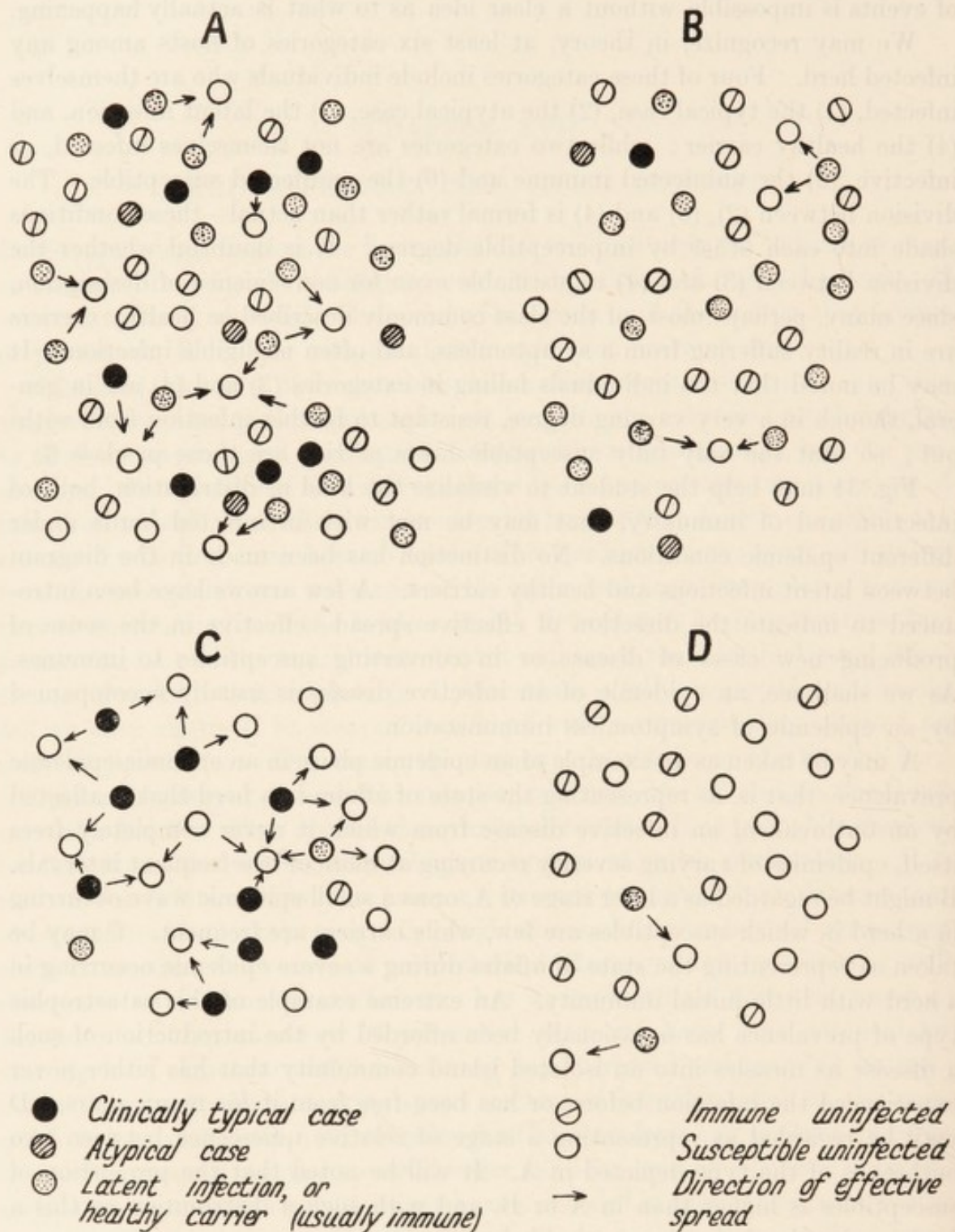


FIG. 34.

indication that an individual has something over 0.01 A.U. of antitoxin per c.c. of circulating blood, and also that he will produce further antitoxin briskly and effectively in response to any entry of toxin into his tissues. By swabbing throats and noses, and testing the virulence of any morphologically typical

diphtheria bacilli recovered, we can determine with some approach to accuracy the distribution of the parasite among the hosts at risk; and we are thus in a position to give a description of the course of events in any particular community that is vastly more informative than we can at the moment supply for most other diseases.

It has of course long been recognized that the diphtheria bacillus is not confined to those who are suffering from the clinical disease in its typical form. It is frequently isolated from cases of mild sore throat associated with an epidemic of typical diphtheria, less frequently from healthy contacts, and still less frequently from non-contacts.

Kober (1899) records the isolation of diphtheria bacilli from 70 per cent. of 139 contacts who were themselves suffering from mild sore throat, and from 8 per cent. of 123 contacts who had apparently normal throats. Closeness and continuity of contact, here as in other diseases, have a considerable influence on the carrier rate. The collected figures recorded in the Medical Research Council's monograph on diphtheria (see Monograph 1923) show a 15 per cent. carrier rate of virulent diphtheria bacilli among 610 contacts in barracks or hospital wards, a 7 per cent. carrier rate among 10,883 home contacts, and a 0.6 per cent. carrier rate among the general non-contact population.

As a measure of the risk to which an ordinary urban population is submitted we may take the carrier rate of virulent diphtheria bacilli among children attending the elementary schools in and about London. During the last 10 years or so it has fluctuated between 2.5 per cent. and 5 per cent. (see Dudley 1923, Forbes 1927).

The events that follow the passage of virulent diphtheria bacilli from the throat of a case, or of a carrier, to the throat of a non-infected person will depend on the immunological condition of the recipient. If he is susceptible (Schick-positive) he will either respond by developing antitoxin and so becoming more resistant, and eventually Schick-negative, or else he will develop clinical diphtheria and become a case. Hence Schick-positive carriers of virulent diphtheria bacilli are very rare. They are not non-existent—they cannot be if we accept the view that the transition from the susceptible to the immune class is usually the result of latent infection—but we may take it that a person who is carrying virulent bacilli in sufficient number to be detectable by an ordinary swabbing is (a) immune (Schick-negative), or (b) undergoing rapid immunization to the Schick-negative level, or (c) incubating the disease.

If, then, we take the case of a relatively isolated community, such as a large boys' school, and trace the spread of infection and the development of immunity during an epidemic of diphtheria we shall observe the following sequence of events.

At the start we may suppose that we have 50 to 70 per cent Schick-negative immunes and 30 to 50 per cent. Schick-positive susceptibles. Among these boys there will be a certain number of carriers of virulent diphtheria bacilli, say 3 per cent.; these will be immunes.

When these bacilli spread to uninfected boys they may obtain lodgment either in a susceptible or in an immune; and, depending on the dose of bacilli transferred, the exact degree of immunity of the recipient and many other factors of which we have as yet no knowledge, they may bring about any of the following transformations.

Frequently :

- (a) Schick-positive susceptible → case (mild or severe).
- (b) Schick-positive susceptible → Schick-negative immune
 - (a) without detectable carrying,
 - (b) with detectable carrying.
- (c) Schick-negative immune → Schick-negative immune carrier.

Very rarely :

- (d) Schick-positive susceptible → Schick-positive carrier.
- (e) Schick-negative immune → mild "bacteriological" case.

As an illustrative example of the findings that have actually been recorded in work of this kind, we may cite the illuminating studies of Dudley (1923, 1926, 1932) on diphtheria prevalence during a series of years in the Royal Naval School at Greenwich, a school of some 1,000 boys.

Between January and May 1919—the period at which Dudley's record starts—there were 65 cases of diphtheria. From then onwards until May 1921 there were no cases. Between May and July of that year 20 cases were recorded, and in the September-to-December term the outbreak reached epidemic proportions, with 77 cases. Between January and April 1922, 831 of the boys were Schick-tested. The results obtained are summarized in Table XXVI.

TABLE XXVI

SHOWING RELATION BETWEEN SCHICK REACTION AND PREVIOUS EXPERIENCE AMONG BOYS TESTED JANUARY TO APRIL 1922.

Previous Residence in School.	Experienced 1919 Epidemic.	Experienced 1921 Epidemic.	Per cent. Immune (Schick-negative).
None (new boys)	No	No	58
6 months-2 years	No	Yes	85
Over 3 years	Yes	Yes	95

The relation between experience and immunity is obvious. Moreover, the fluctuations in the immunity rate as plotted by terms of entrance were discontinuous. For entrants during the four terms September 1917 to September 1918 inclusive the percentage giving negative reactions in 1922 were 100 per cent., 92 per cent., 100 per cent. and 98 per cent. respectively. For the entrants during the period May 1919 to May 1921 inclusive the rates by terms of entrance were 82 per cent., 85 per cent., 83 per cent., 82 per cent. and 86 per cent. as compared with the 58 per cent. in boys joining in January 1922. It would appear that there had been two waves of immunization in the school, coinciding with the waves of clinical infection.

A sample swabbing of the school during the latter part of the 1921 epidemic showed 28 carriers of diphtheria bacilli in addition to the 77 cases. During the period January to July 1922 the carrier rate for virulent diphtheria bacilli was 2.2 per cent., during September to December of the same year it rose to 4.5 per cent., and during this period cases of diphtheria were still occurring. But immunization of susceptibles was proceeding synchronously with the spread of clinically detectable infection. Of 88 boys who had given positive

reactions on first testing 24 reacted negatively when retested 3 months later. Similar findings are recorded for later years, with carrier rates of virulent diphtheria bacilli varying from 1.0 to 4.5 per cent., and with recurrent cases of diphtheria that never attained the epidemic frequency of September to December 1921. Thus of 35 Schick-positive entrants in March 1924, 13 had become negative within 4 months; and 2 years' residence in the school had increased the 58 per cent. immunity rate of the new boys entering in January 1922 to 84 per cent. It may be noted that this rapid immunization was confined to the boarders, forming the population of about 1,000 to which all the above figures refer. There were at the school some 120 day boys, who did not share in the continuous exposure to risk of infection experienced by the boarders. Among these the rate of immunization was relatively slow. Over a 4-months' period only one of 24 Schick-positive day boys became Schick-negative.

The increasing ratio of carriers to cases as a herd becomes more and more immune is well illustrated by the case rates and carrier rates in one school company, of some 160 boys, during the winter term of 1921 and the winter term of 1922.

	Case Rate.	Carrier Rate.
1921 . . .	7 per cent.	2 per cent.
1922 . . .	0.6 " "	6 " "

In 1921 during a severe epidemic following on a period in which the school had been free from diphtheria for 2 years, the spread of virulent bacilli produced 3.5 cases to one carrier; in the winter of 1922 after this severe exposure and in spite of the entrance of 43 new boys during the post-epidemic period, the spread of infection produced 10 carriers to one case.

In assessing the significance of such figures we must remember that the real carrier: case rate over any considerable interval of time is always higher than that given by comparing the recorded case rate with the recorded carrier rate over that period—a point that has recently been stressed by Dudley (1932). All clinical cases of diphtheria will be recorded. A case rate of 3 per cent. per annum in a particular community means that 3 persons in every hundred of those exposed to risk develop diphtheria during the year in question. But a carrier rate of 7 per cent. does not mean that 7 per cent. become carriers during the year; it means that at any one swabbing of an adequate sample 7 persons in each hundred are, on the average, found to be harbouring diphtheria bacilli. The total number of persons that become carriers during the period in which the seven cases of diphtheria occur will be much higher. Thus, over one yearly period, Dudley records an average carrier rate of 6.6 per cent. with small fluctuations above or below this level; but repeated swabbing (7–8 times) of a large sample of boys showed that at least 40 per cent. were harbouring the diphtheria bacillus at one time or another during this period.

The general significance of the picture presented by such studies as these is clear enough; but we must remember that the two categories—immunes and susceptibles—that are divided from one another by simple Schick testing present a very incomplete picture of the gradations in resistance that actually exist in a herd at risk. Glenny (1925) defines five grades of immunity, which are set out in Table XXVII. These are, of course, not exhaustive; nor are they sharply demarcated from one another.

TABLE XXVII

SHOWING VARYING GRADES OF RESISTANCE TO DIPHTHERIA. (After Glenny.)

	Group.				
	1	2	3	4	5
Schick test	—	+	+	+	+
Antitoxin in blood .	> 1/30 A.U.	< 1/30 A.U.	0	0	0
Earlier stimuli . . .	Many	Many	Many	Few	None
Response to further stimuli	Rapid	Rapid	Rapid	Slow	Slowest
Description	Immune	Immune	Potentially immune	Sub-immune	Fully susceptible

If we could elaborate our study of a population at risk to the point of estimating at regular intervals the exact amount of antitoxin in each person's circulating blood; and if, dealing with a very large population, we could withdraw an adequate sample at frequent intervals and determine the response of each Schick-positive person to small injections of toxoid, we should be able to build up a far more detailed picture of what was really happening. Adopting Glenny's classification, we should be able to detect the transformation from the fully susceptible to the sub-immune, and thence through the Schick-positive immune to the fully developed Schick-negative immune, as well as transference from the Schick-positive to the Schick-negative class.

Our rare Schick-positive carriers would be found in the immune or potentially immune sub-groups, and would rapidly pass into the Schick-negative class. We should almost certainly find that severe cases tended to arise from the fully susceptible Schick-positives, mild "bacteriological" cases from the sub-immunes or potentially immunes.

A point of great importance is the reaction of the Schick-immunes to the reception of virulent diphtheria bacilli. We know that they very rarely contract clinical diphtheria, and then only in a mild form. We know that many of them become carriers. Is a point reached in natural immunization at which a person resists carrier infection as well as infection in the clinical sense? Can we grade our Schick-immunes as we grade our Schick-susceptibles, putting at the lower end of the scale those who have just attained to the Schick level of immunity, and at the upper end of the scale those who are so resistant that virulent diphtheria bacilli are unable to gain lodgment in their throats? If a human herd is rendered 100 per cent. Schick-negative, either by natural immunization or by prophylactic inoculation, will infection with the diphtheria bacillus be eliminated in the bacteriological sense, or will a freedom from clinical diphtheria be associated with a persistent carrier rate not much below that of the population at large, and perhaps with carrier epidemics? Is complete immunity, of the kind that would render a herd entirely free from infection, likely to be attained by immunization of this kind, however far it is pushed?

We cannot yet answer these questions. There is a suggestion in some of Dudley's recent studies (Dudley 1932) that Schick-immunes who have been exposed to risk for long periods carry virulent diphtheria bacilli less frequently than Schick-immunes with a shorter experience in the infected environment. But as yet we have no statistically decisive figures. In so far as we are dealing

with a purely antitoxic immunity—and this is the case in our artificial Schick-immunes—we should not expect freedom from the carrier state unless, as seems unlikely, successful lodgment in the throat depends on a local toxic effect. In so far as our natural Schick-immunes possess an antibacterial as well as an antitoxic immunity we must remember that the diphtheria bacillus is separable into many antigenically different types, though all types produce the same toxin (Durand 1918, 1920, Havens 1920, Smith 1923, Eagleton and Baxter 1923), so that we should expect any antibacterial immunity to be type-specific. Moreover, it is very doubtful whether the mechanisms discussed in Chapter VIII would have any appreciable effect in preventing a localized tonsillar infection.

We should, then, be inclined to guess that immunity to clinical diphtheria would be attained much more readily than immunity to carrier infection, and that an immune herd, in the former sense, might provide a persistent focus of infection.

Scarlet fever is another disease in which, by the aid of the Dick test and the examination of throat swabs for hæmolytic streptococci, we can obtain information of the same general kind as that now available in the case of diphtheria; but we have not yet any records comparable in detail and duration with those afforded by Dudley's work at Greenwich. So far as our fragmentary records go they look like pieces of a very similar picture, with one essential difference. The production of the erythrogenic toxin forms a relatively small part of the pathogenic potentialities of hæmolytic streptococci; and an antitoxic immunity that will suffice to prevent the occurrence of the clinical syndrome that we label scarlet fever will not suffice to prevent the spread of tonsillitis, or of other clinically obvious streptococcal infections (see Okell 1932). For effective immunity against all the clinical manifestations of infection with hæmolytic streptococci we need an antibacterial as well as an antitoxic immunity; and this antibacterial immunity will almost certainly be type-specific.

Turning to diseases in which antitoxic immunity, in the strict sense in which we are using that term, plays no apparent part, we are not in a position to give any adequate description of the course of events in a naturally infected herd. This is because we have no such simple tests as the Schick and Dick reactions by which to separate our immunes from our susceptibles. There are good reasons for believing (see p. 320) that by an adequate survey, including a careful study of the distribution of the infecting organism and tests for the presence of specific antibodies in the blood of the hosts at risk, we could add greatly to our knowledge of what is happening during an endemic or epidemic prevalence. But we have not got that knowledge yet.

Some things we do know. We know that in certain diseases such as cerebrospinal meningitis, the ratio of carriers to cases is very high (see for instance Glover 1918), so that it seems probable that the immunes greatly outnumber the susceptibles. Preceding an epidemic outbreak the carrier rate among the population at risk reaches a high level; and it is probable that any environmental factors that tend to increase this rate, such as overcrowding, play a large part in determining the occurrence of an outbreak of

the clinical disease. The same general relationship—a widespread carrier epidemic associated with relatively few clinical cases—seems to hold in certain virus diseases, such as poliomyelitis and encephalitis lethargica (see pp. 255, 256).

In other diseases, such as typhoid fever, the ratio of carriers to cases is not so high (see Klinger 1906, Minelli 1906, Prigge 1909, Rosenau *et al.* 1909, Gill 1927). But we know that atypical cases of typhoid fever occur during an epidemic (see for instance Billet *et al.* 1910), and that many carriers of typhoid bacilli give no history of ever having suffered from a typhoid-like disease (see Klinger 1909). It is a fairly safe assumption that an endemic or epidemic prevalence of typhoid fever is associated with the occurrence of sub-clinical immunizing infections. We know quite certainly that such a prevalence leaves behind it infected healthy carriers who may be a potent source of further spread. For histories of such classical carriers as the Strassburg baker's wife, the Folkestone milker, and Typhoid Mary, reference may be made to the excellent monograph of Ledingham and Arkwright.

The Experimental Study of Herd Infection and Immunity.

It is clearly possible, by selecting a convenient host species and a parasite that spreads naturally among them, to submit problems of the kind we have considered above to direct experimental study—initiating an epidemic of a particular disease among our test population, and studying the reactions of new entrants, of old members of the herd, or of migrants from one herd to another, by any available means. We can, under such conditions, observe the effects of any intentional interference we choose, splitting a herd into smaller units, reaggregating these units after any selected interval, varying the rate of immigration of susceptibles, actively immunizing some or all of our animals by vaccines of different kinds administered by different routes, and so on as new problems suggest themselves for attack. In this way we gain enormously in an increased control over many of our unknown variables; but we lose, in so far as we wish to argue from our experimental herds to happenings in the natural world outside our cages, by having to rely on analogies that are certainly incomplete, and may well be misleading. Because our control is still far from complete we must use large numbers of animals and make constant use of statistical methods in trying to assess the meaning of our results. We must, therefore, use a small, easily controlled and relatively inexpensive animal, and the mouse fulfils these requirements. Experiments of this kind have been carried out during the last decade, in this country and in America, on mouse typhoid—caused by infection with *Bact. aertrycke* or less frequently with *Bact. enteritidis*—, on mouse pasteurellosis, and on a virus disease known as ectromelia (see Marchal 1930). The American workers have also studied the spread of infection among rabbits and fowls housed in laboratories, or maintained as breeding stock by dealers. It will be convenient to consider the data thus obtained in direct relation to the observations on human herds, noting carefully the points at which particular caution must be exercised in applying the argument from analogy, and in generalizing from an isolated experience.

Is there, in the spread of infection among mice, the same distribution of typical cases, atypical cases, latent infections and carriers, and the same natural immunization associated with natural infection, that occurs among human herds?

In the particular case of mouse typhoid—and almost certainly in other epidemic infections—it is quite certain that the great majority of the mice at risk are infected by the bacterial parasite at some period or other during a prolonged epidemic prevalence.

In a particular experiment (Topley, Ayrton and Lewis 1924) 5 mice were fed on a culture of *Bact. aertrycke*. As soon as they were found to be excreting the bacillus in their faeces, 20 normal mice were added, and thereafter 1 normal mouse was added to the cage each day for 115 days. The faeces of every mouse were examined on 6 days a week during the course of the experiment; each mouse that died was examined bacteriologically, and at the termination of the experiment all surviving mice (55 in number) were killed, cultures were taken from their spleens, and their blood was tested for agglutinins. The existence of non-fatal, and apparently mild or trivial, infections was clearly demonstrated. Some mice that excreted *Bact. aertrycke* on several occasions remained in apparent health during the whole period of their residence in the cage. When these were killed at the end of the experiment some gave negative spleen cultures and showed no agglutinins in their blood, others gave negative spleen cultures but possessed agglutinins, others gave positive spleen cultures with or without agglutinins in the serum. Taking, as criteria of infection, death from the infective disease, excretion of the organism in the faeces, and the isolation of the organism, or the demonstration of agglutinins, in the surviving mice, it was found that of 128 mice which had resided in the cage for periods of 14 to 115 days, 122 had become infected. Of these infected mice 46 were surviving in apparent health on the day when the experiment terminated, but 25 of them (54·3 per cent.) were found to be harbouring *Bact. aertrycke* in their spleens. The collected figures for eleven different epidemics of mouse typhoid, lasting from 60 to 117 days, show that of 267 surviving mice 190 (71·2 per cent.) were latently infected (Topley 1926).

The way in which the bacterial parasites are distributed among the hosts at risk is then of the same general kind as obtains in natural epidemics in man, differing mainly in the fact that fewer hosts escape infection. Is there any evidence that the spread of infection results in immunization as well as, or in place of, the production of overt disease? That survivors from an epidemic are, on the average, more resistant than new-comers to an infected herd is certain; for they live longer—usually much longer—when exposed to a subsequent wave of mortality (Topley 1921, Amoss 1922).

This increase in resistance with increasing herd experience under epidemic conditions can be studied in greater detail by constructing life tables for the mice submitted to risk of infection during a long-continued prevalence of such a disease as mouse typhoid. It is convenient in calculating the expectation of life to limit it to 60 days, regarding any mouse living for longer periods as dying on the 60th day, since the use of the unlimited expectation of life gives undue weight to the relatively few mice that live for much longer periods, and also because we have a normal standard of comparison for the shorter period, but none for the latter.

Employing this value (${}_{60}E_x$) we find in a particular experiment, in which 3 normal mice were added to an infected herd each day from March 6th, 1921, to June 30th, 1923, that the limited expectation of life drops slightly (from 22·49 to 21 days) during the first week

of herd life and then rises, at first slowly, but more steeply after the 15th day, until by the 33rd day it reaches the figure of 34.75 days. It fluctuates round this figure up to the 100th day of herd residence; after which the numbers of survivors are too small to give a reliable average. In general then, we may say that a mouse that has survived about 30 days' exposure to risk will live more than half as long again as a new-comer to the cage (Greenwood and Topley 1925).

We can ask the same question in another way, using a direct experimental comparison instead of a life table. In a particular experiment (Greenwood, Newbold, Topley and Wilson 1926) in which mouse pasteurellosis was the infection selected for study, two parallel epidemics (A and B) were initiated on November 11th, 1924, and were maintained by the addition of normal mice until December 1925. At regular intervals groups of mice that had survived for 10, 20, 30, 40, 50 days or more in Herd A were transferred to Herd B, and with them were added a numerically equal group of normal mice that had had no previous experience of pasteurellosis. Averaging the results, the figures shown in Table XXVIII were obtained:

TABLE XXVIII

Length of Life in Herd A.	Expectation of Life in Herd B ($60E_x$).
Nil (normal entrants to B).	22.37 $\pm$ 0.36 days
10 days	21.34 $\pm$ 1.10 „
20 „	25.50 $\pm$ 1.13 „
30 „	32.55 $\pm$ 1.28 „
40-45 days	33.08 $\pm$ 1.52 „
50-60 „	37.39 $\pm$ 1.69 „

Here, again, survival through a testing period of 30 days or more added some 50 per cent. to a mouse's expectation of life under severe epidemic conditions.

But clearly we cannot, in the absence of further evidence, assume from such results that the mice have been actively immunized. We are dealing with fatal diseases, and natural selection has certainly been at work. In the mouse typhoid experiment, of every 1,000 mice living on day 0 (their day of entry to the herd) only 256 survived for 30 days. Death had by then removed some 75 per cent. of those at risk; and, if no active immunization had occurred, the surviving 25 per cent. would have a higher average resistance than new-comers to the herd, assuming only that the natural resistance of mice differs *inter se*, and that this difference plays some significant part in determining survival during the first 30 days—an assumption that may be very safely made. Webster and his colleagues (see Webster 1923*a, c, d*, 1924*a, b*, 1926, Pritchett 1925, 1926), consider that this natural, inborn resistance is the dominant factor in determining death or survival during an epidemic. That it plays some part there can be no reasonable doubt, but there are reasons for doubting its entire dominance, and for believing that active immunization is exerting an effect similar in kind, if less in degree, to that exerted in the spread of diphtheria in man.

Thus (Greenwood *et al.* 1926, 1928, Newbold 1927), if the method of partial correlation is applied to the data for mouse typhoid and mouse pasteurellosis, and correlation coefficients are calculated for:

(A) length of after-life and length of previous exposure, keeping constant the death rate during previous exposure and the death rate during after-life, and

(B) length of after-life and death rate during previous exposure, keeping constant the length of previous exposure and the death rate during after-life, we get the figures set out in Table XXIX.

TABLE XXIX

Coefficient.	Nature of Epidemic.		
	Pasteurellosis. (1)	Pasteurellosis. (2)	Mouse Typhoid.
A	0.194 $\pm$ 0.016	0.150 $\pm$ 0.015	0.307 $\pm$ 0.014
B	0.029 $\pm$ 0.017	- 0.018 $\pm$ 0.016	- 0.282 $\pm$ 0.014

Coefficient A is positive and significant in all cases, indicating that the longer a mouse has survived within limits under constant conditions as regards death rate, the longer it will continue to survive under continued exposure to infection. Coefficient B is insignificant in the two pasteurellosis experiments, significant but negative in the mouse typhoid experiment, indicating that, with equal lengths of exposure to risk, the survivors from a period of high mortality have no significant advantage over the survivors from a period of lower mortality. This latter finding clearly suggests that the elimination by death of the more susceptible mice is not a very important factor in raising the average resistance of the survivors, while the advantage derived from prolonged exposure at a constant death rate is compatible with the view that active immunization is important. The exact significance of these results is, however, uncertain. It is probable that some at least of the mice emerging from a period of high mortality will already be suffering from an infection which will cause their death in a few days or weeks, apart altogether from any subsequent risks, while it may be that fewer of the mice emerging from a more prolonged exposure to a lower rate of herd mortality are in this particular position.

The probability that active immunization is an important factor is, perhaps, increased by the observation that, in the transfer experiment with pasteurellosis, mice that had lived in herd A only during periods of low death rates—less than one-third the average death rate for the whole epidemic period—still showed a marked advantage over normal mice on transference to herd B. The limited expectation of life of those normal immigrants that entered herd B on the same days as these particular groups of migrants from herd A was 20.9 ± 0.49 days, for those migrants that had spent 50 days in herd A it was 34.6 ± 4.10 days.

It may be noted also that such evidence as is available (Topley, Wilson and Lewis 1925, Greenwood and Topley 1925) indicates that the increased resistance gained by survival in an infected herd, or after experimental infection by feeding or inoculation, is specific in the ordinary bacteriological sense. A mouse that has survived infection with *Bact. aertrycke* appears to be no more resistant than normal mice to infection with *Pasteurella muriseptica*. This, of course, accords well with epidemiological experience in general, but not with the view that the determining factor in increasing the average resistance of a herd at risk is the selection by the sieve of death of those individuals possessing an innate non-specific resistance. If differences in innate resistance are of major importance it would appear that they must either be specific or must be differences of immunizability rather than that of immunity.

Whether or not active immunization is the main factor that determines the increased average resistance of surviving mice, the grade of antibacterial immunity produced can excite little enthusiasm.

As stated above, the expectation of life limited to 60 days was selected in these and subsequent experiments in part because there is an available standard of reference for

this period (Greenwood, Topley and Wilson 1931*b*). On October 4th, 1929, 20 normal mice were assembled in a cage of the same type as that used in all epidemic experiments. From then onwards until January 17th, 1930, daily additions of normal mice were made, and the herd was then observed until May 27th, 1930. The 329 mice of this experiment were living under exactly the same conditions as the infected herd, except that no infection was present at any time during the period of observation. Of these 329 mice 56 died. The limited expectation of life, as was to be expected, remained practically constant at all cage ages from day 0 to day 170—the last day for which figures were available. The lowest ${}_{60}E_x$ figure was 56.69 days, the highest 59.27 days. The figure rose slowly from just under 57 days on the day of entry to over 58 days on the 40th day of cage life—presumably as the animosities of first acquaintance were replaced by the toleration of later herd life and fighting grew less frequent. Thereafter it hardly varied.

Taking a figure of 58 as our normal ${}_{60}E_x$, we note that the best that natural immunization against mouse typhoid or mouse pasteurellosis can do—so long as surviving mice are exposed to a continuous risk of heavy infection—is to raise the expectation of life from a little under a half to a little over two-thirds of the normal figure. This is a very different picture from that presented by our study of diphtheria in man—even allowing for the fact that in the latter case we are dealing with a disease of relatively low fatality. Either mice differ in some fundamental way from men; or the conditions in mouse cages differ fundamentally from those in schools and institutions, an obvious possibility; or natural immunization against enteric infection or against pasteurellosis is less efficient than natural immunization against diphtheria or scarlet fever. Perhaps, though by no means certainly, this might be expanded to the conclusion that natural antitoxic immunization is much more effective than natural antibacterial immunization.

There is no known disease of mice that presents any close analogy with such toxæmic human infections as diphtheria and scarlet fever; but the description by Marchal (1930) of a natural virus disease of mice—ectromelia—that gives rise to severe and fatal epidemics under experimental conditions has afforded an opportunity for the study of natural immunization against a virus infection. The results obtained present a striking contrast to mouse typhoid or mouse pasteurellosis.

Adopting the same plan of starting an epidemic in a herd of mice and thereafter adding 3 mice a day, two epidemics of this disease have been observed over a period of some 18 months. Applying the ordinary life-table method it was found in one of these epidemics—the results in the other were entirely analogous—that the limited expectation of life (${}_{60}E_x$) on entry to the infected herd was 30.71 days. It fell until the 7th day when it was 25.39 days, this fall obviously depending on the average time taken to contract infection. From then onwards the ${}_{60}E_x$ value rose; but instead of reaching a maximal value in some 30 days at a figure far below the normal expectation of life it continued to rise until, on the 100th day after entry, it had reached the figure of 54.69 days, and thereafter fluctuated slightly above and below this value over a period of some 15 months. In other words, instead of experience and survival in the infected herd resulting, at the best, in an expectation of life of some two-thirds of the normal span, those mice that lived through some 3 months or more of the epidemic prevalence had an expectation of life little inferior to the normal ${}_{60}E_x$ value of 58 days, though they continued to live in a herd in which the specific infection was causing a high and continuous death rate.

A more prolonged experience of the same epidemic prevalence has shown that waves of exceptionally high mortality occur during which many of the

old survivors succumb; but this fact does not abolish the significance of the marked difference in behaviour between a bacterial and a virus infection over epidemic periods that last through a large part of the lifetime of a normal mouse. It seems fairly clear that, even under conditions of severe and continuous exposure to infection, a mouse can develop a relatively effective immunity to ectromelia.

How far may the course of events in an infected herd be modified by the artificial immunization of some or all of the hosts at risk?

We shall discuss in a later chapter the evidence with regard to the efficacy of prophylactic vaccination in human herds. So far as experimental epidemiology is concerned we can, at the moment, attempt an answer only in the particular case of mouse typhoid. It is quite certain that active immunization with a killed culture of *Bact. aertrycke* will increase the resistance of mice to that organism; and although some of the earlier observations (Loeffler 1906, Wolf 1908, Yoshida 1909, Brückner 1922, Webster 1922, Ornstein 1922, Neufeld 1924, Lange and Yoshioka 1924) suggest that the immunity obtained in this way is relatively poor and inconstant, repeated trials with considerable numbers of mice, and with carefully adjusted doses of vaccine and of living culture, indicate that a quite significant increase in resistance can regularly be obtained (Topley 1929, Topley, Wilson and Lewis 1925).

Thus, in one experiment, 50 mice were immunized by two intraperitoneal injections of 5×10^8 *Bact. aertrycke* killed by formalin and heat. A week after the second injection these 50 immunized mice, together with 50 normal controls, were injected intraperitoneally with 1,000 living *Bact. aertrycke*. The results are shown in Table XXX.

TABLE XXX

	No. Inoculated.	No. Died in 28 Days.	Average Time to Death of Mice that Died.	No. Survivors with Positive Spleen Cultures.
Normal	50	50	5.0 days	—
Vaccinated . . .	50	29	18.3 „	12

This vaccine had, therefore, produced the following effects: a proportion of the immunized mice showed a partial but ineffective immunity in that they lived on the average far longer than the controls but succumbed to the disease during the period of observation; 24 per cent. showed a more effective resistance by living in apparent health throughout the period of observation, but post-mortem examination showed that they were suffering from a latent infection; 18 per cent. appeared to have been rendered completely immune to this dose of bacteria, in that they remained in apparent health for 28 days and showed no evidence of infection when killed and examined by spleen culture. These results may be taken as representative of a considerable number of similar experiments.

We should not perhaps expect an immunity of this order to be very effective as a prophylactic measure in herds submitted to a continuous risk of infection, and actual trial confirms our lack of faith.

An epidemic of mouse typhoid was started on January 1st, 1928, and was continued until September 17th, 1929, by the addition of 3 normal mice a day (Greenwood, Topley and Wilson 1931a). Every 7th day 10 additional normal mice, and several groups, each of 10 mice, that had been immunized with different vaccines were added to the cage. The results, as they concern the relative efficacy of different antigenic components, have already been noted on p. 131. We are concerned here only with the results obtained with the most effective vaccines employed.

The limited expectation of life ($_{60}E_x$) of the normal mice on entry was 26.26 ± 0.641 days; for the four immunized groups that had received vaccines containing the smooth surface antigen the $_{60}E_x$ figures were 32.10 ± 0.432 , 34.97 ± 0.845 , 35.06 ± 0.845 and 33.71 ± 0.853 days respectively. Thus by active immunization with a killed vaccine an increase in average resistance was obtained of the same order as that attained naturally by normal mice after living for some 30–50 days in an infected herd under the joint influence of active immunization and mortuary selection.

After 60 days' residence in the cage the $_{60}E_x$ figures for the vaccinated and unvaccinated groups have, as we should expect, largely levelled up, though the vaccinated mice still show a slight advantage. The figure for the unvaccinated group has risen to 36.47 days, those for the vaccinated groups to 40.17, 37.39, 36.90 and 47.54 days respectively.

Very similar results were obtained in another experiment in which a comparison was made between the effect of administering a killed *Bact. aertrycke* vaccine by the mouth, or by intraperitoneal inoculation (Greenwood, Topley and Wilson 1931c). A larger dose was used for *per os* than for intraperitoneal immunization ($5,000 \times 10^6$ as against 500×10^6 bacilli). In each case two doses of vaccine were given with a week's interval between them, and the mice were added to the infected herd 1 week after the second dose of vaccine. The experiment lasted from December 12th, 1929, to June 26th, 1930. The death rate in this epidemic was rather lower than in those referred to above, and the expectation of life of the normal mice on entry was therefore somewhat longer. The $_{60}E_x$ figures on the day of entry were as follows: normal mice 31.18 ± 1.04 days, mice vaccinated *per os* 35.30 ± 1.22 days, mice vaccinated intraperitoneally 38.19 ± 1.17 days.

The evidence so far recorded may conveniently be summarized in tabular form (Table XXXI). The $_{60}E_x$ figures for the surviving mice have been recorded after that period of exposure (30–100 days) when the full advantage appears to have been attained, and the limited expectation of life has reached relative stability. For the uninfected standard herd the figure of 58 days has been taken both for new entrants and for survivors at all periods. The two sets of figures for the vaccinated mice in the second mouse typhoid experiment refer to the least and most effective of the four antigenically similar vaccines employed.

The significance of the figures is obvious. With such bacterial infections as mouse typhoid and pasteurellosis residence and survival in an infected herd increases resistance, as measured by expectation of life, but never raises it to the normal level. However long a mouse has lived in such a herd it is never indifferent to the risk that it continues to run. There is no solid immunity.

In the particular case of mouse typhoid, active immunization, by the intraperitoneal injection of two massive doses of a vaccine with an optimal antigenic character, will induce an increase in resistance of the same order as that attained after long residence in the herd. This increased resistance on entry is augmented by life in the infected herd; but vaccination followed by exposure to risk never raises the resistance to the normal level. Here, again, solid immunity is not attained. The effect of *per os* immunization is similar in kind to that of intraperitoneal immunization, though the resistance induced is slightly inferior.

TABLE XXXI

SHOWING LIMITED EXPECTATION OF LIFE ($_{60}E_x$), IN DAYS, FOR VARIOUS GROUPS OF MICE SUBMITTED TO EPIDEMIC INFECTION.

Infection.	Normal.		Vaccinated.			
			I.P.		P.O.	
	N.E.	S.	N.E.	S.	N.E.	S.
None	58.00	58.00	—	—	—	—
Mouse Typhoid	22.49	34.75	—	—	—	—
Mouse Typhoid	26.26	36.47	32.10 35.06	40.17 36.90	—	—
Mouse Typhoid	31.18	42.40	38.19	49.52	35.30	44.87
Pasteurellosis	22.37	37.39	—	—	—	—
Ectromelia	30.71	54.69	—	—	—	—

I.P. = Intraperitoneal.
N.E. = New Entrants.

P.O. = *Per os*.
S. = Survivors after 30-100 days.

In the case of the virus disease, ectromelia, the natural immunizing response is far more effective. The immunity attained is not complete—old survivors sometimes die of typical ectromelia, even though they have passed through a characteristic attack many months earlier—but it is of a high order.

There are as yet no recorded data with regard to the effect of active immunization in pasteurellosis or in ectromelia.

It may also be noted that in the case of mouse typhoid, and with immunization by two doses of an optimal vaccine, the immunization of *all* entrants to a herd will not suffice to suppress the infection, or even to ameliorate appreciably its final effects.

In an experiment designed to test this point (Greenwood, Topley and Wilson 1931c) 3 normal mice were added daily to an infected herd from March 15th, 1929, to May 12th, 1929. The limited expectation of life on entry of these mice was 27.00 ± 1.019 days. From May 13th, 1929, to October 20th, 1929, 3 immunized mice were added daily instead of the 3 normal mice, and from October 21st, 1929, to June 29th, 1930, 1 immunized mouse was added daily to see whether slowing the rate of immigration would have any significant effect on the epidemic prevalence. The $_{60}E_x$ figure for the immunized mice added during the three-a-day period was 34.92 ± 0.684 days, for those added during the one-a-day period it was 36.38 ± 0.938 days—results entirely comparable with those recorded above. But mouse typhoid was spreading and killing as actively in June 1930 as it was in March 1929. The mice at risk lived a little longer, but that was all.

It would, of course, be quite wrong to conclude—even if we may justifiably argue from mice to men—that active immunization of such a kind is useless. As has been noted above, it is capable of completely protecting a proportion of mice against a single experimental infection with a dose of living bacilli that is fatal to unvaccinated controls. Under conditions in which exposure

to risk is slight, transient or intermittent, a resistance of this order may make all the difference between escape and infection, or between life and death. The lesson, so far as it is applicable, would appear to be that such resistance does not allow its possessor to ignore the risk of infection. We could not, by universal immunization of such a kind, stamp out a disease irrespective of the sanitary environment.

The Rôle of Bacterial Variation in Herd Infection and Immunity.

This problem, which may well be of major significance for the student of infective disease, is at the moment in a rather curious position. Judging from much of the current epidemiological literature one would assume that the primary question—Do changes in bacterial characters play an important part in epidemic happenings?—had been answered clearly and decisively on a basis of incontrovertible evidence. There is free reference to "epidemic" strains of particular bacteria, to loss and gain of virulence and infectivity during an epidemic prevalence, to secular changes in the pathogenic powers of a particular parasite. But nearly all such writing is really only the writer's way of saying that the disease in question was more prevalent, or more fatal, or spread more rapidly at one time than at another. The difficulty, which has seldom been faced, is that a description of epidemic events is, of necessity, a description of a fluctuating equilibrium between parasite and host. For an increase in bacterial virulence we can substitute a decrease in host resistance, or *vice versa*; the effect, *on balance*, is the same. If our descriptions are to be used as evidence in solving this particular problem the rise in virulence, or the fall in resistance, must be proved, not assumed; and the proof is no easy matter.

That changes in host resistance are quite certainly of primary importance will be obvious from our consideration of the natural history of diphtheria or scarlet fever in man, and of the happenings in herds of mice. Is there any evidence that changes in the infectivity or virulence of the bacterial parasite plays an equally significant part?

Of indirect and suggestive evidence there is plenty. We know that bacteria frequently undergo variation in the laboratory; and we know that avirulent or atoxigenic variants may be isolated from man or animals (see for instance Reimann 1927, Wadsworth and Sickles 1927, Okell 1929). We know also that antibodies acting on the surface antigens of the smooth virulent forms of bacteria are in many cases capable of inducing the S→R variation (see for instance Griffith 1923). We know that many epidemic diseases have displayed wide secular fluctuations in their fatality. Taking our biological and epidemiological knowledge as a whole, the conclusion that bacterial variation plays an important part in epidemic happenings seems so probable that an experimental answer in the opposite would certainly be suspect—suspect to the extent that we should want to be very sure that our experiments had been properly planned, and that our conclusions had not been arrived at by an unjustifiable inductive generalization from a few particular instances. Experiment, combined with carefully controlled bacteriological and immunological observations in naturally occurring epidemics, must, of course, be our final court of appeal; but neither from this source nor from any other should we accept hasty judgments.

The experimental data at present available may be summarized as follows.

Webster and his colleagues (Webster 1923*a, b, c, d*, 1924*a, b, c, d*, 1925, 1926, 1927, 1928, 1930*a, b, c*, Webster and Burn 1926, 1927*a, b*) have shown quite clearly that different strains of the same bacterium, isolated at different periods of the same epidemic prevalence, may possess an approximately equal virulence as judged by direct inoculation tests, including inoculation into the stomach through an oesophageal tube. This, indeed, was their constant finding with the strains they examined. They conclude that changes in bacterial virulence play no part in the fluctuations in mortality that may be observed at different stages of a long-continued epidemic prevalence. These they would regard as due to changes in the average host resistance, and particularly to innate differences among the hosts at risk, or to seasonal or dietetic factors, that affect resistance in some non-specific way. They have, however, recorded differences in virulence among strains of the same bacterial species isolated from different epidemic prevalences of the same infective disease (Webster 1930*d*, Hughes 1930, Hughes and Pritchett 1930, Pritchett, Beaudette and Hughes 1930*a, b*), and they would regard these differences in virulence as determining certain observed differences in behaviour between one epidemic and another—for instance, differences in the proportion of carrier infections to fatal cases of the disease.

We have so far considered variations in *virulence*; but virulence may not be the only factor concerned. If we assume the possible existence of another factor, labelling it *infectivity* and defining it as the *capacity to spread from one host to another under any specified conditions of exposure to risk of infection*, we may inquire whether infectivity is so highly correlated with virulence that we may regard them as synonymous terms.

The evidence available (Topley, Greenwood, Wilson and Newbold 1928) suggests, as would be expected, that those strains of *Bact. aertrycke* that are capable of spreading rapidly among a herd of mice, giving rise to severe epidemics of mouse typhoid with a high mortality rate, are always of relatively high virulence as judged by direct intra-peritoneal inoculation, and that strains of low virulence are incapable of giving rise to severe epidemics, or of spreading widely among the herd at risk, although they may persist for long periods in the tissues of the originally infected hosts, and may give rise to a few latent infections, or even to an occasional death, among the normal mice exposed to risk.

The correlation between virulence and the power to induce severe epidemics by contact infection does not however hold with all strains of *Bact. aertrycke*. A particular strain of this organism, isolated originally from an infected mouse, was found to possess an unusual combination of characters (Topley, Greenwood and Wilson 1931). It was of moderately high virulence, killing in repeated tests some 65–75 per cent. of mice inoculated with 100–1,000 bacilli, but it possessed little power of producing severe epidemics by contact infection. In six closed epidemics, in each of which 100 normal mice were exposed to infection from 25 infected companions, the average limited survival time of the exposed mice ($_{60}E_x$) was 51.27 days—not far short of the normal limit. The proportion of the survivors harbouring *Bact. aertrycke* in their spleens at the end of the period of observation was however high—an average of 31 per cent. for the six epidemics. Data were available for two other groups of epidemics, initiated with two strains of *Bact. aertrycke* of low virulence as tested by direct inoculation. In one group the average $_{60}E_x$ was 52.62 days, in the other 52.21 days—figures almost identical with those obtained with the more virulent strain—but the percentage of infected survivors was 9.7 in one group and 9.0 in the other, less than one-third of the figure recorded for the relatively virulent strain. This strain had therefore the following combination of characters: a relatively high virulence when injected into the tissues, a considerable power of contact spread with the production of latent infections, but a relatively slight power of producing fatal contact infections.

It was of obvious interest to test the resistance to contact infection, with a highly

virulent and infective strain, of survivors from these epidemics of latent infection, and to compare it with the resistance of survivors from more fatal epidemics and with that of artificially immunized mice. An experiment carried out along these lines gave the results shown in Table XXXII.

TABLE XXXII

	Average $60E_z$.	Per cent. Surviving on 60th Day.
Normal mice	37.49 ± 2.613	36.7
Vaccinated mice	41.95 ± 1.529	53.0
Survivors of severe epidemics	48.31 ± 1.836	66.0
Survivors of mild epidemics	48.75 ± 1.861	69.0

Thus the survivors from these mild epidemics were much more resistant than normal mice, significantly more resistant than vaccinated mice, and just as resistant as the survivors from epidemics of greater severity, involving a more stringent mortuary selection. The strain that gave rise to these mild epidemics might, perhaps, be regarded as one in which the natural immunizing activity was greater than the natural killing power. Clearly, if a strain of this character made its appearance during an epidemic prevalence it *might* exert a significant influence on the course of events. But we cannot disregard the *if*.

In recent unpublished experiments results have been obtained that seem to afford a satisfactory proof that changes in infectivity do occur during the epidemic spread of a bacterial parasite. Certain strains of *Pasteurella* that originally possessed little power of contact infection, though they were of moderately high virulence as judged by direct inoculation into mice, have, during their slow and limited spread in an experimental herd, given rise to variants in which the capacity for rapid and fatal spread was well developed. These experiments, like that recorded above, do not indicate a complete correlation between virulence and infectivity. If we adopt a term that is very commonly, though a little loosely, employed in current literature, and speak of an *epidemic strain* of a particular bacterial species, defining it as a *strain that has the capacity to spread naturally among a herd at risk giving rise to a severe and fatal epidemic*, we might hazard the guess that an epidemic strain is characterized by two attributes, high virulence and high infectivity, and that it may lose its epidemic character by losing either attribute. On what infectivity, as apart from virulence, depends we have as yet no knowledge. It does not depend primarily on an ability to multiply *in the tissues*; for a strain may be highly virulent but not highly infective. It must presumably depend on adaptations that enable it to meet those environmental changes that are associated with transfer from host to host, and to establish in the recipient host a primary focus of infection from which subsequent tissue invasion can occur. But to say this is merely to define our problem a little more closely.

In summary; variations in virulence in a bacterial parasite during an epidemic prevalence have often been sought for, in most cases with negative results. There is experimental evidence that different strains of the same bacterial parasite, possessing different degrees of virulence or of infectivity,

produce very different results when they are allowed to spread by natural infection among a susceptible herd. There is evidence that a bacterial parasite may vary in infectivity during an experimental epidemic; but there is as yet no evidence from which we can judge how often such variations occur, or how important a part they play under natural conditions.

The Rôle of other Factors in Herd Infection and Herd Immunity.

All those factors that affect the resistance of the individual will affect the average resistance of the herd of which he is a member. Those factors that have been adequately studied have been considered more or less briefly in earlier chapters.

There are, as we have said, special factors, such as the spatial distribution of the hosts at risk, the duration or the intermittency of contact, and other conditions of arrangement or environment, that affect herds but not individuals. Some of these have been studied, in a tentative and preliminary fashion, by direct experiment. Many have been studied in far greater detail but with less control under field conditions. Some day we may be able to work these factors into our immunological picture in terms of the effects produced by changes in the rate at which infection is received, or in the rate at which immunization proceeds (see for instance Dudley 1923); but that day has not come yet.

SUMMARY

Both observation and experiment show that the spread of infective disease is a highly complex process, in which the overt cases form only a small part of the changes occurring in the total reacting system—the infected herd, the infecting parasites, and their mutual environment.

Endemic and epidemic diseases are associated with endemic and epidemic immunization. Sometimes one aspect of infection predominates, sometimes the other. Such natural immunization is sometimes very effective, as in human diphtheria, sometimes, as in experimental mouse typhoid, it is of little avail. So far as the evidence goes at present, the purely toxæmic infections and many of the virus infections appear to be more potent immunizers than are many bacterial infections.

Vaccination with killed bacterial suspensions enables us, in the particular case of mouse typhoid, to confer on normal mice a partial immunity of the same order as that attained by exposure to risk in an infected herd. We can, that is, produce by artificial immunization an effect of the same kind as that produced under natural conditions by the combined action of immunization and mortuary selection. We have as yet no data in regard to the efficacy of artificial immunization in other epidemic diseases of mice. The efficacy of prophylactic immunization in human herds will be considered in Chapter XIX.

In attempting to solve any problem of mass prophylaxis *the herd must be considered as a whole*. The shift in equilibrium produced in the reacting system by any intentional interference must be examined from this point of view,

not solely in relation to those individuals whom the interference immediately concerns.

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CHAPTER XVIII

THE PRACTICAL APPLICATION OF IMMUNITY IN DIAGNOSIS

IN this and the following chapters we shall consider a few examples of the application of immunological methods in the diagnosis, prophylaxis and treatment of disease. These examples have been selected from the multitude that present themselves for study because they illustrate one or other of the principles that must be observed in all work of this kind. For details of technique and for a discussion of the many diagnostic tests and prophylactic or therapeutic methods of which no mention can here be made the student is referred to one of the many textbooks dealing specifically with this branch of practical medicine. In the present chapter we shall deal with problems of diagnosis.

THE DIAGNOSIS OF INFECTION

It should be clearly recognized as a general principle that no indirect method of immunological diagnosis can afford evidence of infection as certain as that obtained by the direct isolation of the specific causative organism from the patient's blood, tissues or excreta.

Taking the superiority of direct bacteriological diagnosis as axiomatic, the next point to emphasize is that a clear appreciation of the limitations to which any particular immunological test is subject is of primary importance in attempting to assess the significance of the results obtained.

Here again we shall be wise to think in terms of odds, or probabilities. We cannot often give these odds in the form of numerical values ; but, in the better documented reactions at least, we can define orders of probability, differentiating between those that approach certainty, those that afford significant diagnostic indications, and those to which little importance can be attached. Such differentiation is very necessary. Except in those cases where the result of a laboratory test is unequivocal it will form only a part of the evidence on which a diagnosis is based, and the clinician should know just how far he is to weight his clinical observations by the findings of the laboratory worker.

Among the methods commonly employed in immunological diagnosis are the following.

The Demonstration of Specific Antibodies in the Blood.

Any of the specific sensitizing antibodies may be selected as an indicator of infection, that reaction being chosen which is most likely to reveal the

presence of the antibody in question. Of the reactions in common use, agglutination perhaps holds pride of place, and will usually be employed when the specific antibodies are likely to be present in sufficiently high titre. It may, therefore, be considered first.

Agglutination.—The best illustrative example of the use of this method is that afforded by the group of enteric fevers—the typhoid and paratyphoid infections.

It will be convenient to discuss the factors that must be considered in performing this test, and in interpreting the results obtained, in the form of answers to a series of questions that, *mutatis mutandis*, we must ask ourselves when faced by any problem of this kind.

(1) *Is the clinical condition that we are attempting to diagnose a bacteriological as well as a clinical entity?*

(2) *If not, how many different bacterial species or types are able to produce this particular clinical condition, and how are they related to each other antigenically?*

(3) *If the number of species or types is large, is it necessary to include them all in our test, or may we be satisfied with a more limited range?*

(4) *If the agglutination of any one species or type may be brought about by different antibodies acting on different antigens, have these antibodies a different diagnostic value, so that we must determine the presence and titre of each apart from the other?*

(5) *Must our determination be quantitative as well as qualitative? If so, how far should our reagents and methods be standardized?*

(6) *At what stage of infection are agglutinins likely to appear, and when do they attain their highest titre?*

(7) *What proportion of cases develop agglutinins at each stage of the disease, and what proportion give a negative reaction throughout?*

(8) *What are the frequency and limits of titre of the antibodies with which we are concerned in the blood of normal members of the population, or of subgroups of the population that have been differentiated by some particular experience?*

(9) *Is any advantage to be gained by studying fluctuations in antibody titre, in addition to determining the titre of a particular antibody at a particular time?*

When we have answered these questions we shall be in a better position to assess the significance of our findings in any particular case.

THE AGGLUTINATION TEST IN ENTERIC FEVER.

(1) *Is enteric fever a bacteriological as well as a clinical entity?*

It is not.

(2) *How many different bacterial species or types may cause enteric fever, and how are they related to each other antigenically?*

So far as we know at present there are some twelve species or types within the salmonella group that are capable of causing a continued fever in man, though the majority of these more commonly give rise to acute gastro-enteritis. It is probable that other types, not yet recognized, will be found to have this faculty, and that some of the types which have up to the present been found

only in association with acute gastro-enteritis will be found capable of causing an illness of the enteric type under exceptional conditions.

In Table XI, p. 103, the antigenic structure of these twelve types has been set out in tabular form. Of these twelve types, *Bact. typhosum*, *Bact. paratyphosum A*, *Bact. paratyphosum B* and Hirschfeld's bacillus are by far the most important causal organisms in enteric fever; but their geographical distribution is very different. *Bact. typhosum* has an almost world-wide distribution. The frequency of *Bact. paratyphosum A* infections would seem to vary rather widely from time to time and from place to place. At the present time *Bact. paratyphosum B* is, in this country and in many other parts of the world, a very frequent cause of enteric fever. Hirschfeld's bacillus is particularly frequent in Eastern Europe, parts of Asia and in British Guiana (see Giglioli 1930). The monophasic Western type of *Bact. paratyphosum C* has now been recorded as the infecting agent in several cases of enteric fever occurring in Western Europe, but its frequency does not seem to approach that of the types mentioned above. The remaining types have, up to the present, been isolated only in sporadic cases or in small outbreaks. *Bact. aertrycke*, a natural pathogen of rodents, usually causes acute gastro-enteritis in man, very rarely a continued fever of the enteric type. The Dublin type is of interest, in that it is associated with an intestinal infection in cattle as well as with a continued fever in man (Smith and Scott 1930). *Bact. enteritidis*, when it infects man, usually gives rise to acute gastro-enteritis. There are records in the literature which suggest that it may sometimes cause infection of the enteric type; but in view of its close antigenic relation to the Dublin type such records must be treated with reserve.

We are not yet in a position to give any adequate description of the distribution of the less well-known members of the salmonella group as human pathogens in various parts of the world. The differentiation of this group by adequate methods of antigenic analysis is recent history, and the application of such methods to a study of continued fevers, especially in tropical and sub-tropical countries, is as yet in its infancy.

(3) *Is it necessary to include all these types or species in our list?*

For routine purposes it is neither necessary nor possible.

Considering first the H, or flagellar, antigens, it will be seen from Table XI, p. 103, that a relatively small number of bacterial suspensions would enable us to detect the presence of flagellar agglutinins acting on the various types or species concerned, *if we could assume that group as well as type agglutinins are always produced in response to infection with diphasic species*. This is a large and unwarranted assumption, but such figures as are available suggest that both types of agglutinin are commonly produced and that we shall detect many, perhaps most, cases of infection with a diphasic species if we include a suspension of one of them in the group phase.

Suppose then that we selected the following series of formalized cultures for the detection of flagellar agglutinins:—

- (1) *Bact. typhosum*.
- (2) *Bact. paratyphosum A*.
- (3) *Bact. paratyphosum B* (type phase).
- (4) Hirschfeld's bacillus (type phase).
- (5) *Any diphasic species in the group phase*.

It will be seen from the table that we shall be able to detect the flagellar agglutinins produced in all the common types of enteric infection, and in some uncommon forms. If the infection is typhoid fever, or an infection with *Bact. paratyphosum A*, *Bact. paratyphosum B* or Hirschfeld's bacillus, we shall obtain an indication of the fact. If it is caused by the Western European type of *Bact. paratyphosum C* or by some diphasic species other than *Bact. paratyphosum B* or Hirschfeld's bacillus, we shall have to go further before we can identify the actual infecting species.

But even this amount of information is more than we usually need for routine purposes, *i.e.*, for the primary examination of specimens of blood sent to the laboratory from suspected cases of enteric fever. We shall usually not be ignorant of the types of enteric infection that are prevalent in the districts in which we are working, and if we limit ourselves to these types the odds will be high that we shall detect the majority of infections.

In this country, for instance, and at the present time, the great majority of all enteric infections are caused by *Bact. typhosum* or by *Bact. paratyphosum B*. We shall be at a very slight disadvantage if we omit a type suspension of Hirschfeld's bacillus, especially as we shall probably detect cases of Hirschfeld infection, without identifying its exact type, by demonstrating the presence of group agglutinins. Whether we can safely omit a suspension of *Bact. paratyphosum A* is a matter of opinion.

In other countries, and probably in this country at some future time, it will be necessary to alter our selection; but it is generally true that, given a knowledge of the prevailing types of infection, it is possible to select a small number (3-5) of formolized suspensions that will in the great majority of cases give us all the information we are likely to obtain by searching for flagellar agglutinins.

(4) *Is it necessary to determine the presence of somatic as well as of flagellar agglutinins?*

It is. The necessity for doing so has been emphasized in particular by Felix (1924*a, b*, 1930), who has studied the development of H and O agglutinins in typhoid and paratyphoid infections as part of a more general investigation into the immunological significance of these two kinds of antibody. The detection and measurement of both kinds of agglutinin is of particular value in circumstances that will be considered in Section (6). For the moment it is sufficient to note that a proportion of typhoid patients develop somatic but not flagellar agglutinins, so that these cases will be missed by the laboratory worker if he looks for flagellar agglutinins only.

This important finding has been confirmed by many subsequent workers (Stuart and Krikorian 1928, Felix and Olitzki 1928, Pijper 1930, Gardner *et al.* 1930). It would appear that the proportion of cases showing O agglutinins only is much higher in some localities than in others. Thus Pijper in South Africa (see Felix and Olitzki 1928) noted 34 persistently negative reactions in 120 cases of undoubted typhoid fever, when testing for H agglutinins only. Some cases of this type, at least, are infected with O variants of the typhoid bacillus (Olitzki 1928, Gardiner *et al.* 1930).

It is equally true that particular samples of serum obtained from typhoid or paratyphoid cases may show flagellar but not somatic agglutinins. Smith (1932) records that 6 of 28 cases of typhoid fever showed H but not O agglutinins when the cases were first examined, while 11 of 42 cases of paratyphoid B fever gave a similar result. Whether a case ever passes

through all its stages developing H but no O agglutinins is as yet uncertain, though it seems unlikely. In any event the lesson is clear—both H and O agglutinins should be searched for in each specimen of blood examined.

A curious feature of the somatic agglutinins in typhoid and paratyphoid fever is that they fail to show the specificity that would be expected from the antigenic structure of *Bact. typhosum*, *Bact. paratyphosum A* and *Bact. paratyphosum B* as determined by analysis with antisera prepared in the rabbit (see Table XI). Thus Felix (1924a) records that, of 28 cases of typhoid fever proved by the isolation of *Bact. typhosum* from the blood or faeces, 10 gave somatic agglutination with *Bact. paratyphosum A* and 21 with *Bact. paratyphosum B*, as well as with *Bact. typhosum*. Of 8 proved cases of infection with *Bact. paratyphosum A*, 6 gave somatic agglutination with *Bact. typhosum* and 6 with *Bact. paratyphosum B*. Of 11 proved cases of infection with *Bact. paratyphosum B*, 6 gave somatic agglutination with *Bact. typhosum* and 2 with *Bact. paratyphosum A*.

Smith (1932) records similar observations. The sera of 21 of 28 cases of typhoid fever showed O agglutinins for *Bact. typhosum* (the sera were collected at all stages in the disease, sometimes very early). Six cases showed O agglutinins for *Bact. paratyphosum B* (1 case was not tested against this organism). Of 42 cases of paratyphoid B fever, 25 showed O agglutinins for *Bact. paratyphosum B* and 20 showed O agglutinins for *Bact. typhosum*.

In general it has been found that the O titre for the infecting organism is higher than for the heterologous organism; and Gardner and Stubington (1932) have shown that, using standardized suspensions and applying an appropriate reduction factor to the observed titres, it is possible in a majority of cases to form a correct opinion with regard to the type of infecting organism. This problem needs much fuller investigation before we can draw any detailed and definite conclusions with regard to the relationships between the somatic antigens of bacilli of the enteric group as a whole and the corresponding antibodies in the blood of infected persons. For the moment we must note that, while the detection and measurement of O agglutinins is a valuable and often necessary step in the diagnosis of enteric infection, it appears to be of much less value in differentiating between one type of enteric infection and another.

(5) *Must our determination be quantitative as well as qualitative, and how far should our reagents and methods be standardized?*

For reasons that will become apparent in later sections it is always desirable to determine the amount of agglutinin in a serum, as well as its mere presence or absence.

The results of agglutination tests are commonly expressed as titres, i.e., as the highest dilution of a given serum that will produce agglutination of a given bacterial suspension under specified conditions of time and temperature. We shall, in all cases, wish to determine the real end-point; so that we must specify conditions that will allow this end-point to be obtained. Somatic agglutination, for instance, is slower than flagellar flocculation. It is usual to employ a geometrical series of dilutions, such as 1:20, 1:40, 1:80, 1:160 and so on, and the observed titre is thus subject to a wide margin of error unless some additional precautions are taken. A rough approximation may be made by noting the last tube to show detectable flocculation of the bacilli. By adopting an arbitrary degree of agglutination as a standard, and applying an interpolation table to the actual readings obtained, a much greater degree of accuracy can be attained (see Dreyer and Inman, 1917).

But our difficulties do not end here. All suspensions of the same bacterial species are not equally sensitive to agglutination; and if we tested a single sample of serum against a dozen suspensions of *Bact. typhosum* prepared from different strains in different ways we might get widely divergent answers. If there were no gross antigenic variations in our strains there would be a limit to such divergence—we should not often find that

the titre given by any one suspension was more than, say, two or four times that given by another; but this is a wide margin of technical error.

A valuable lead was given by the work of Dreyer and his colleagues during the late War (Dreyer, Walker and Gibson 1915, Dreyer and Walker 1916, Dreyer and Inman 1917), and the Standards Laboratory instituted in the Department of Pathology at Oxford under the auspices of the Medical Research Council has since performed an important service in preparing and issuing standard agglutinable suspensions, not only of the flagellated bacilli of the enteric group, but of non-flagellated variants of the same species, and of various other bacteria that are commonly employed in agglutination tests (see Gardner 1920, 1921, 1929). A sensitive suspension of a particular bacterium is adopted as a standard, and any subsequent suspension is compared with this by duplicate testing against an agglutinating serum. The new suspension is then labelled with a number that allows the titre observed with any given serum to be reduced to the titre that would have been observed had the "standard" suspension been employed. Whether this corrected titre is used, or the reduction factor is employed to give an answer in terms of "agglutinin units" per c.c., is a minor question of convention. The significant gain is that comparable figures can be obtained by those working in different places, or by the same worker carrying out tests at different times. How far this gain is an important one depends on circumstances. In general the titres to which diagnostic importance are attached are high, or the differences as compared with normal limits are large. Provided that a sensitive suspension is employed the significance of a single test is in most cases not greatly increased by correcting for this particular technical error; but the value of the data with regard to the frequency and titres of normal agglutinins that we quote in the next section would be greatly increased if a standardized technique were universally adopted, and in connection with the fluctuations discussed on p. 306 such standardization—even if it only takes the form of using a single sensitive suspension for successive tests made on the same patient—is absolutely necessary for reliable results.

For these reasons among others it is obviously better to work with killed and preserved rather than with living suspensions. It is a common practice to use formalized broth cultures for the detection of flagellar agglutinins, and suspensions from solid media after extraction with alcohol—which renders the flagellar antigens insensitive to agglutinins—for the detection of somatic agglutinins. Alternatively, any suitable suspension of an O variant may be used for the latter purpose (Gardner 1920, 1929, Felix and Olitzki 1928).

(6) *At what stage of enteric infection are agglutinins likely to appear, and when do they attain their highest titre?*

Reference to Figures 24, 25, 26, pp. 179, 180, will give a sufficient answer. Flagellar agglutinins usually appear sometime during the 1st week of illness, and commonly attain their maximal titre between the 16th and 21st day; though they may occasionally develop later, or go on rising longer. After the 28th day or so they usually fall; at first rather rapidly, then more slowly. There is some evidence suggesting that somatic agglutinins may appear earlier than flagellar agglutinins.

It follows that little importance can be attached to a negative agglutination test on a sample of blood taken during the 1st week of illness. It does *not* follow that a test made at this time is useless. It is often of the greatest value. If positive, at a reasonably high titre, it will afford an immediate diagnosis; if negative, or positive at a titre too low to be diagnostic, it affords a most valuable base line for studying later fluctuations should these occur (see p. 306).

(7) *What proportion of cases develop agglutinins at each stage of the disease, and what proportion give a negative reaction throughout?*

The answer to this question is rendered somewhat difficult by the fact

that the greater part of the available data refers to tests carried out by the old microscopic technique, or by other methods that fail to distinguish between the different antigenic components involved.

Park and Williams (1925), using the microscopic technique, record the proportion of cases of typhoid fever giving positive agglutination reactions as follows :

1st Week	.	.	.	.	.	.	.	.	.	20 per cent.
2nd "	.	.	.	.	.	.	.	.	.	60 " "
3rd "	.	.	.	.	.	.	.	.	.	80 " "
4th "	.	.	.	.	.	.	.	.	.	90 " "
2nd Month	.	.	.	.	.	.	.	.	.	75 " "

They note that, in those cases in which repeated tests are possible, 98 per cent. show the presence of agglutinins at some time during the course of the disease.

Using modern methods and titrating the O as well as the H agglutinins, it is probably very exceptional for a case to pass through all its stages without showing a significant rise in the titre of agglutinins.

(8) *What is the frequency and concentration of H and O agglutinins for the bacilli of the typhoid-paratyphoid group in the serum of normal members of the population, or of subgroups of the population, that have been differentiated by some particular experience?*

We may, for our present purpose, neglect the older records and confine our attention to recent studies. These studies have been carried out since the War; so that all investigators have been dealing with populations that include, mainly among the adult males, a relatively high proportion of a differentiated subgroup—those who have received one or more inoculations of T.A.B. vaccine while on active service. For this reason it is important where possible to differentiate between the inoculated and uninoculated, and where this cannot be done the grouped results must be regarded as applying only to a particular population at a particular, and exceptional, time.

A valuable record of the London population in 1921 is given by Rosher and Fielden (1922), who examined 1,000 samples of serum sent to the laboratory for a Wassermann test, *i.e.*, from patients in whom there was no suspicion of an existing typhoid infection.

These sera were tested against formolized suspensions of *Bact. typhosum*, *Bact. paratyphosum A*, *Bact. paratyphosum B* and certain other members of the salmonella group at dilutions ranging from 1:20 to 1:640 or more. As our present knowledge with regard to the diphasic variation of the flagellar antigens was not then available it is impossible to be certain which type of agglutinin was concerned in the flocculation of the diphasic species. Taking the results for the three more important species, with this reservation as regards *Bact. paratyphosum B*, we obtain the figures set out in Table XXXIII.

In 330 instances it was possible to obtain a reliable history as to whether the person had or had not been inoculated. The figures for this sub-sample, again taking the titres of 1:20 or over, are set out in Table XXXIV.

It is clear that the relatively high proportion of agglutinating sera among the London population as a whole was, in 1921, determined by the presence of a large number of recently inoculated persons.

TABLE XXXIII

Bacterial Suspension.	Percentage of Sera Agglutinating at 1 : 20 or over.
<i>Bact. typhosum</i>	29
<i>Bact. paratyphosum A</i>	17
<i>Bact. paratyphosum B</i>	23

A similar study was carried out in Manchester a few years later by Smith, MacVie and Newbold (1930); 302 specimens of sera sent to the laboratory for Wassermann tests during the years 1925 and 1926 were tested against formalized suspensions of various bacteria of the salmonella group. In this case type suspensions of the diphasic species were employed, together with a single group suspension to detect the group agglutinins, so that a complete picture of the flagellar agglutinins was obtained over the range of types examined.

TABLE XXXIV

Bacterial Suspension.	Inoculated.		Uninoculated.	
	No. of Sera.	Per cent. +	No. of Sera.	Per cent. +
<i>Bact. typhosum</i>	149	89	181	3
<i>Bact. paratyphosum A</i>	149	58	181	0
<i>Bact. paratyphosum B</i>	149	71	181	4

Taking first the Manchester population as a whole—and including the results with the type phase of Hirschfeld's bacillus, the type phase of *Bact. aertrycke* and the group suspension—the percentages giving agglutination at 1 : 20 or over are set out in Table XXXV.

TABLE XXXV

Organism.	Percentage of Sera Agglutinating.
<i>Bact. typhosum</i>	14.9
<i>Bact. paratyphosum A</i>	5.9
<i>Bact. paratyphosum B</i> (type)	5.9
Hirschfeld's bacillus (type)	0.3
<i>Bact. aertrycke</i> (type)	5.3
"Group" Suspension	6.3

Comparing these figures with those of Rosher and Fielden they show a drop to about a half in the case of *Bact. typhosum*, to about a third in the case of *Bact. paratyphosum A* and to about a quarter in the case of *Bact. paratyphosum B*. Agglutinins for the type phase of Hirschfeld's bacillus appear to be very uncommon in the normal English population. No information was available with regard to the state of inoculation of the persons from whom these blood samples were obtained; but a division into males and females (see Table XXXVI) affords highly suggestive evidence, since the proportion of females inoculated during the War was relatively trivial.

TABLE XXXVI

Organism.	Percentage of Sera Agglutinating.	
	Males.	Females.
<i>Bact. typhosum</i>	23.3	4.7
<i>Bact. paratyphosum A</i>	11.6	0
<i>Bact. paratyphosum B</i> (type)	9.7	2.0
Hirschfeld's bacillus	0.0	0.6
<i>Bact. aertrycke</i> (type)	5.7	4.7
"Group" Suspension	7.0	4.7

It will be noted that the figures of Smith, MacVie and Newbold for females agree fairly closely with those of Rosher and Fielden for uninoculated persons of both sexes, so far as *Bact. typhosum*, *Bact. paratyphosum A* and *Bact. paratyphosum B* are concerned. In the case of the latter organism Rosher and Fielden's figure of 4.0 per cent. must be compared with both the paratyphoid B and group figures of Smith, MacVie and Newbold. That the disparity between the male and female groups is due to inoculation is confirmed by comparing the figures for Hirschfeld's bacillus and *Bact. aertrycke* in the type phase—neither of which are present in the ordinary T.A.B. vaccine with those for the other suspensions.

The fall in the frequency of flagellar agglutinins among the population as a whole is almost certainly due to a wearing-off of the effect of inoculation. Thus, in the case of *Bact. typhosum* Rosher and Fielden found 42 per cent. of agglutinating sera among adult males, compared to the 23.3 per cent. noted by Smith, MacVie and Newbold some six years later.

When we consider titres, as well as the mere presence or absence of agglutinins, the figures provided by Smith, MacVie and Newbold for females (see Table XXXVII) afford a useful guide to the titres we may expect to find in uninoculated adults.

TABLE XXXVII

SHOWING THE PERCENTAGE OF SERA OBTAINED FROM 146 FEMALES NOT SUSPECTED OF ENTERIC INFECTION GIVING AGGLUTINATION AT THE SPECIFIED TITRE OR HIGHER.

Organism.	Titres.					
	1:20	1:40	1:80	1:160	1:320	1:640
<i>Bact. typhosum</i>	4.7	2.7	0.7	0.7	0	0
<i>Bact. paratyphosum A</i>	0	0	0	0	0	0
<i>Bact. paratyphosum B</i> (type)	2.0	1.3	0	0	0	0
Hirschfeld's bacillus	0.6	0.6	0.6	0	0	0
<i>Bact. aertrycke</i> (type)	4.7	4.0	2.0	0	0	0
"Group" Suspension	4.7	2.0	1.4	1.4	0.7	0

Titres of over 1:160 are clearly very exceptional. Below this level there are considerable differences in the frequency of flagellar agglutinins for the different organisms.

As regards the somatic agglutinins we have relatively little information. Felix (1924a, b, 1930) considers that O agglutinins acting on this group are normally present in human sera, and may reach a titre of 1:100. Gardner (1929) records observations on 47 normal persons believed never to have been inoculated. Of these 24 gave no agglutination at a dilution of 1:20, 22 (46.8 per cent.) gave agglutination at a titre between 1:20 and

1 : 50, and 1 (2.1 per cent.) gave agglutination at a titre between 1 : 50 and 1 : 200. Wyllie (1932) failed to detect O agglutinins at a titre of 1 : 25 in 23 of 28 normal persons with no history of enteric infection nor of prophylactic inoculation. The remaining 3 sera gave O agglutination at 1 : 50.

Pending fuller information we may take it that O agglutinins, at least to low titre (1 : 20–1 : 50), are more common than H agglutinins among the normal uninoculated population.

A point of great interest is the relation between H and O agglutinins in inoculated persons. Felix believes that O agglutinins are not produced in response to the ordinary T.A.B. inoculation, and hence that the presence of O agglutinins at a titre of 1 : 100 or over is as reliable as indication of active infection in an inoculated as in an uninoculated person. This view, in its entirety, has not been supported by the results of subsequent workers.

Thus Gardner (1929) found that of 6 persons with a history of inoculation in past years 1 gave O agglutination with *Bact. typhosum* at a titre of over 1 : 200 and of 11 persons inoculated 4 weeks before testing 3 gave titres of this order. To check these findings a healthy adult, who had been inoculated some 14 years earlier, was given 2 inoculations of T.A.B. vaccine and his agglutinins were determined at frequent intervals during the following month. The flagellar agglutinins were raised from a titre of 1 : 185 to a titre of 1 : 1,500; the O agglutinins were raised from zero to 1 : 50; both thereafter sank.

Smith (1932) examined the sera of 17 normal persons who had been injected with typhoid vaccine between the years 1915 to 1929. All 17 showed flagellar agglutinins for *Bact. typhosum* at titres varying from 1 : 50 to 1 : 200; two of them showed O agglutinins, one at a titre of 1 : 50, the other at a titre of 1 : 200. Wyllie (1932) examined the sera of 22 persons who had received prophylactic injections of typhoid vaccine between 1928 and 1931; 13 showed no O agglutinins at 1 : 25, 9 reacted at or above this titre, the highest titre reached being 1 : 100. He adds the interesting observation that of 24 persons who had received intravenous injections of T.A.B. vaccine between October 1929 and September 1930—as a therapeutic measure for rheumatic or other infections—19 gave O agglutination at titres ranging from 1 : 50 to 1 : 250 when examined in July and August 1931.

It seems clear that inoculation with typhoid or T.A.B. vaccine induces a much higher titre of H than of O agglutinins, and that the O titre, at least after the ordinary prophylactic injection, sinks back to the normal level long before the H agglutinins have disappeared. We may then conclude that a relatively high titre of O agglutinins has a far greater diagnostic significance than a relatively high titre of H agglutinins in an inoculated person, especially if that person has not been inoculated very recently. When *very high* H titres are found they become significant even in inoculated persons. It is, for instance, very unusual to find a H titre of much over 1 : 1000 for *Bact. typhosum*, or of much over 1 : 500 for *Bact. paratyphosum A* or *Bact. paratyphosum B* at any period later than 6 months after inoculation.

So far we have considered only the subgroup of our general population that is composed of inoculated persons. There is another subgroup—persons suffering from diseases other than enteric fever—about which we clearly need information. In general it may be stated that experience with the agglutination test in typhoid fever, lasting now over nearly 40 years and extending in all over enormous numbers of cases, has shown quite clearly that the reaction may be regarded as specific within a small margin of error, provided that we are dealing with uninoculated persons and that agglutination at low titre

(less than 1:50 or thereabouts for flagellar agglutinins or less than 1:100 for somatic agglutinins) be excluded.

It would appear, however, that typhoid agglutinins are more frequent in certain infections than in others, or than in normal persons. Thus, for example, Madgwick and Partner (1932) examined the sera of 73 uninoculated asylum inmates suffering from pulmonary tuberculosis; 27.3 per cent. showed H agglutinins for *Bact. typhosum*, 13.6 per cent. for *Bact. paratyphosum A* and 24.6 per cent. for *Bact. paratyphosum B*, in most cases to relatively low titre. Of 100 inmates not suffering from tuberculosis, 4 showed H agglutinins for *Bact. typhosum*, none for *Bact. paratyphosum A* and 2 for *Bact. paratyphosum B*, all to low titre. We cannot therefore apply our figure for the population at large to sick persons of all kinds. More information is required with regard to the frequency and titre of typhoid and paratyphoid agglutinins in persons suffering from various febrile conditions.

(9) *Is any advantage to be gained by studying fluctuations in titre, in addition to determining the titre of a particular agglutinin at a particular time?*

A very great advantage is gained, particularly in inoculated persons, as has been pointed out by Dreyer and his colleagues and by others (Dreyer, Walker and Gibson 1915, Dreyer and Walker 1916, Dreyer and Inman 1917, see also Perry 1918, Topley, Platts and Imrie 1920).

As regards the flagellar agglutinins reference to Chapter XI will show the time relations of the response to the inoculation of a killed culture and the response to active infection. When an inoculated person becomes infected, the response to infection is superimposed on the residual effect of the earlier response to inoculation, and we get the usual "infection" curve, starting not from the zero line, but from a base line representing the residual "inoculation" agglutinins. Since the rise in titre is more rapid than the fall, the most significant information will be obtained by repeated tests carried out during the first 3 weeks of infection; but the early part of the fall from the maximal titre attained is usually steep enough to show a significant drop in titre during the early weeks of convalescence.

The significance of flagellar agglutination to a titre of 1:20 in a non-inoculated person during the first week of a suspected enteric infection is very doubtful. The demonstration of a titre of 1:150 7 days later makes the diagnosis practically certain—not only, or so much, because 1:150 is at, or a little above, the diagnostic limit as because we know that the titre has risen to nearly ten times its previous value during the 7 days. A titre of 1:50 in an inoculated person has no significance, a rise in titre from 1:50 to 1:400 within 10 days or so points strongly to an active infection.

As regards the inoculated person, however, we must not forget the possibility of the non-specific stimulation of antibody production—the fresh output of typhoid agglutinins in response to some quite different infection.

The evidence on this point is conflicting. As regards the effect of enteric infection itself, it would appear that a person who has been inoculated with T.A.B. vaccine and subsequently develops typhoid fever usually responds by a renewed production of typhoid agglutinins, while the titres of agglutinins for *Bact. paratyphosum A* and *Bact. paratyphosum B* remain quite steady, or show trivial fluctuations. In some cases, however, a person who has been inoculated with triple vaccine and subsequently develops an infection due to one of the three organisms against which he has been vaccinated produces agglutinins not only against that organism but also against one or both of the heterologous species.

In regard to other diseases there is a conflict of opinion. Felix (1924*b*) considers that this non-specific response is of frequent occurrence, particularly as the result of infection with exanthematic typhus but also with many other infections, and that the demonstration of a rise or fall in agglutinin titre in an inoculated subject suffering from a febrile disease has thus no diagnostic significance. It is quite certain (see Perry 1918, Topley, Platts and Imrie 1920) that inoculated persons may suffer from febrile infections of many kinds without showing any detectable fluctuations in the titre of flagellar agglutinins, and the application of the "agglutinin curve" as a diagnostic criterion during the War indicated its usefulness. But, in view of Felix's findings a rise or fall in titre cannot be regarded as certainly diagnostic of active enteric infection. We badly need more detailed information of such changes in titre as may occur in the course of various infective diseases.

On the basis of these answers we may now attempt to frame some general rules for the interpretation of agglutination tests in a suspected case of enteric fever.

(1) A negative reaction against all the suspensions tested may be due to any of the following causes: (a) The patient may not be suffering from enteric fever. (b) The specimen may have been taken during the early stages of the disease, before agglutinins have appeared in the blood. (c) The type of enteric fever may be an unusual one, caused by infection with an organism not antigenically represented in the range of suspensions employed.

A decision between (a) and (b) can be made by repeating the test after a suitable interval. Alternative (c) must, if necessary, be further investigated by additional serological tests and, above all, by repeated attempts to cultivate the causative organism.

In general, a completely negative reaction in the first week of disease has little significance except as a basis for the interpretation of later tests. A completely negative reaction in the 2nd week indicates a balance of odds (2 to 1 or more) against the patient's being infected with any organism included in the test. A completely negative reaction in the 3rd week raises the odds to 10 to 1 or more. Repeated negative tests make it very unlikely (50 to 1 against or more) that the tentative clinical diagnosis is correct.

(2) A positive reaction at any time against any suspension must be considered in relation to:

- (a) The known distribution of agglutinins among the normal population.
- (b) The stage of disease at which the sample was taken.
- (c) The history of the patient as regards inoculation.

If an *uninoculated* person in this country shows the presence of flagellar agglutinins to *Bact. typhosum* at a titre of 1:20 the odds are about 20 to 1 against his being a normal member of the population, at a titre of 1:40 these odds increase to about 36 to 1, at 1:160 to about 140 to 1. These figures do not differ significantly for the "group" suspension. For *Bact. paratyphosum B* the odds against normality are rather higher. With *Bact. paratyphosum A* and the type phase of *Bact. paratyphosum C* the odds are so high, among an English population, that the presence of H agglutinins at 1:20 is strongly suggestive of infection.

As regards the O agglutinins we cannot at the moment give such definite

figures. A positive reaction at 1:20 has perhaps not much significance, especially with a typhoid O suspension. At a titre of 1:50 the odds are probably in favour of infection. Accepting Gardner's observations and Smith's findings on inoculated persons, we might guess that they were of the order of 10 to 1 or more. At 1 in 100 the odds increase still further and at 1:200 they become almost certainly diagnostic of enteric infection.

We shall clearly be influenced in our conclusions, especially in the case of the flagellar agglutinins, by the period of disease at which the blood sample was taken. We know that cases of typhoid and paratyphoid infection commonly develop H agglutinins to a titre much over 1:20—a titre of well over 1:1000 is quite common. A titre of 1:20—1:80 in the first week of the disease would be highly suggestive, though by no means diagnostic, of infection. A similar titre in the third week would be of much less significance.

In inoculated persons—or when previous inoculation is likely though uncertain—the case is quite different.

Flagellar agglutinins to titres below 1:200 or so must be neglected—in recently inoculated persons even higher titres may be due to this cause. Somatic agglutinins, except in persons inoculated within the past few weeks or months, have almost the same significance as in the uninoculated. High H titres (1:1000 or over) have considerable diagnostic significance in inoculated persons, limited by the possibility of some infection other than enteric having non-specifically stimulated the renewed production of agglutinins originally formed in response to inoculation.

(3) The diagnostic value of any single test will be greatly increased by the demonstration of a subsequent significant rise above the level originally observed—say a rise to more than twice or four times the original titre. In an uninoculated person such a rise will be almost decisive evidence of infection. In an inoculated person it will tell strongly in its favour—how strongly, we cannot tell, in any particular case, until we have more detailed information with regard to the fluctuations that occur in inoculated persons who are suffering from febrile infection of other kinds.

A marked fall during the early stages of convalescence will have a similar significance, but such a fall often occurs much more slowly and is hence more difficult to demonstrate.

THE AGGLUTINATION TEST IN *BRUCELLA* INFECTION.—The agglutination test within recent years has been extensively applied to the diagnosis of undulant fever—an infection that may, as is now known, be caused by *Br. abortus*, the common causative organism of epidemic abortion in cattle, as well as by the classical *Br. melitensis*. Since the data that have been collected serve to illustrate important general principles we may consider them very briefly under the same headings as we employed above.

(1) Undulant fever in man is not a bacteriological entity in the strictest sense, since *Br. melitensis* can be distinguished from *Br. abortus* by appropriate means, and the latter species, or variety, can itself be separated at least into the bovine and the porcine types.

(2) These species or types are very closely related antigenically. The

most striking antigenic differences have been detected between strains labelled *Br. melitensis* and *Br. abortus* on the one hand and *Br. paramelitensis* and *Br. paraabortus* on the other.

Ross (1927) suggested that the para-strains were rough variants of the normal smooth forms, and the careful and detailed studies of Pandit and Wilson (1932) have made it clear that this is the case, and have emphasized the need for using completely smooth strains in diagnostic tests. The antigenic relationship between *Br. melitensis* and *Br. abortus* in the completely smooth form has been studied by accurate quantitative methods by Wilson and Miles (1932), who find that the differences involved are quantitative rather than qualitative—the two types appear to possess the same antigenic components, developed to a very different degree.

(3) In the light of our present knowledge it would not appear to be necessary to use more than a single smooth bacterial suspension for diagnostic purposes, since it will respond, though perhaps to a slightly different titre, to the antibodies developed in response to infection with any of the prevalent types of *Brucella*.

(4) There is no evidence of the existence of separate agglutinating antibodies corresponding to the H and O agglutinins acting on the bacteria of the salmonella group. *Br. abortus* and *Br. melitensis* have been reported by almost all observers to be non-motile and non-flagellated.

(5) Our determinations here, as in all other cases, should be quantitative. The same principles of standardization apply as in all other agglutination tests.

(6) and (7) The stage of infection at which agglutinins appear has not yet been determined with the same accuracy in the case of undulant fever as in that of enteric infection. The time relations as measured from the initial infection are probably not very different in the two diseases; but relapses in enteric fever are relatively uncommon, while undulant fever is a characteristically relapsing disease. Each relapse will provide a fresh stimulus to agglutinin production, and it often happens that it is a relapse that first raises a clinical suspicion of the real nature of the infection. At this stage a high titre of agglutinins may occasionally be attained—up to 1:5000 or more. On the other hand, some cases never produce agglutinins to high titre, and it would appear that a minority of cases—probably a very small minority—fail to form agglutinins in detectable amount.

(8) The data with regard to the frequency and distribution of brucellar agglutinins among the population at large form the most interesting part of the story from our present point of view. The same method of assay has been followed as in the search for typhoid agglutinins—the examination of sera sent to the laboratory for Wassermann tests.

Among the earliest records is that of Harrison and Wilson (1928) who examined 998 specimens of serum with the results shown in Table XXXVIII.

TABLE XXXVIII

SHOWING THE PERCENTAGE OF SERA AGGLUTINATING *Br. abortus* TO THE SPECIFIED TITRE OR HIGHER.

Titre	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280
Per cent.	5.41	3.71	1.80	0.70	0.40	0.20	0.20	0.10

It will be noted that titres of 1:80 or over are very uncommon.

The figures of Harrison and Wilson refer to the population in and around Manchester. Cruickshank and Barbour (1931) record similar figures for North-East Scotland, based on the testing of 1,522 Wassermann sera. They found that 2.36 per cent. gave agglutination at 1:20, and 0.72 per cent. at 1:40 or over. Smith (1932), working in the same district and examining 1,446 sera, reports 2.08 per cent. as giving agglutination at 1:25 or over, and 0.49 per cent. as agglutinating at 1:50 or over. Morgan (1932) in Cardiff records 6 of 1,325 sera (0.46 per cent.) as agglutinating at 1:40 or over. In America, Martin and Myers (1931) report 4.3 per cent. of 1,000 sera as giving agglutination at a titre of 1:20, and Sasano *et al.* (1931), testing blood samples from 1,000 unselected patients in a sanatorium, found that 7.8 per cent. gave agglutination at 1:15 and 4.1 per cent. at 1:45 or over. Olin (1931) at Stockholm examined 3,003 sera from persons with no history of undulant fever; 4.1 per cent. gave agglutination at 1:40 or over, 1.5 per cent. at 1:80 and 0.56 per cent. at 1:160.

These figures are not dissimilar from those recorded for agglutinins to *Bact. typhosum* among a normal sample of the population. There are, however, additional and very interesting data with regard to the age and occupational distribution of brucellar agglutinins. Harrison and Wilson (1928) noted that 6 of 7 sera that agglutinated *Br. abortus* at 1:80 or over came from males between the ages of 19 and 56 years. Smith (1932) noted that all of 8 sera agglutinating at 1:100 or over came from males between the ages of 23 and 55 years; and that 3 of these males were farm servants and one a butcher. Thomsen (1931) examined sera from 61 persons in no way connected with cattle; none agglutinated *Br. abortus*. Sera from 8 veterinary students all reacted negatively. Of 65 sera from veterinary surgeons in rural practice 17 (26.2 per cent.) gave agglutination—a much higher percentage showed the presence of complement-fixing antibodies. Huddleson and Johnson (1930) examined sera from 49 veterinary surgeons and found that 28 (57.1 per cent.) gave agglutination at 1:50 or over while 13 (26.5 per cent.) gave agglutination at titres between 1:100 and 1:500. Only 3 of these persons gave a past history of undulant fever. Dible and Pownall (1932) tested the sera of 100 slaughtermen in Liverpool and as a control the sera of 100 other males showing a similar age distribution. At a dilution of 1:10 there was no difference between the percentage of positive reactions in the two groups; but at a dilution of 1:40 12 slaughtermen gave agglutination as against 2 persons in the control group.

When we come to interpret our results, we are in somewhat of a dilemma. If we obtain a positive agglutination at a titre of 1:10 the odds are something between 8:1 and 20:1 against the patient's coming from a random sample of the normal population, so that agglutination at this low titre lends some support to a clinical diagnosis of undulant fever; though the support at this level is of little moment. When our titre reaches a level of about 1:50 the result is much more significant. The odds are about 100 to 1 against the patient's being uninfected. At still higher titres the odds rise rapidly; but—and here may come our trouble—it is quite clear that any specialized group that runs a relatively high risk of contracting undulant fever will also be particularly liable to contract latent infections, and will for this reason tend to show a high frequency of agglutinins even in the absence of clinically overt disease. If, for instance, we are faced with a case of pyrexia in a veterinary surgeon practising in a rural area, his occupation will increase the probability that he is suffering from an infection with *Br. abortus*; but our knowledge of the distribution of brucellar agglutinins among his occupational group tells us that it is about an even chance that we shall find agglutinins active at a titre of 1:50, whatever the nature of his present infection may be, and about a

1 in 4 chance that we shall obtain agglutinins at a titre between 1:100 and 1:500. With slaughtermen or butchers—another group liable to develop undulant fever—the chances are apparently about 1 in 10 that we shall detect agglutinins at a titre of 1:40 in the absence of active infection.

When, therefore, the clinical diagnosis is uncertain, so that the immunological findings form an important part of the evidence on which a diagnosis must be based, we must be careful to correlate our results with the known distribution of agglutinins *in the age and occupational group to which the patient belongs*.

Complement Fixation.—It may be taken as a general rule that when sensitizing antibodies are developed of a kind, and to a titre, that will give easily detectable flocculation of bacteria *in vitro* the agglutination test will be the diagnostic method of choice. It happens not infrequently, however, that sensitizing antibodies can be detected by the complement-fixation technique when agglutination tests give negative results. In such cases we are forced to employ the more delicate method. For illustrative purposes we may refer briefly to the complement-fixation test in tuberculosis and in gonococcal infections.

Before doing so it is necessary to mention the Wassermann reaction, which, as we have already noted, occupies a somewhat anomalous position among the diagnostic serum reactions.

The Wassermann test (Wassermann, *et al.* 1906) was originally performed with a watery extract of the liver of a syphilitic foetus as antigen, and was at first supposed to be specific in the bacteriological sense, since such livers were known to be rich in *Trep. pallidum*. It was soon found however (Marie and Levaditi 1907) that extracts of normal liver gave equally good results from the diagnostic point of view, and that the reacting "antigen" was contained in the alcohol-soluble, acetone-insoluble, lecithin fraction of the tissue lipoids (Porges and Meier 1908). The "antigen" most commonly employed at the present time is a lecithin extract obtained from heart muscle, that has been rendered more sensitive, as regards its reaction with syphilitic sera, by the addition of a solution of cholesterol. The reaction depends on the fact that syphilitic infection induces the production of a serum constituent, the "Wassermann antibody," that reacts with a lipid antigen of this type giving fixation of complement, or, as in the Kahn (1928) or other flocculation tests, visible precipitation. Its basis is essentially empirical. It has been found in practice to be one of the most useful and reliable methods of laboratory diagnosis, but its significance depends entirely on an observed correlation with clinical findings, for which there is as yet no satisfactory explanation. For the latest and most authoritative data with regard to the diagnostic value of the Wassermann test and the precipitin tests that have derived from it see Report (1929).

COMPLEMENT FIXATION IN TUBERCULOSIS.—Here, and in the following section, the available data are more conveniently considered together than under separate headings. The human and bovine types of tubercle bacilli form a single homogeneous antigenic group (Tulloch *et al.* 1924, Cumming 1925, Wilson 1925, Furth 1926), so that we have only one antigenic component or group of components to consider. That specific complement-fixing antibodies are frequently present in the sera of patients suffering from tuberculous infections there is no doubt. There is, however, equally little doubt that positive results may not infrequently be obtained with sera from apparently

healthy persons, or from patients suffering from infections other than tuberculosis. The antigens employed in the test have been legion—bacillary suspensions and extracts prepared in a variety of ways—so that the very numerous reports by different observers cannot be clearly correlated with one another. The kind of results that have been very generally obtained are sufficiently illustrated by the findings of Rieux (1926) summarized in Table XXXIX.

TABLE XXXIX
COMPLEMENT FIXATION IN TUBERCULOSIS

Type of Disease.	Per cent.
Pulmonary tuberculosis with positive sputum	85
Clinically tuberculous pleurisy	46.6
Tuberculous peritonitis	80
Tuberculous meningitis	Rarely positive
Glandular tuberculosis	38
Bone and joint tuberculosis	50
Renal tuberculosis	90
Healthy persons	10-15
Persons suffering from non-tuberculous diseases	10

In severe disease of long standing the test is usually positive; but in such cases diagnostic help of this kind is seldom required. A test that gives 1-6 negative reaction, in 10 in proved cases of tuberculosis, and about 1 positive reaction in 10 in persons not suffering from an active tubercular infection, is of somewhat doubtful service.

The tubercle bacillus is rich in lipoids; and it has long been recognized that misleading positive reactions may occur due to the "Wassermann antibody" present in syphilitic sera. Witebsky and Klingenstein (1932) have recently reported that the complement-fixing antibodies present in tuberculous sera may be differentiated from the "Wassermann antibody" by a difference in their heat-stability—the former resist heating at 63° C., the latter does not. It seems improbable, however, that any refinement of this kind will improve the sensitivity and specificity of the reaction to the point where the laboratory worker will be able, in a high proportion of clinically doubtful cases, to decide that it is long odds in favour of or against the patient's being actively infected with tuberculosis. And that is the ultimate practical requirement of any laboratory test. (For further reports on this reaction see Caulfield 1911, Fraser 1913, Calmette and Massol 1914, McIntosh, Fildes and Radcliffe 1914, Dudgeon, Meek and Weir 1914, Meek 1914, Radcliffe 1915, von Wedel 1920, Watkins and Boynton 1920, Cooke 1921, Urbain and Fried 1921, Sellers and Ramsbottom 1922, Brocq-Rousseau, Urbain and Cauchemez 1923, von Gutfeld and Weigert 1924, Kalcher and Sonnenfeld 1924, Douglas and Meanwell 1925, Salomon and Valtis 1925, Valtis 1925, McIntosh and Whitby 1930.)

COMPLEMENT FIXATION IN GONOCOCCAL INFECTION.—The gonococcus is not an entirely homogeneous species as regards its antigenic structure (Torrey 1907, Pearce 1915, Hermanies 1921, Jötten 1921, Torrey and Buckell 1922, Tulloch 1923, Atkin 1925); but the differentiation into serological groups is not sharp (see, in addition to the above, Wollstein 1907, Vannod 1907, Thomson and Vollmand 1921, Cook and Stafford 1921, Warren 1921). Tulloch (1923), for instance, could find no evidence of serological grouping among 100 strains of gonococci by direct agglutination, but by absorption he was able to differentiate one main group, containing 72 strains, and 4 subgroups containing 3 to

7 strains apiece, leaving 8 strains unclassified. We have, then, more than one antigenic component to consider, but these components have not yet been mapped out with sufficient accuracy for us to be able to specify which of them we must include in our test, and there would appear in the light of experience to be no need to put up separate tests using different serological strains. The difficulty is commonly met by using a polyvalent antigen—a suspension prepared from several different strains; though the need for this precaution is not universally conceded.

There appears to be good evidence that the test yields information of diagnostic value, when the results are properly interpreted.

(See Müller and Oppenheim 1906, Watabiki 1910, Schwartz and McNeill 1911, 1912, Keyes 1912, Kolmer and Brown 1914, Irons and Nicoll 1915, Dixon and Priestley 1919, Magner 1920, Smith and Wilson 1920, Herrold 1921, Torrey and Buckell 1922, Torrey *et al.* 1922, Walker 1922, Rubenstein and Gauran 1923, Price 1931.)

There seems to be general agreement that positive results are seldom if ever obtained in uninfected persons if all reagents are properly standardized and controlled. The proportion of positive results commonly obtained in the various types of gonococcal infection are set out in Table XL, which has been compiled from several of the papers referred to above.

TABLE XL
COMPLEMENT FIXATION IN GONOCOCCAL INFECTION

Type of Infection	Per cent.
Acute Gonorrhœa	48
Subacute and Chronic Gonorrhœa	61
Epididymitis.	82
Prostatitis	80
Vesiculitis.	88
Metritis	68
Salpingitis	77
Vulvo-vaginitis	50
Arthritis	82

It will be noted, as in the case of tuberculosis, that the higher percentages of positive results are obtained in long-standing infections; and it is infections of this type that present the greatest diagnostic difficulties both to the clinician and to the bacteriologist. The complement-fixation test in gonorrhœa is, in fact, most likely to give positive results in just those cases where a serological test is most useful.

This point is well illustrated by comparative figures given by Tulloch (1929) for the results of direct microscopical examination of films and of complement-fixation tests in 1,548 persons examined for gonococcal infection. In 433 cases gonococci were detected in the film and the complement-fixation test was positive. In 737 cases both tests were negative. In 215 cases gonococci were detected microscopically, but the complement-fixation test was negative: 175 of these cases were of less than 14 days' standing, 19 had lasted between 2 weeks and 2 months. In 163 cases no gonococci were detected microscopically but the complement-fixation test was positive: the majority of these cases were subacute or chronic infections, 84 in females and 79 in males.

In interpreting the results of this test we must bear these points in mind :

- (1) The reaction does not become positive for at least 2 weeks after infection.
- (2) It is generally negative throughout in simple cases of anterior urethritis.
- (3) It is generally positive (80 to 90 per cent.) in subacute and chronic cases in which infection has spread beyond the urethra.

(4) A single negative test cannot exclude infection. Repeated negative tests obtained at intervals over a period of 4 to 6 weeks greatly increase the odds against the existence of gonococcal infection, though we have no adequate data to give as a basis for numerical computation.

(5) It is commonly stated that a positive reaction, once established, tends to remain positive until about 3 months after clinical cure, and that a change in the reaction from positive to negative after this period is evidence of the absence of latent infection.

(6) Treatment by the injection of a gonococcal vaccine—which may itself stimulate the production of complementing-fixing antibodies—invalidates the results of this test.

The Precipitin Reaction.—Except as an alternative to the Wassermann reaction the precipitin test has not come into common use as a method for demonstrating the presence of antibodies in an infected person.

Testing for Antigens.

Immunological methods are not commonly employed as primary tests for the presence of bacterial antigens in infected persons though they are in frequent use for the final identification of an organism isolated by culture.

A particular case, in which precipitation or complement fixation affords the readiest method of primary detection and identification of antigenic material, is the differential diagnosis of smallpox and chicken-pox by testing the extracts of the scab against specific antisera (see p. 264).

Testing for Allergy.

The classic example of the diagnostic allergic reaction is, of course, the tuberculin test in one or other of its modifications. The subcutaneous test of Koch (1890, 1891), depending on a generalized constitutional reaction, is seldom employed; in cattle it is less easy to interpret than other tests of hypersensitiveness (see Report of Medical Research Council Tuberculin Committee 1925, 1928) and in man is by no means free from danger. The conjunctival test of Calmette (1907) and of Wolff-Eisner (1907) has also been largely abandoned, as has also the percutaneous or inunction method of Moro (Moro and Doganoff 1907, Moro 1908). The tests which have been most widely employed are the cutaneous test of von Pirquet (1907) in which tuberculin is applied to a small scarified area of skin, and the intracutaneous test of Mantoux (1908) in which 0.1 c.c. of a suitable dilution of tuberculin (usually 1:1000 or 1:10,000) is injected intradermally. In either case a positive reaction takes the form of an erythema, or erythematous infiltration, reaching its maximum development in about 48 hours and persisting for a day or two thereafter. Either of these tests can be made quantitative by using several

different dilutions of tuberculin. Until recent years the von Pirquet method was the more widely employed, and formed the basis on which most of the earlier data were collected; but since the War there has been a growing consensus of opinion that the intradermal test is more accurate and reliable, and it has now come into general use.

For a fuller discussion of the diagnostic significance of these tests reference may be made to Hart (1932*b*) and, for a shorter summary, to Gloyne (1932). Here we can only deal very briefly with certain of the more important points at issue. It will be convenient to discuss them under headings very similar to those we employed in considering the *in vitro* antigen-antibody diagnostic reactions.

(1) *Is tuberculin a single antigenic, or "atopic," substance or does it contain a variety of active components, the effect of each of which must be considered separately? In particular is there any difference between human and bovine tuberculin?*

Koch's "old tuberculin"—the material employed in the allergic tuberculin test—is a crude product, obtained by concentrating a 6–8 weeks' culture of tubercle bacilli in glycerine veal broth to one-tenth of its volume at 100° C., and freeing it from bacilli by filtration. For a discussion of the chemical nature of its varied constituents and of their immunological significance, see Tytler (1930) and Hart (1932*b*). For our present purpose it is sufficient to state that the active allergic substance appears to be associated with the protein fraction of tuberculin, and that we may regard it as an immunological entity. There is no evidence that the tuberculin produced by the human type of tubercle bacillus differs in any significant way from that produced by the bovine type (Gauvain 1917, Tisdall and Brown 1926, Stewart and Collins 1927, Calmette 1928, Okell 1930).

There is, however, another factor to be considered—the skin reaction of tuberculous and non-tuberculous subjects to the non-specific constituents of crude tuberculin. Many observers have controlled the tuberculin test by the simultaneous inoculation at another site of glycerinated veal broth. The injection of this material in very low dilutions may lead to a delayed non-specific reaction; but, as Hart (1932*a, b*) has pointed out, tuberculous subjects appear to develop an increased sensitivity to the non-specific constituents of crude tuberculin as well as to the active principle itself, so that skin reactions having all the characters of the typical tuberculin reaction may be obtained by the injection of glycerine veal broth in suitable strength, though such reactions are not obtained in non-tuberculous controls. This non-specific sensitivity is never so marked as that towards the specific active principle, as shown by the fact that far larger amounts of the non-specific constituents are required to elicit a typical response.

(2) *Should we regard the difference in skin sensitivity between tuberculous and non-tuberculous subjects as qualitative or quantitative, and, if the latter, how are we to obtain quantitative measurements?*

Whether non-tuberculous subjects would show any skin sensitivity to the purified active principle of tuberculin we do not yet know, because this principle has not yet been isolated in a chemically pure form. So far as crude tuberculin is concerned we should certainly regard the reaction as quantitative rather than qualitative (see Long 1928, Tytler 1930, Hart 1932*a, b*).

Using the intracutaneous test, Hart (1932*b*) recommends as an initial test the injection of 0.1 c.c. of a 1 : 10,000 dilution of tuberculin. All persons reacting negatively to this dose should be given a further injection of 0.1 c.c. of a 1 : 1000 dilution (which may be used as the initial dose in cases where the possibility of a severe reaction can be excluded). The injection of this amount of tuberculin is commonly regarded as an adequate diagnostic test, and is probably sufficient for routine purposes. If, however, there remains any doubt as to the clinical diagnosis in a patient giving a negative reaction, or if there is any reason to suspect depressed reactivity (see Section 3) a further test with a 1 : 100 dilution should be carried out, and in some cases it is desirable to follow this, if the reaction is still negative, with a test at a 1 : 10 dilution. With dilutions of 1 : 100 or greater, control injections of glycerine veal broth are unnecessary, but with a 1 : 10 dilution they should always be included.

(3) *At what stage of tuberculosis does sensitivity develop, and when is it most marked? What proportion of cases give a negative tuberculin reaction throughout the whole course of their infection?*

The problem here at issue differs fundamentally from that presented by an acute self-limited disease of the type of enteric fever. Clinical tuberculosis, omitting for the moment sub-clinical infection, is a protean disease, presenting all grades of severity and all rates of progression. We cannot even summarize the mass of data on which the answers to these two questions must be based, but must deal with them didactically, drawing largely on the recent monograph by Hart (1932*b*), whose findings are in general accord with our whole body of evidence.

Any tuberculous infection, however slight, is apparently associated with the development of skin sensitivity to tuberculin, so that almost all cases that present clinical signs or symptoms show a positive reaction. This sensitivity, however, varies in degree. A negative response to the injection of 0.1 c.c. of a 1 : 10,000 dilution of tuberculin does not exclude tuberculous infection—in Hart's series the error due to false negatives at this level was 12 per cent. A negative reaction at 1 : 1000 considerably increases the odds against tuberculous infection—in Hart's series the error was 4 per cent. Increasing the dose in repeated tests to 1 : 100, and if necessary 1 : 10 dilution, lowers the error to about 2 per cent. A completely negative test therefore indicates odds of about 50 to 1 against a person's being infected with tuberculosis.

In advanced pulmonary tuberculosis sensitivity is depressed, but seldom abolished. With 1 : 10,000 and 1 : 1000 dilutions of tuberculin many patients in the final stages of the disease fail to react; but with lower dilutions (1 : 10 or 1 : 100) all but 3.5 per cent. give positive reactions.

In general, however, a positive reaction tells us nothing at all about the severity of the infection. It does not differentiate clinical from sub-clinical infection. It does not tell us that the particular condition from which a patient is suffering is tuberculous in nature; it merely tells us that he has some kind of tuberculous infection. Obviously, then, the significance that we attach to a positive reaction will depend on the frequency and distribution of latent tuberculosis among the population at large. This leads us directly to our final question.

(4) *What are the frequency and distribution of tuberculin sensitivity among the not-clinically-tuberculous members of the population or among populations at large?*

The cutaneous test has been widely exploited by von Pirquet and others from this point of view with highly interesting results. Table XLI (modified from von Pirquet 1909), indicates the frequency of positive reactions among different age groups in Vienna.

TABLE XLI

SHOWING THE PERCENTAGE OF CHILDREN IN DIFFERENT AGE GROUPS GIVING A POSITIVE CUTANEOUS TUBERCULIN REACTION.

	Age in Years.													
	0-1	1-2	2-3	3-4	4-5	5-6	6-7	7-8	8-9	9-10	10-11	11-12	12-13	13-14
No. of cases	388	89	75	87	62	66	61	39	72	45	44	45	36	22
Per cent. Positive	0	0	14	14	16	34	35	41	46	56	71	69	64	91

It will be seen that positive reactions are very rare during the first 2 years of life, after which they rapidly increase so that about half the children react positively at the end of their second decade, and over 90 per cent. react positively before attaining the age of 15.

This indicates an almost universal tuberculization of the population during childhood and puberty. The findings of other observers are in general conformity with those of von Pirquet (see Mantoux 1910, Calmette *et al.* 1911, Opie and McPhedran 1926, Hart 1932b). There are, however, geographical and racial differences, and if we widen our range to include uncivilized as well as civilized peoples, these differences are striking. Thus Fig. 35 (see Cummins 1911-12) shows the percentage of positive reactors among certain primitive races compared with those found in Paris, Lille and Vienna.

We should hardly accept the results of tuberculin tests as adequate evidence of this widespread tuberculization of mankind if they were unsupported by other evidence; but this is not the case. Careful and detailed pathological studies on *post-mortem* subjects, dying from all causes, have yielded results which are in entire accord with those obtained by tuberculin tests in the living.

Table XLII summarizes the findings of Naegeli (1900) in Zurich, and Table XLIII the findings of Burkhardt (1906) in Dresden.

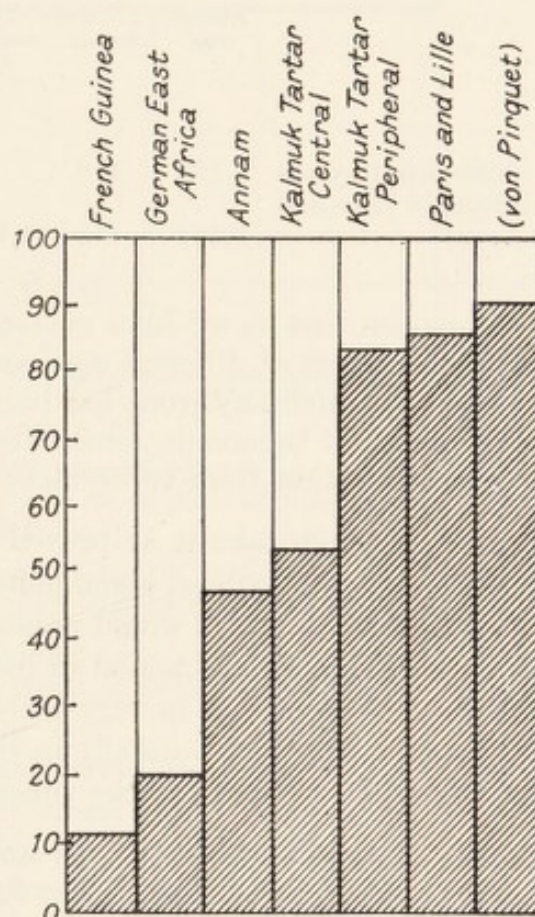


FIG. 35.

Comparative percentages of positive cutaneous tuberculin reactions in primitive and civilized peoples. (After Cummins.)

TABLE XLII

SHOWING THE FREQUENCY OF TUBERCULOSIS (Tb.) OF ALL FORMS IN POST-MORTEM SUBJECTS AT ZURICH. (Modified from Naegeli 1900).

	Autop-sies.	Tb. not Found.	Tb. found.	Percent-age Tb.	Of Tb. Patients.			
					Died of Tb.	Percent-age.	Non-lethal Tb.	Per-centage.
Children 0-18 years	88	73	15	17.04	11	73.3	4	26.7
Adults 18 years and over . . .	420	29	391	93.11	110	28.13	281	71.87

TABLE XLIII

SHOWING THE FREQUENCY OF TUBERCULOSIS (Tb.) OF ALL FORMS IN POST-MORTEM SUBJECTS AT DRESDEN IN 1900-01. (Modified from Burkhardt 1906).

	Autop-sies.	Tb. not found.	Tb. found.	Percent-age Tb.	Of Tb. Patients.			
					Died of Tb.	Percent-age.	Non-lethal Tb.	Per-centage.
Children 0-18 years	190	118	72	38	35	48.6	37	51.4
Adults 18 years and over	1,262	113	1,149	91	466	40.6	683	59.4

Moreover, just as we have evidence that the frequency of Schick and Dick negative reactors at different ages are intimately associated with the risk of infection to which any group has been subjected, so we have evidence that the development of tuberculin sensitivity is more rapid in children that are subjected to infection from tuberculous parents than in those that are not.

We may then take it as proved that the great majority of us—those at least that live in civilized communities—are infected with tuberculosis before we reach adult life. It would appear that the results of infection vary very widely according to the period of life at which infection becomes established. Infections in infancy or in very early childhood are certainly more frequently and more rapidly fatal—usually in the form of miliary tuberculosis associated with meningitis—than are the tuberculous infections of later years.

This correlation between age and severity of infection is illustrated by Fig. 36 taken from Naegeli. The figures of 100 per cent. for the fatality of tuberculous infection in infancy and for the incidence of tuberculous infection of all types in adult life must be regarded as estimates of probability based on a particular experience; certainly they do not hold true for all times and places, even if we confine ourselves to civilized communities. It is, however, generally true that the case fatality of tuberculosis is very high in infancy, falls rapidly during later childhood and puberty, fluctuates during early and

middle adult life, largely in association with the frequency of the pulmonary form of the disease, and then sinks slowly again during and after the decade 30-40 years.

Clearly, then, the significance of the cutaneous tuberculin test—remembering that it is positive in latent as well as in overt tuberculosis—is very different from that of the diagnostic reactions we have considered above. We cannot do better, in summarizing the present position with regard to the diagnostic value of the test, than to quote the relevant passages from the conclusions of Hart (1932b).

(1) The positive reaction has its chief clinical value in infancy. An infant under 2 years showing a positive reaction, but without symptoms, should be kept under observation for years, on account of the possible development of clinical tuberculosis. If obscure and persistent symptoms (*e.g.*, unexplained pyrexia) are present, a positive reaction should be regarded as important evidence, but not proof, that these symptoms are tuberculous in origin, and

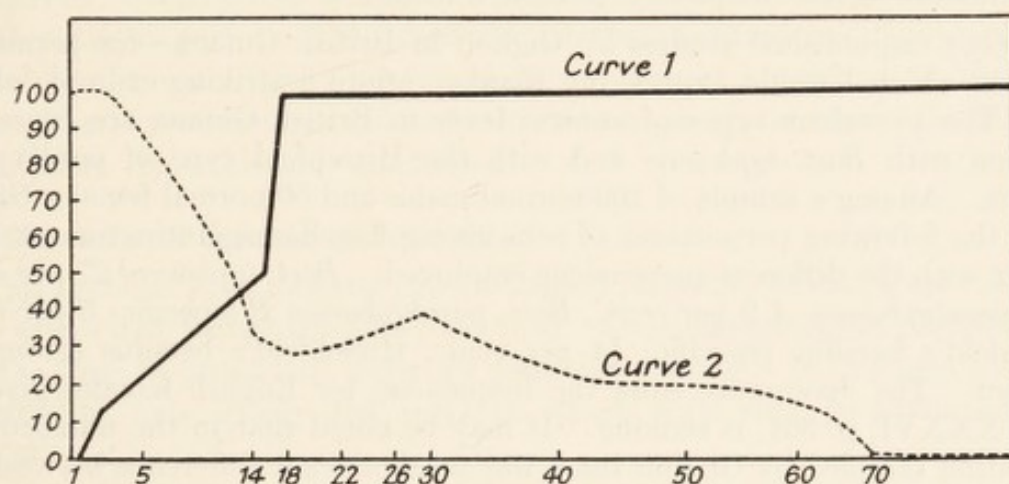


FIG. 36.

Curve 1 = frequency of tuberculosis in every 100 autopsies of each age group.
 Curve 2 = frequency of fatal tuberculosis in relation to all cases of tuberculosis.
 (After Naegeli.)

a serious view should be taken until time shows this assumption to be unwarranted.

(2) A positive reaction in a young child aged 2 to 5 should suggest that such symptoms are tuberculous in origin.

(3) The principal application of the tuberculin test in clinical practice is in the negative diagnosis, *i.e.*, the exclusion of tuberculosis. A negative intracutaneous reaction excludes this disease with a high degree of accuracy, which is almost the same at every age. (For quantitative criteria in assessing a negative reaction, see above.)

We have in the above summary confined our attention to the diagnosis of tuberculosis in man; but one of the common sources of human infection, particularly in childhood, is the consumption of milk, and one of the most important applications of the allergic skin reaction is in the detection of tuberculosis in cattle. The general findings in dairy herds do not differ essentially from those in herds of men, but it would take us too far

to consider them in detail. For further information with regard to the technique of the test in cattle and the significance of the results obtained, reference may be made to the Reports of the Medical Research Council Tuberculin Committee 1925, 1928.

THE DIAGNOSIS OF HERD INFECTION

In the preceding sections we have taken "diagnosis of infection" as meaning "the diagnosis of infection in a particular person suspected on clinical grounds of suffering from a particular disease." But while considering this problem we have set out data that can be used for another purpose—the diagnosis of the frequency and distribution of a particular kind of infection among a particular sample of the general population. We have noted, for instance, the frequency of flagellar agglutinins for members of the typhoid-paratyphoid group among two samples of the English population, and have seen how agglutinins for the *Brucella* group vary from one occupational herd to another.

There is little doubt that the frequency of agglutinins in any herd affords an indication of the frequency of herd infection.

Recent unpublished studies by Giglioli in British Guiana—for permission to quote which I would express my thanks—afford a striking example of this fact. The prevalent types of enteric fever in British Guiana are caused by infection with *Bact. typhosum* and with the Hirschfeld type of paratyphoid bacillus. Among a sample of 100 normal males and 50 normal females Giglioli found the following percentages of sera giving flagellar agglutination at 1:20 or over with the different suspensions employed. *Bact. typhosum* 27 per cent., *Bact. paratyphosum A* 9 per cent., *Bact. paratyphosum B* (specific) 5 per cent., Hirschfeld's bacillus (specific) 14 per cent., Hirschfeld's bacillus (group) 30 per cent. The divergence from the frequencies for English females given in Table XXXVI, p. 304, is striking. It may be noted that in the uninoculated population sampled by Giglioli there was no significant difference between the frequencies in the male and female subgroups. With these figures as our only data we could have foretold with confidence (a) that enteric fever as a whole was far commoner in British Guiana than in England, and (b) that infection with Hirschfeld's bacillus was common in British Guiana but extremely rare in England.

Analogous evidence, indicating the value of agglutinin frequency as an index of herd infection among human beings, has recently been recorded by Havens and Mayfield (1931).

Whatever may be our views with regard to the mode of origin of the "normal" antibodies there is now no doubt that the different frequencies observed in different countries, age groups, social classes, occupational groups, etc., is highly correlated with the risk of infection to which these groups are exposed. We shall, for instance, certainly not err if we draw, from the fact that Group A shows a 10 per cent. frequency of agglutinins to *Bacillus X* and Group B a 0.2 per cent. frequency, the conclusion that infection with that organism is common among the population of which Group A is a sample, rare among the population exemplified by Group B. It should perhaps be emphasized that the *difference* is the most significant point. If we found, in a large number of

samples of adults drawn from many different countries and many different occupational groups, that the frequency of a particular antibody at a particular titre was always in the neighbourhood of say 5 per cent., we should in the present state of our knowledge be hard put to it to determine whether this indicated uniform frequency of the infection in question, or whether we were dealing with some non-specific effect. Surveys of this kind are becoming increasingly common, and serve a very useful purpose. An example in the realm of virus diseases has been referred to on p. 256. It is often important to map out infected areas from the administrative point of view; and a knowledge of the infections prevalent in his district is of considerable help to the bacteriologist faced with the diagnosis of a clinically atypical fever—from this view-point this particular method of survey might be exploited far more widely than it is, especially in tropical countries.

The same use may, as we have seen, be made of an allergic reaction *in vivo*. By means of the intracutaneous test we can assess the frequency of infection in different countries and among different races, in different age groups in any one area, and in persons of the same age groups submitted to different environmental conditions. With this knowledge we are in a position to study the effect of various environmental and other factors on the course of the infection as it occurs under natural conditions.

The Diagnosis of Sources of Infection.

This problem is, in its more general aspects, a matter of diagnostic bacteriology, with which we are not here concerned. But immunological methods have come to form so important a part of detective technique in this field that it is desirable to note briefly the ways in which they may be advantageously applied.

(A) THE DETECTION OF CARRIERS.—If one is faced with the problem of detecting the source of infection in an outbreak of infective disease in a closed or semi-closed community—say an outbreak of diphtheria in a school, or an outbreak of enteric fever in an asylum—one of the possibilities to be kept constantly in view is the presence in the community of one or more “healthy carriers,” *i.e.*, persons who are suffering from latent infections with the specific organism. The final identification of the carrier demands the actual isolation of the organism, but a preliminary application of the ordinary immunological methods may save a great deal of time and work. A carrier of typhoid or paratyphoid bacilli will usually, though not always, show the presence of agglutinins for the organism he is carrying. A carrier of diphtheria bacilli will almost always be found in the Schick-negative group. By a preliminary sorting of this kind we can narrow the field of bacteriological search to more manageable proportions.

(B) THE IDENTIFICATION OF “INFECTING” AND “CARRIER” STRAINS.—There are many problems in which it is of particular importance to narrow bacteriological identification within finer limits than can be achieved by morphological, cultural or fermentation tests. Serological methods have, indeed, a leading place in the routine of the bacteriological laboratory, and the final

identification of any member of, for instance, the salmonella group depends largely on a recognition of its precise antigenic structure.

The use of such methods is, however, constantly extending; and a good illustration of the kind of information that they yield is afforded by recent studies on various types of acute streptococcal infection.

Puerperal fever, in its more severe and fatal form, is commonly caused by infection with a hæmolytic streptococcus; and one of the problems at issue is the way in which the puerperal patient becomes infected—in particular, whether infection is extrinsic (derived from some outside source) or intrinsic (derived from a pre-existing focus of infection in the patient herself). To prove extrinsic infection it would not be sufficient to demonstrate the presence of hæmolytic streptococci in some contact or attendant, since these bacteria are not uncommon in the normal throat; but the hæmolytic streptococci are an antigenically heterogeneous group, and if it can be proved that the strain isolated from a patient is antigenically identical with that present in the doctor, nurse or attendant who has been in close contact with her the odds on infection having occurred by that path are very greatly increased. Smith (1931) has carried out a study along these lines, using the method of agglutination and agglutinin absorption. In 15 of 18 cases of streptococcal uterine infection he was able to trace the source of infection. In only 2 of the 15 cases was the hæmolytic streptococcus responsible for infection of intrinsic origin—in one case it was recovered from the patient's nose, in the other from a septic focus on her hand. In the other 12 cases an antigenically identical strain was recovered from the throat or nose of the doctor, nurse or student in attendance. The figures are obviously too small to give any reliable indication of the relative frequency of intrinsic and extrinsic infection; but they indicate the lines along which the essential data can be gained.

The studies of Griffith and Gunn (Griffith 1926, 1927, Gunn and Griffith 1928) and of Glover and Griffith (1931) on scarlet fever and tonsillitis afford further examples of a similar kind. By an extended application of these methods it should be possible to describe the course of events in epidemics among particular communities with an accuracy and detail that has hitherto been impossible, and so to assess the importance of various factors that contribute to their maintenance and spread.

THE DIAGNOSIS OF IMMUNITY

In relation to preventive, as opposed to curative, medicine, the diagnosis of immunity is often as important as the diagnosis of infection. If we are trying to control in any herd the morbidity or mortality associated with a particular infective disease, our work will obviously be simplified if we can, as a preliminary, sort the members of the herd into immune and susceptible subgroups. Having done so we can, perhaps, shield our susceptibles from serious risk of infection. If this is impossible, or over-troublesome, we may be able to turn them into immunes by some appropriate method of immunization.

The methods available are of the same general kind as those we have considered above.

The Demonstration of Specific Antibodies in the Blood.—In the diagnosis of immunity it is the antitoxins rather than the antibacterial sensitizing antibodies that have so far proved to be serviceable criteria of immunity.

The most striking example is afforded by diphtheria, and here the practical utility of this method has been enormously extended by the introduction of the Schick reaction, in which the presence of circulating antitoxin is inferred from the absence of the characteristic skin reaction to the intradermal injection of a suitable dilution of toxin. The Dick test, in the case of scarlet fever, provides a similar, if somewhat less precise, method of differentiating susceptibles from immunes. The results that have been obtained with each of these tests have been referred to in connection with many of the problems discussed in earlier chapters, and there is no need to repeat them here.

In how far the demonstration of antibacterial antibodies may, in the future, prove a useful method of diagnosing immunity it is impossible to say. For one thing we do not yet know with any exactitude how far the presence of these antibodies in the blood may be taken as evidence of increased resistance. In a country in which enteric fever is endemic, with a high rate of morbidity and hence a high rate of natural immunization by subclinical infection, it might be useful—particularly if the prevalent types of enteric infection were well known—to test for agglutinins as a preliminary to any attempt at mass immunization. We should, on general principles, pay more attention to O than to H agglutinins as evidence of immunity, though we should not disregard the presence of the latter. We should certainly, if we had to choose, immunize first those who showed no agglutinins at all.

In virus diseases the presence of neutralizing antibodies, and probably the presence of complement-fixing or precipitating antibodies where such *in vitro* tests are available, afford evidence of immunity as well as of infection. Under certain limiting conditions tests like these may in the future play a considerable part in the control of herd infection.

Allergic Reactions.—We have referred in Chapter XII to the fact that infection is associated with a relative resistance to re-infection—the condition of infection immunity. Thus the cutaneous tuberculin reaction may be taken as indicating not only the presence of latent tuberculosis, but as evidence of a relative resistance to re-infection with the development of more serious disease. We have little exact information with regard to the real effectiveness of this increased resistance but certain observations are suggestive.

Thus Heimbeck (1927, 1928) noted the tuberculin reaction of 337 probationer nurses entering on duty at Oslo Municipal hospital. Of these 185 gave negative reactions, presumably because they came in the main from country districts or small towns and had not been exposed to any high risk of infection. During the following 3 years almost all these girls became von Pirquet-positive, and 24 per cent. of them developed clinical tuberculosis, or 19 per cent. if we exclude 9 cases in which the only clinical evidence of tuberculosis was erythema nodosum (see Hart 1932*b*). Of those probationers who gave positive reactions on entry only 3 per cent. developed clinical disease. If these observations are

confirmed it will obviously be desirable to limit the nursing of patients with open tuberculosis to those who show, by an allergic reaction, that they have already contracted a sub-clinical infection.

SUMMARY

(1) Immunity reactions may be employed for the diagnosis of infection in a particular patient, for determining the frequency and distribution of infection among a herd, for the detection of sources of infection and paths of spread, and for the diagnosis of immunity.

(2) In the diagnosis of infection no immunity reaction has the same decisive significance as the isolation from the patient, or carrier, of the organism with which he is suspected to be infected.

(3) The methods employed in the diagnosis of infection are (*a*) the detection of specific antibodies in the patient's blood, usually by agglutination or by complement fixation; (*b*) the demonstration of an allergic response to a particular antigen, usually by an intracutaneous test, and (*c*) exceptionally, the demonstration of a particular antigen in material derived from the patient.

(4) In performing any diagnostic antigen-antibody reaction, we must know (*a*) the nature and number of the antigens and antibodies that may be concerned; (*b*) how many of these antigens we must include in our test suspensions, and how far we need differentiate one from the other, and (*c*) the experimental conditions that will allow us to obtain accurate quantitative results.

(5) In interpreting the results of such tests we must know (*a*) the proportion of cases of the suspected disease in which the antibodies in question are produced; (*b*) the period of the disease at which they appear, and the time at which they reach their maximum titre; (*c*) the frequency and titre of these antibodies among the population at large, and among special groups of the population differentiated by age, occupation, protective inoculation, or some other factor that would render them more likely than the average person to show the presence of the antibody in question.

(6) It is often possible to obtain more significant information by demonstrating a fluctuation in antibody titre, than by detecting the presence of an antibody to a particular titre at a single examination.

(7) In performing and interpreting an allergic reaction we require information of the same kind as that set out under (4) and (5).

(8) In the diagnosis of immunity the method most commonly employed is the detection of circulating antitoxin in a person's blood. This is usually tested indirectly by injecting an appropriate amount of toxin into the skin and noting the absence of the characteristic reaction that follows such an injection in a susceptible person. In the case of those bacteria that do not produce a soluble toxin it is possible that antibody reactions may, in the future, be used for this purpose; but this field is at the moment unexplored.

An allergic reaction may be taken as indicating a relative immunity as well as the presence of a latent or overt infection ; but we have no reliable information as to the effectiveness of immunity of this kind.

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CHAPTER XIX

THE PRACTICAL APPLICATION OF IMMUNITY IN PROPHYLAXIS

IN assessing the value of any prophylactic measure the final test must always be empirical—does it work in practice? There may be excellent *a priori* reasons for expecting that a particular method of immunization will lower the morbidity or mortality due to a particular infective disease; but until the method in question has been tested under field conditions we can never be sure what its actual effect will be. We have discussed in Chapter II the ways in which the results of such a field trial may be assessed, and have seen that it is not so easy as might be supposed to reach a decisive conclusion. Until we have obtained a statistical answer from the field, we must act on a balance of probabilities, and in estimating that balance we must consider all the available data with regard to the proposed method in the light of our knowledge of immunity reactions in general.

If our opinion were sought on the probable value of any method of immunization as applied to a particular problem in preventive medicine, we should require answers to such questions as the following before we could offer a fair summing-up on the evidence.

(1) *Does the immunizing reagent on which the method depends contain antigens, or antibodies, that might reasonably be expected to confer active, or passive, immunity against the infective disease in question?*

(2) *Can this reagent be standardized so that we can measure, at least approximately, the dose that we are giving?*

(3) *Is there any evidence that the reagent—antigen or antibody—does in fact confer active or passive immunity against experimental infection?*

(4) *Has the method been tested under field conditions, and, if so, with what results?*

(5) *If the method has been shown to have a definite protective value under a particular set of field conditions, are the conditions existing in the particular problem with which we are faced so similar that we are as likely to get good results in the one case as in the other?*

It will be useful to consider a few illustrative examples drawn from methods that are in common use or that have been tested on an adequate scale; and it will be convenient to consider separately those methods that involve active, and those that involve passive immunity, and to separate as far as we are able antitoxic from antibacterial effects.

The standardization of immunological reagents is considered more fully in

Chapter XXI. Here we must be content, for the most part, to give summary answers to our second question. It will also be convenient to combine the answers to questions (4) and (5), since the same data frequently fall for consideration under both heads. Much of the evidence on which our answers rest has been set out in earlier chapters and need not be repeated in detail. For the rest, in dealing with the superabundant literature of applied immunity, quotation must be parsimonious and often arbitrary.

It would have been reasonable to add to our five questions another—*How long does the immunity last?* But in no instance could we have given a precise answer, and such data as are available can best be considered under headings (4) and (5). This factor of duration is, however, of primary importance in regard to the relative advantages of active and passive prophylactic immunization in particular circumstances, and in this aspect it is referred to briefly on page 348.

ACTIVE ANTITOXIC IMMUNIZATION

Active Immunization against Diphtheria.

(1) The immunizing reagent consists of diphtheria toxin, so modified as to be wholly or relatively non-toxic, while retaining its antigenic power. We know (see below) that this reagent is capable of stimulating antitoxin production in animals or man, and our knowledge of antitoxic immunity in diphtheria would lead us to expect that such antitoxin production would result in an effective immunity.

(2) The reagent can be standardized with a reasonable degree of accuracy (see p. 394).

(3) As early as 1892 von Behring and Wernicke had shown that susceptible animals might be safely immunized by inoculating them with increasing doses of living culture after a protective dose of antitoxic serum. Six years later Nikanaroff reported successful immunization by the inoculation of a few doses of toxin neutralized with antitoxin; and Dreyer, in 1900, proposed the use of toxin-antitoxin mixtures during the early stages of antitoxin production in horses. The long lag in the application of similar methods to man appears to have been in part due to the fact that the reported results of toxin-antitoxin mixtures in the horse were unfavourable, while unmodified toxin was clearly too dangerous a reagent for use in human subjects. More recent work (see for instance Glenny and Waddington 1928) has shown quite clearly that laboratory animals, such as the guinea-pig, may be rendered immune to diphtheria toxin, as judged by the intradermal test, by a few injections of toxin-antitoxin or of toxoid.

(4) and (5) The introduction of the Schick test has provided us with an indirect method of assessing immunity in man without observing the result of actual exposure to risk of infection. The validity of this test depends, of course, on the fact that observation of large samples of natural Schick-immunes has shown that such persons are very unlikely to contract diphtheria. Even so, we should never accept the proof that active immunization will convert a Schick-positive to a Schick-negative reactor as final evidence that this pro-

cedure will protect against natural infection. We must give due weight to the evidence with regard to the induction of Schick-immunity, and the stimulation of antitoxin production as determined by direct titration, but much more to the observed effect of active immunization on the incidence of the disease. It will be convenient to deal with both questions together.

Von Behring (1913) reported the successful use of a toxin-antitoxin mixture in the immunization of children; but the exact nature of his preparation was not described. From 1913 onwards the use of such mixtures for the active immunization of children developed very rapidly, particularly in America and owing largely to the work of Park and Zingher (Park 1913, 1918, 1922, Park and Zingher 1915, 1916, Zingher 1921, 1922). Although these toxin-antitoxin mixtures have proved effective and are entirely safe as long as they are accurately prepared and are stored and used under optimal conditions there are latent possibilities of danger if these conditions are not fulfilled.

In one instance, involving 5 deaths and 40 serious reactions (see Forbes 1927), it was found that the toxin had been added in fractions to obtain the correct toxin-antitoxin ratio, in forgetfulness of the Danysz phenomenon. In another instance it was found that a particular phial of toxin-antitoxin mixture that had been frozen had caused severe constitutional reactions, while other phials of the same batch, which had not been frozen, had been used without any untoward results. Further investigations showed that the freezing of such mixtures frequently resulted in a marked rise in toxicity. It has been shown by Pope (1927) that this phenomenon is dependent, in part at least, on a separation of phenol during freezing; this leads to a local concentration greatly in excess of the 0.5 per cent. or so added for preservative purposes, and to a consequent destruction of antitoxin, which is more sensitive than toxin to phenolization. Observations recorded by Robinson and White (1928) indicate that, even in the absence of added phenol, dissociation of toxin-antitoxin mixtures may occur on freezing, the degree of dissociation apparently depending on the concentration of the mixture.

There are obvious advantages in using a prophylactic in which the chance of such a dissociation is eliminated, and recent investigations have shown that various forms of toxoid, or of toxoid-antitoxin mixtures, are highly efficient antigens (see Hartley 1926, Watson and Langstaff 1926, Glenny and Pope 1927, Glenny and Waddington 1928). Ramon in 1924 employed, under the name of "anatoxin," a toxoid prepared by adding 0.4 per cent. formalin to a toxic filtrate and incubating for 1 month at a temperature of 37° C., and this "anatoxin" has been extensively used as a prophylactic in many countries. There is another advantage in using toxoid rather than toxin-antitoxin or toxoid-antitoxin mixtures. It has been shown (see p. 209) that the injection of these toxin-antitoxin mixtures induces or increases hypersensitiveness to horse serum as judged by the usual intracutaneous tests; and it would appear (Gordon and Creswell 1924, Veldee *et al.* 1931) that the widespread use of such mixtures for the immunization of children may considerably increase the incidence of serum sickness when antiserum derived from the horse is later administered for therapeutic purposes in any disease.

We can, with any of these reagents, convert the great majority of Schick-positive to Schick-negative reactors (see Park and Zingher, above, Ramon 1926, Lagrange 1926, Kundratitz 1927, Progulski and Redlich 1927, Martin, Loiseau and Laffaile 1928, Parish and Okell 1928, van Boeckel 1928, Ramon, Debré *et al.* 1931, Ray 1931, Seligmann 1931 and many others). Using one of the recognized methods of immunization, such as the injection of three doses of formolized toxin (0.5 c.c., 1.0 c.c. and 1.5 c.c.) with an interval of 2-3 weeks between the injections, it is possible to induce Schick-immunity in 88-98 per cent. of Schick-susceptibles within 6-8 weeks. The results obtained in some of the trials have given us valuable hints with regard to the probable duration of the immunity so induced. Thus (Parish and Okell 1928) 440 children who were Schick-positive when first tested were rendered Schick-negative by immunization. When retested 1-7 years later approximately 5 per cent. had become positive again. But these "relapsed"

Schick-positives still showed the effect of immunization by rapidly producing circulating antitoxin in response to very small amounts of diphtheria toxin; they would fall into the "sub-immune" or "potentially immune" classes of Glenn's classification (see p. 280).

In considering the results of field trials as judged by changes in morbidity or mortality we must remember Question (5), and try to define as nearly as we may the conditions to which these results apply. We may, for instance, ask how far active immunization with toxoid protects the individual against the risk of infection under varying conditions; whether it has proved an effective means of herd immunization in small closed or semi-closed communities; and whether it has been shown significantly to reduce the incidence of diphtheria in the general community, when applied on the largest practicable scale.

In examining the evidence with regard to the first question it must be noted that immunization is often, but not always, preceded by Schick testing, only the Schick-positive reactors receiving the subsequent immunizing injections of toxoid. In such cases the "treated" group consists of natural and artificial immunes, and the "control" group consists, in general, of a sample of the community having approximately the same age distribution as the treated group, but neither Schick-tested nor immune. In other cases the comparison is between those who have been artificially immunized and those who have not, irrespective of the distribution of natural immunity in the untreated groups.

Bieber (1920) reported the after history, from 1913, of 1,097 immunized and 3,275 non-immunized children in villages near Magdeburg. Of the former, 52 (4.8 per cent.) had contracted diphtheria; of the latter, 493 (15.1 per cent.). Park (1922) records the history over a shorter period of 90,000 immunized or naturally immune children and 90,000 controls. During the period of observation 14 of the former contracted diphtheria as against 56 of the latter. Adams (see Fitzgerald 1928) records the history during the years 1926, 1927 of 11,000 children who had received two immunizing doses of toxoid, and of 9,000 non-immunized controls. Among the immunized the incidence of diphtheria was 1.55 per 1,000, among the non-immunized it was 11.44 per 1,000. Isabolinski and others (1931) describe the results of immunization in Smolensk. Of 4,185 children, immunized with toxoid, 0.14 per cent. contracted diphtheria during the eleven months following immunization; of 19,000 control children in the same town 1.5 per cent. contracted diphtheria during the same period. McKinnon and others (1931) and Fitzgerald and others (1932) record the results of immunization in Toronto. Among a group of 36,189 children who had passed through the immunization centres and had been found to be Schick-negative, or had received one or more immunizing doses of toxoid, or had been found to be sensitive to a preliminary injection of toxoid and so received no further treatment, there were 120 cases of diphtheria during the period of observation (December 1926-June 1929). As a control the prevailing rates of diphtheria morbidity at ages among the non-immunized population were applied to each age group of the immunized. This gave 478 as the expected number of cases. Of the 36,189 children, 16,829 had received three doses of toxoid. Among this group 222 cases of diphtheria would have been expected; only 23 occurred. There are many other records of an analogous kind; for a recent summary see Forbes (1932).

The answer to our first question does not seem to be in doubt. Schick testing, followed by the immunization of susceptibles, or immunization without preliminary testing, will afford a very valuable degree of protection, but not

absolute immunity. The odds against contracting diphtheria would seem on the available evidence to be increased four to ten times.

Turning to the control of diphtheria among isolated or semi-isolated communities, we find that Schick testing and artificial immunization has proved highly successful. Fever hospitals supply an environment in which there is a particularly high risk of infection, and the history of attempts to protect the nursing and domestic staff is correspondingly instructive (see for instance Benson 1928, Woods 1928, Harries 1930).

The figures given by Harries (1930) for the Birmingham City Fever Hospital are set out in Table XLIV.

TABLE XLIV

SHOWING THE DIPHTHERIA INCIDENCE AMONG THE NURSING AND DOMESTIC STAFF AT BIRMINGHAM CITY FEVER HOSPITAL. (Modified from Harries.)

Year.	Diphtheria Incidence per cent.		
	Nursing.	Domestic.	Combined.
1919	15.6	9.4	13.5
1920	24.5	16.3	21.8
1921	13.0	11.1	12.4
1922 (a)	6.5	6.7	6.5
1923 (b)	3.7	8.9	5.2
1924	0.93	11.1	3.9
1925 (c)	—	—	0.77
1926	—	—	0.76
1927	—	—	0
1928	—	—	0
1929	—	—	0.6

(a) Schick testing of nursing staff commenced.

(b) Active immunization of nursing staff commenced.

(c) Active immunization of domestic staff commenced.

The significance of such figures cannot be in doubt. The decline of diphtheria among the nurses coincident with the commencement of Schick testing and immunization, the persistence of diphtheria among the domestic staff until similar measures were applied to them, and the subsequent lowering of incidence until, during 3 years, only 1 case of diphtheria occurred among a staff increasing from 130 to 166, indicate clearly that adequate immunization of all susceptibles will reduce to insignificance the incidence of this disease among a community exposed to particularly severe risks.

The application of similar methods to schools and other closed or semi-closed communities, where all new entrants can be tested and if necessary immunized and the herd immunity thus maintained at its highest attainable level, has given equally satisfactory results. Numerous examples are on record, and the history of events at the Royal Naval School at Greenwich as recounted by Dudley (1923, 1926, 1932) affords an admirable illustration.

We may interpolate here a brief description of the method of controlling an outbreak of diphtheria in a school, or similar institution, advocated by

Okell, Eagleton and O'Brien (1924). The steps that should be taken directly a case of diphtheria occurs are as follows :

- (1) Perform a Schick test on all inmates and take swabs from the nose and throat.
- (2) Next day take a preliminary reading of the Schick tests, and isolate in one ward all negative reactors. The results of the swabs, based on morphological appearances, will be available on this day. Those who are Schick-negative—swab-positive should be regarded as carriers and retained in isolation. Those who are Schick-negative—swab-negative are uninfected and immune, and can be released from isolation. Those who are Schick-positive—swab-positive (they will be a very small group, if they are found at all) should be regarded as probably incubating diphtheria: they should be kept under close and constant observation, and treated with antitoxin immediately any clinical signs develop. Those who are Schick-positive—swab-negative are uninfected but susceptible: they should be kept under observation and swabbed again if any signs of infection develop. The immunization of all susceptibles should be commenced at once.
- (3) All cultures of morphologically typical *C. diphtheriae* should be tested for virulence. Those Schick-negative persons who are harbouring avirulent diphtheria bacilli may be released from isolation, leaving those immunes who are carriers of virulent bacilli and who must be regarded as potential disseminators of infection until they cease to carry. Any susceptible person who was, at the time the swabs were taken, harbouring virulent diphtheria bacilli will probably have developed the disease before the results of the virulence tests are known. Those Schick-positives who are harbouring avirulent bacilli may be regarded as uninfected susceptibles.

When we turn to our final question—Will Schick testing and immunization significantly reduce, or altogether eliminate, the incidence of diphtheria in the community at large if applied on the widest possible scale?—it must be realized that the emphasis is on the final limiting clause. There is no reason to doubt that, if all susceptibles in the community could be tested and if necessary immunized, the reduction in the incidence of diphtheria would be of the same order as that attained in closed or semi-closed communities. But we are dealing with practical politics, and what we want to know is the probable effect of an administratively possible policy. We are here faced with a problem of great statistical difficulty. It will not do to cite a few instances in which the inauguration of mass-immunization of school children has been associated with a decline in the general diphtheria morbidity rate, because we know quite well that most infective diseases are subject to secular trends in frequency, and a falling rate for any one of them might well have no significant association with any particular preventive measure adopted at the time the fall occurred.

Woods (1928), for instance, has pointed out that the fall in the diphtheria-morbidity rate which followed the introduction of mass immunization in New York in 1918 cannot be accepted as certainly significant. The rate was already falling before the immunization began; and if a straight line is fitted to the trend of mortality for the years prior to 1917, and is then extrapolated beyond 1918, this extended line approximates very closely to that which would describe the actual course of events during the following decade.

Godfrey (1932), in an admirable review of the available evidence, discusses the possible reasons for such divergent results as those recorded in New York City and Philadelphia, where immunization was followed by a marked decrease in morbidity, and in Newark, Detroit and Buffalo, where similar measures failed. Tracing in detail the course of events in eleven different districts in New York State he arrives at the conclusion that the crucial point is to be found in the immunization of the child of pre-school age (the age group 1-5). The earlier attempts at mass immunization were, for obvious administrative reasons, confined mainly to children of school age (the age groups 5-9 and 9-14). Godfrey can

find no evidence that the immunization of 50 per cent. or over of the children in these age groups produces a definite fall in the incidence of diphtheria in the community as a whole. When, however, the immunization of 30 per cent. or more of the age group 1-5 is superimposed on that of the later age groups there is an immediate and striking decline in the general prevalence of the disease. Godfrey found only two exceptions to this rule in the eleven areas from which his data were obtained.

The same point is made by Lee (1931) who has studied the trend of diphtheria mortality in Philadelphia and in New York City from 1870 to 1930. Fitting an exponential curve to the recorded death rates, he finds that for neither city was there any significant departure from the general trend between the end of the period (1890-1900), when the use of antitoxin as a therapeutic measure was becoming general, and 1929. But in 1930, following the extension of the immunization campaign to children of pre-school age, there was a dramatic change in both cities. In Philadelphia the expected death rate on the basis of the previous trend would have been 10.5 per 100,000, with odds of 20:1 that it would fall between 8.1 and 13.6. Actually it was 2.86. In the case of each city the odds against the observed difference between the actual and the expected death rates being due to chance are of an astronomical order.

It is possible that the effect of immunizing children of school age alone may be offset, so far as its influence on the *general* morbidity is concerned, by the creation of healthy carriers who may infect the younger susceptibles in their homes (see p. 280). We have, here, an admirable example of the necessity for considering the factors that determine herd immunity, as well as those that determine immunity in the individual.

In summary, we have in the modern method of antidiphtheria immunization a measure that is based on well-attested immunological principles, involves the use of a reagent of proved potency, is supported by the results of numerous animal experiments, has been shown to be effective in raising the resistance of the individual and in controlling the incidence of the disease in isolated or semi-isolated communities, and is almost certainly capable of greatly reducing the incidence in the community as a whole, if due attention is paid to herd reactions.

Active Immunization against Scarlet Fever.

(1) The reagent employed for this purpose is a filtrate of a broth culture of a toxigenic strain of hæmolytic streptococcus, and in this respect it is analogous to the filtrate of a toxigenic strain of diphtheria bacillus employed for active immunization against diphtheria. But, while the diphtheria toxin is converted to toxoid or mixed with antitoxin before use, the streptococcal toxin is injected in its unaltered state. The reactions produced, even by a considerable dose of toxin in a susceptible person, do not involve the risk of death that would be associated with the injection of a relatively much smaller dose of unaltered diphtheria toxin, and by suitable gradation of dosage in accord with the susceptibility of the person to be immunized it is usually possible to prevent any serious reactions. It is commonly assumed that the only active antigenic constituent concerned is the erythrogenic or rash-producing toxin; but this assumption may be ill-founded.

Todd (1932) has shown that infections with hæmolytic streptococci, including scarlet fever, are generally associated with a considerable increase in the titre of anti-hæmolysin in the blood, and that the sera of horses immunized against hæmolytic streptococci or

their products often contain anti-hæmolysin to high titre. It is possible, therefore, that the ordinary method of immunization against scarlet fever may stimulate the production of anti-hæmolysin as well as of the so-called scarlatinal antitoxin.

In the following paragraphs we are ignoring these difficulties and limiting ourselves to the antigenic effect of the erythrogenic toxin. Immunization with this reagent may reasonably be expected to produce active immunity against that part of the total scarlatinal syndrome to which it gives rise—the characteristic rash and probably many of the other toxic symptoms. We should not expect any antibacterial immunity in the ordinary sense. There is no reason to suppose that the filtrate injected contains an effective dose of bacterial antigen; and, even if it did, the resulting increase in resistance would only be operative against a single antigenic type of hæmolytic streptococcus, while a variety of types may give rise to typical scarlet fever. There is therefore no *a priori* reason to suppose that this method of immunization will protect against the local proliferation of hæmolytic streptococci in the throat, or their possible spread to other regions; though it might have such an effect if the erythrogenic toxin plays a part in damaging the tissues and allowing the streptococci to proliferate.

(2) The erythrogenic toxin can be standardized after a fashion (see p. 392) but not nearly so precisely as diphtheria toxin, since no satisfactory test animal is available.

(3) There is evidence that an antitoxic serum will protect an animal against a massive dose of toxic filtrate, or against the acute toxæmia that is associated with massive experimental infection (see Parish and Okell 1927); but no animal develops a characteristic scarlatinal syndrome, nor gives a satisfactory skin reaction; and the lethal dose of toxin is so large that quantitative data with regard to the effect of active immunization are difficult to obtain.

(4) and (5) Extensive trials have been made on man. The results are in general accord with the expectations set out under (1) above. The injection of toxin in suitably graded doses will, in a considerable proportion of cases, confer antitoxic immunity, as judged by the conversion of a Dick-positive to a Dick-negative reaction.

It would appear (Benson 1928) that the total dose of toxin required to induce this type of immunity may be relatively large—20,000 skin test doses. For persons who give mildly positive Dick reactions 1,000 skin test doses usually form a safe initial dose, but in those who give a vivid and extensive Dick reaction it is wiser to commence with the injection of 500 skin test doses, or even less. Injections may then be given at weekly intervals, increasing the dose 2 to 5 times at each successive injection until a total of 20,000 skin test doses has been given in the course of 3 to 4 weeks. Dick and Dick (1929) have recommended even larger doses, commencing with 500 and working up to 80,000 to 100,000 skin test doses.

The evidence with regard to the proportion of susceptibles that can be rendered Dick-immune by active immunization is less extensive and less concordant than that available in the case of antidiphtheria immunization.

It has been the experience of several observers that the proportion of successful immunizations is lower (see for instance Toyada *et al.* 1929) and that the relapse from acquired skin immunity to skin sensitivity is more frequent. Moreover (see Okell 1932) the failure to respond to the intradermal injection of the erythrogenic toxin would seem to be a less certain index of effective immunity against scarlet fever than is a negative

Schick reaction of immunity to diphtheria. There is no question that Dick-negative reactors are on the average much more resistant than Dick-positive reactors, but many careful observers have recorded the occurrence of scarlet fever in negative reactors, and it seems certain that the Dick-positive rate during the first few days of the disease falls far short of 100 per cent. (see Toyada *et al.* 1929).

The records of the incidence of scarlet fever in the immunized and non-immunized are as yet too few to afford a safe basis for any final conclusion as to the effectiveness of the procedure under different conditions; but they clearly suggest that it has considerable value.

Dick and Dick (1929), summarizing their records, state that among 11,584 immunized persons in institutions where scarlet fever was epidemic, no case of scarlet fever occurred, and that of 1,191 nurses or interns immunized before commencing work in fever hospitals, none contracted the disease. Toyada and others (1929) record a scarlet fever incidence of 1 per 1,000 among fully immunized persons, 18.1 per 1,000 among those partially immunized, and 62.5 per 1,000 among non-immunized controls.

The experience at the Edinburgh City Fever Hospital (Benson 1928) has also been favourable. Table XLV records the effect of Dick testing and immunization on the incidence of scarlet fever among the nursing staff to 1927, and gives comparative figures for the results of immunization against diphtheria.

TABLE XLV

ANNUAL INCIDENCE OF DIPHTHERIA AND SCARLATINA AMONG THE NURSING STAFF OF THE EDINBURGH CITY FEVER HOSPITAL.

Year.	Total Staff.	Diphtheria Incidence per cent.	Scarlatina Incidence per cent
1919	145	10.34	4.82
1920	148	6.75	9.46
1921	146	9.58	10.27
1922 ¹	147	8.84	6.12
1923	137	3.65	4.38
1924	128	3.12	8.59
1925 ¹	161	3.10	9.31
1926	153	1.30	3.52
1927	148	0.67	0.67

The figures for 1928, 1929, 1930 and 1931, as given in the Annual Reports of the Medical Officer of Health for Edinburgh, show that the very low incidence of diphtheria and scarlet fever among the staff has been maintained.

It may be noted that the experience of active immunization in man is in general accord with the view that the protection afforded is less effective against the invasive than against the toxæmic effects of hæmolytic streptococcal infection. Thus Kinloch and others (1927) note an increase in the frequency of streptococcal tonsillitis among the nursing staff of a fever hospital after immunization, suggesting that the typical scarlatinal syndrome had been replaced by an infection confined to the throat. Benson (1928), on the other hand, has recorded a slight diminution in the incidence of tonsillitis among the nursing staff after immunization; but the diminution was insignificant in comparison with the much greater decrease in the incidence of scarlet fever among the staff over the same period (see, however, the effect of antitoxic sera in lowering the frequency of complications in scarlet fever, p. 364).

¹ Schick testing and immunization commenced September 1922. Dick testing and immunization commenced October 1925.

ACTIVE ANTIBACTERIAL IMMUNIZATION

Active Immunization against Typhoid Fever.

(1) The prophylactic reagent consists of a suspension of typhoid bacilli, killed by heating to 55°–60° C. and adding 0.5 per cent. phenol. Often, in order to protect against infection with *Bact. paratyphosum A* and *Bact. paratyphosum B*, these bacteria are added to the suspension, constituting the ordinary T.A.B. vaccine. A usual bacterial content is 1,000 million *Bact. typhosum*, 750 million *Bact. paratyphosum A* and 750 million *Bact. paratyphosum B* per c.c., and a usual dose is 0.5 c.c. subcutaneously, followed by 1 c.c. after an interval of 7–14 days. Provided that the vaccine is prepared from a smooth strain, containing an adequate amount of the smooth somatic antigen, it is reasonable to believe that it will afford some degree of protection.

(2) The method of standardization at present employed is confined to stating the bacterial content in numbers per c.c. (see p. 395).

(3) There is evidence (see pp. 130, 131) that the injection of vaccines prepared from smooth cultures of *Bact. typhosum*, or of related organisms, confers a significant, but by no means an absolute immunity against experimental infection and a much less effective protection against continuous exposure to contact infection in an experimental herd.

(4) and (5) Antityphoid vaccination in man was initiated by Wright in 1897 (see Wright and Semple 1897, Wright 1902, 1908)—though the observations of Pfeiffer and Kolle (1896) have been held by some to establish a claim to priority—and has since been tried under field conditions on the largest scale. The recorded results strongly support the view that it significantly reduces the incidence of enteric infection in environments in which such infection is particularly likely to occur.

The most impressive figures are those collected by the Antityphoid Committee (Rep. 1913), referring to the incidence of typhoid fever among inoculated and uninoculated troops stationed abroad. These figures have been analysed in a valuable paper by Greenwood and Yule (1915), to which reference should be made by all those who are interested in the general question of the interpretation of data of this kind. Employing the method of the fourfold table, and including as inoculated all those who had received typhoid vaccine at the time of the last available return, the data are set out in Table XLVI.

TABLE XLVI

	Not Attacked.	Attacked.	Total.
Inoculated	10,322	56	10,378
Not inoculated	8,664	272	8,936
Total	18,986	328	19,314

$$\chi^2 = 180.38.$$

$$P = \text{less than } 0.0001.$$

The value χ^2 is a measure, devised by Pearson, of the probability that the distribution actually observed might have arisen as the result of chance; as χ^2 increases, this probability decreases. The value P gives this probability in the usual numerical form (*i.e.*, in this particular case the odds are greater than 9,999 to 1—actually they are much greater—against the observed distribution having arisen as the result of chance).

War conditions provide an admirable breeding ground for typhoid and paratyphoid infections; and it might have been expected that 1914-18 would have yielded a final proof of the efficacy of antityphoid inoculation, and a close measure of its exact protective value. They did, in truth, greatly strengthen the presumptive evidence in its favour—we cannot reasonably suppose that the whole of the vast difference between the deadly rôle of typhoid in the Boer War (105 cases per year per 1,000 of strength) and its relative insignificance among the huge armies on the Western Front 15 years later (2.35 cases per year per 1,000 of strength—see Harvey 1929) was wholly due to better sanitary conditions. But the exigencies of war, the impossibility of getting complete figures for the inoculated and the uninoculated exposed to risk, and the fact that the proportion of inoculated men rose, in the British Army, to well over 90 per cent. by the end of 1915, rendered impossible the collection of mass figures of a kind suitable for detailed statistical analysis. The general trend of the evidence is not, however, in doubt. Thus (see Harvey 1929) the year 1915 provides figures for the percentage of inoculated men in the Army in France and the cases of typhoid in the inoculated and the uninoculated, from which the incidence in each group can be calculated. The incidence among the inoculated was 9.5 per 10,000, among the uninoculated 103.52 per 10,000. During this period the men were inoculated against typhoid only. The incidence rates for paratyphoid fever were, among the inoculated 21.53 per 10,000, among the uninoculated 39.84 per 10,000. This suggests some degree of group protection against paratyphoid infection—a result that would hardly have been expected on immunological grounds—but the wide difference in the incidence ratios (10.90:1 in favour of the inoculated in the case of typhoid and 1.85:1 in the case of paratyphoid) provides additional support for the view that the recorded figures do in fact represent a saving in morbidity due to the typhoid vaccine.

There is evidence, as might reasonably be expected, that the decrease in morbidity was associated with a decrease in case fatality, at least so far as typhoid fever was concerned. The case-fatality rate among 1,728 inoculated and infected men in the British armies in France was 4.57 per cent.; among 703 uninoculated men it was 18.35 per cent. Curiously enough this finding was not paralleled in paratyphoid infection: among 1,357 infected men who had been inoculated with T.A.B. vaccine the case-fatality rate was 1.25 per cent.; among 2,694 men who had not been inoculated with T.A.B. vaccine it was 1.34 per cent. The difference in the case fatality of typhoid in inoculated and uninoculated men is borne out by the figures recorded by Pfeiffer (1922) for the German Army: 12-20 per cent. among the uninoculated, 2-3 per cent. among the inoculated.

Ancillary evidence, which may perhaps be adduced in support of the efficacy of anti-typhoid vaccination, is provided by the curious change in the sex incidence of enteric infection in the years following the War that has been noted by many observers (see Chauffard, Achard and Sergeant 1921, Weigmann 1926, Hahn 1926, Knorr 1927). Before the War the disease tended to fall more heavily on males, after the War it tended to fall more heavily on females; and, in some instances at least, this shift was most noticeable in those age groups that contained the men who were of military age during 1914-18. The value of this evidence is at best doubtful. In one instance (Weigmann 1926) the incidence of paratyphoid as well as of typhoid fever was lower in males than in females during the post-war years, though vaccine containing paratyphoid as well as typhoid bacilli had not been in general use among the German troops. There is also the possibility that the greater exposure to infection during 1914-18 and not the antityphoid inoculation was the cause of the greater resistance of males of military age in the post-war years.

In the chapter on herd immunity we described experiments on mice; and arguing, with the necessary caveat, from mice to men, we drew the tentative conclusion that antityphoid inoculation, carried out by the best technique at present available, would form a poor substitute for the ordinary methods of sanitary control as applied to a population exposed to a severe and continuous

risk of infection. There seems no reason to modify this view in the light of the recorded results on man. Inoculated men appear to do much better than inoculated mice, but they are never exposed to such severe and continuous risk. Even with their advantages in this respect there is no evidence that the inoculation of all those at risk will stamp out the disease, though it will greatly reduce both its incidence and its fatality. As a method of decreasing the chances of infection or death run by persons who are exposed to a serious but not overwhelming risk for a limited period of time, antityphoid inoculation must be regarded as a valuable prophylactic measure.

Active Immunization against Lobar Pneumonia.

(1) The immunizing reagent is a suspension of killed pneumococci. Our knowledge of antipneumococcal immunity indicates that the essential antigenic component is the type-specific polysaccharide. It is reasonable to expect that adequate immunization with a smooth strain of Type I pneumococcus will increase resistance against Type I pneumonia, and so with each of the other types.

(2) The only method of standardization commonly employed is the enumeration of the bacterial content.

(3) The records of antipneumococcal immunization in laboratory animals are copious, and their significance is unequivocal. It is clear that susceptible animals, such as rabbits and mice, can be immunized with almost uniform success against experimental infection by the usual routes.

More pertinent perhaps from our point of view are the results obtained by Cecil and Blake (1920) and Cecil and Steffen (1923, 1925), who attempted to immunize monkeys against intratracheal inoculation of virulent pneumococci. They found that the subcutaneous injection of killed Type I pneumococci, in doses comparable to those usually employed in man, failed to protect them completely against the subsequent intratracheal inoculation of the living organism. Such vaccination did, however, modify the course of the disease. Invasion of the blood stream was slighter and the proportion of recoveries was higher in the treated than in the untreated animals. When a much larger dosage was employed the results were far more satisfactory. The subcutaneous inoculation of three large doses of Type I or Type II vaccine, or of a vaccine prepared from a particular untyped strain, protected monkeys against the intratracheal inoculation of the homologous pneumococcus. With Type III strains the protection was less complete, only 4 of 8 thrice-vaccinated monkeys proving refractory to infection.

(4) and (5) The records of field trials on man tell a rather puzzling story. The frequency of lobar pneumonia, though very high in the aggregate, is not concentrated on particular age groups or confined in the main, as are diphtheria and scarlet fever, to schools and institutions. Even if we decided that active immunization was a promising method of prophylaxis we should hardly know where to begin. There are, however, certain environments that entail a particularly high risk of infection, and in some of these trials have been carried out on a considerable scale.

The most extensive data are those provided by Lister's report (1917) on the result of vaccination against pneumonia among the native labourers in the Rand mines. The numbers at risk were large; but over the greater part of the period of trial the natives were not divided into inoculated and uninoculated groups. An earlier trial (see Orenstein

1931), in which 55,900 natives of various tribes had been divided into two equal groups, one inoculated and the other uninoculated, had shown a pneumonia incidence of 16·4 per 1,000 among the inoculated, 20·6 per 1,000 among the controls. As a result wholesale inoculation was commenced in many mines, and the only basis of comparison available is that between the incidence of pneumonia in a particular mine before and after the institution of active immunization. As we have seen, this method of comparison is always liable to error. There was, in fact, a definite fall in the incidence of pneumonia following these mass inoculations; but the pneumonia death rate on the Rand had fallen sharply from the high level of 1907–11 before mass immunization was instituted, and there is no need to assume that the fall in death rate from 1916–20 was other than a continuation of the trend that was already well marked over the period 1911–14. Orenstein (1931) would attribute the whole downward trend to circumstances other than immunization, in particular to the decrease in the recruitment of tropical natives since 1911. If, as seems probable, we can attach little significance to the secular change in incidence or mortality we are left with ancillary evidence concerning the prevalence of particular infecting types in the pre- and post-inoculation periods. Lister had previously classified the strains of pneumococci isolated from the Rand miners into a series of types which he labelled A, B, C, D, E, F, G, H, J and K, with a further undifferentiated group. Of these types A, B and C accounted for more than 70 per cent. of cases. Subsequent investigations have shown that Lister's Type C is identical with the classical Type I, and his Type B with Type II. The vaccine he employed for his earlier immunizations was prepared from these three strains, and three subcutaneous injections were given each containing 2,000,000,000 cocci of each type—in some series even larger doses were employed. In a particular trial 82 cases of pneumonia occurred among some 10,000 inoculated natives; but none of these were due to infection with Type A, B or C. The subsequent history of events on the Rand is, however, very difficult to interpret. In 1917 a change was made in the constitution of the vaccine, each dose containing 1,000,000,000 cocci of each of eight different types (see Ordman 1931). The pneumonia death rate continued to fall till about 1920–22 but since then it has risen; and this rise, though not great, has been most marked in the last 3 years of the period under review—1927, 1928 and 1929. Orenstein (1931) notes that, in 1929, inoculation was discontinued in certain mines, and the subsequent experience in these was compared with the experience in others in which inoculation was continued. The mines in which inoculation was continued had a higher attack rate in 1930 than in 1928, while the mines in which inoculation had been stopped had a lower attack rate. It may be, as Ordman (1931) has suggested, that this recent failure in immunization is due to a change in the type of infection, not only in the prevalent types of pneumococci but in the rôle played by other bacterial species. In so far as this is the case, it illustrates the difficulty of immunizing against the clinical entity "pneumonia," which after all is the practical problem to be faced.

Environmental conditions particularly favourable to the occurrence of lobar pneumonia were provided in some of the concentration camps and dépôts during the War. Cecil (1925) reviews the results of a considerable series of mass inoculations carried out in America during the period 1918–19. As in so many of the medical records of the War, military exigencies often prevented the fulfilment of the conditions needed for a satisfactory statistical comparison. At Camp Upton, among 12,519 vaccinated men no case of pneumonia due to Type I, II or III pneumococci occurred during the trial period, while 26 cases occurred among 20,000 unvaccinated controls; but the incidence in the controls is very low, and the significance of the difference is rendered doubtful by the fact that pneumonias due to other types of pneumococci, and streptococcal pneumonia, were also more frequent among the controls than among the inoculated, a result that can hardly be attributed to the inoculation. At another dépôt 38 cases of pneumonia were recorded among 48,849 vaccinated men, an incidence of 83·5 per 100,000; and 83 cases among 49,463 unvaccinated men, an incidence of 168 per 100,000. The types of infecting pneumococci in this series are not specified.

A third large-scale trial has been recorded by Malone (1925) and by King (1925). This was carried out on Indian troops in the Baluchistan district. The vaccine employed contained Type I and Type II pneumococci, 2,500,000,000 of each, and two injections were given. Among 2,996 inoculated men the pneumonia incidence was 9.346 per 1,000; among 5,227 uninoculated controls it was 10.714 per 1,000, a quite insignificant difference. The lack of protection cannot be ascribed to a difference in the incidence of the different pneumococcal types among the inoculated and uninoculated. Of 16 cases of pneumonia among the inoculated, in which the type of infecting organism was determined, 3 were due to Type I, 1 to Type II and 12 to the undifferentiated Group IV. Of 18 cases among the uninoculated, 5 were infected with Type I, 2 with Type II and 11 with Group IV. It is, of course, true that the local prevalence of types was not such as to afford much hope of any great reduction in incidence—23 of 34 typed cases were caused by types against which the vaccine could not be expected to afford protection—but there is no significant evidence of protection against Types I and II.

In summary, it would seem fair to conclude that there is presumptive evidence, based on animal experiments, that immunization with particular types of pneumococci will afford some increase in resistance to infection with these types. This evidence has not been strengthened by the results of recorded trials in man; and it would seem that, with our present methods, the increase in resistance attained is slight at best.

Even if the protection afforded against homologous types were much more effective than it is, our difficulties would not be surmounted. Table XLVII gives the frequency of the three classical types of pneumococci as recorded by different observers.

TABLE XLVII
FREQUENCY OF THE DIFFERENT SEROLOGICAL TYPES OF PNEUMOCOCCI IN LOBAR PNEUMONIA, AS RECORDED BY VARIOUS OBSERVERS.

Observers.	Country or District.	Period.	No. of Cases Examined.	Frequency Percentage.			
				Type I.	Type II.	Type III.	Group IV.
Schorer, Clark and others	U.S.A.	1917-18	101	30.7	14.9	17.8	36.6
Thomas	U.S.A.	1917-21	239	25.1	10.9	14.6	49.4
Cecil, Baldwin and Larsen	U.S.A.	1921-26	1,913	33.6	19.1	13.3	33.3
Griffith	England	1920-22	150	30.6	32.7	6.7	30.0
Glynn, Digby and Jones	England (Liverpool)	1919-21	96	45.8	24.0	2.1	28.1
Ferguson and Lovell	England (Manchester)	1925-27	116	43.1	4.3	0.0	52.6

It will be noted that the proportion of lobar pneumonias due to types other than the classical Types I, II and III vary from 28.1 to 52.6 per cent. of all cases examined.

The recent differentiation by Cooper and her colleagues (Cooper *et al.* 1932) of 29 new types, making 32 types in all, has not rendered the position more hopeful, though it has defined the difficulties. Among 136 "Group IV" strains isolated from lobar or broncho-pneumonia in children, all but three of the new types are represented, the highest number

of strains assigned to any one type is 20, and 13 strains remain unclassified. The problem of successful immunization against pneumonia—even against pneumococcal pneumonia—does not seem to be within sight of solution.

Active Immunization against the Common Cold.

(1) Since there is no agreement with regard to the causal infective agent in the common cold it is clear that no reagent at present available can be regarded as certainly containing antigens that might reasonably be expected to induce active immunity against that prevalent infection. Recent studies (Dochez, Shibley and Mills, 1930, Dochez, Mills and Kneeland 1931*a, b*, Shibley, Mills and Dochez 1929, Long *et al.* 1931) have considerably strengthened the view advanced by Foster (1917) that the ætiological agent in the acute cold is a filtrable virus. Such a view would be in entire conformity with the available records concerning the distribution, in those who have colds and those who have not, of those bacteria, such as the pneumococcus, the hæmolytic streptococcus and the influenza bacillus, that have been suggested as the possible causative agents.

Thus Williams, Nevin and Gurley (1921) studied the nasopharyngeal flora of 77 persons suffering from colds and of 75 normal subjects, and found the following differences in the frequencies of certain pathogenic bacteria: the influenza bacillus, 65 per cent. as against 40 per cent.; the pneumococcus, 39 per cent. as against 26 per cent.; the hæmolytic streptococcus, 16 per cent. as against 9 per cent.

Shibley, Hanger and Dochez (1926) studied the bacterial flora of the nose and throat in 12 persons, over periods varying from 4 to 9 months; during this time several of these persons contracted one or more colds. The results obtained during the early stages of colds did not suggest that a causative rôle could be assigned to any particular bacterial species; it was, indeed, noted that swabs taken during the acute stage of a cold often gave an exceptionally scanty growth. Certain organisms were isolated with some frequency during the later stages of a cold; these included the influenza bacillus and hæmolytic streptococci. Noble, Fisher and Brainard (1928) carried out a detailed study of the bacterial flora of the nose, nasopharynx and tonsils of persons with and without colds. The only significant difference between the two groups was the rather higher frequency of influenza bacilli among persons with colds. Bourn (1932), on the other hand, could find no significant difference in the frequency of influenza bacilli in normal persons and in those suffering from acute infections of the upper respiratory tract; and Gundel and Linden (1931) record that they could find no evidence that any of the potentially pathogenic bacteria commonly found in the throat played any rôle in the common cold.

In an investigation undertaken in Manchester during the period 1926-28 (Blackburn *et al.* 1930) a nasopharyngeal swab was taken each month from 100 persons who volunteered to assist in the inquiry. Additional swabs were taken from those persons whenever they had a cold. Data are thus available for 110 swabs taken from persons with colds, and for 1,731 swabs taken from the same persons when they had no signs of a cold. The results are set out in Table XLVIII. They afford no evidence that the influenza bacillus, the pneumococcus or the hæmolytic streptococcus were acting as causal agents.

There is, of course, no question that many of the complications and sequelæ of the common cold—sinusitis, suppurative inflammation in and about the nasal and post-nasal spaces, and so on—are due to infections with pneumococci, influenza bacilli and hæmolytic streptococci, and Dochez and others (1932) have recently recorded observations which suggest that the occurrence of a cold may be associated with a change of avirulent to virulent types of *H. influenza*; but the ætiology of the acute infective cold remains a mystery unless we tentatively accept the virus hypothesis.

TABLE XLVIII

	With Colds (110 Swabs).		Without Colds (1,731 Swabs).	
	Number. +	Per cent. +	Number. +	Per cent. +
<i>H. influenza</i>	52	47.27	984	56.85
<i>Str. hæmolyticus</i>	19	17.27	229	13.23
Pneumococci	30	27.27	446	25.77

Even if we had reason to believe that the common nose and throat pathogens played a primary rôle, or that their effect as secondary invaders was so important as to justify a serious attempt at active immunization, we should not be hopeful of the results. We have referred above to the antigenic heterogeneity of the pneumococci. The hæmolytic streptococci are at least as heterogeneous. The influenza bacilli have so far defied serological classification. Effective immunization against all probable infecting types would seem a council of despair. The answer to our first question must be that there is no reason to expect that such a vaccine will have any influence as a prophylactic against the common cold.

(2) The vaccine is standardized—if we can call the measurement of irrelevant biological products standardization—by enumerating the bacterial content.

(3) There is no relevant evidence from animal experiments, except that it is possible to induce a type-specific immunity against many of the bacteria commonly included in the vaccine. This would of course be equally true of almost any other mixed bacterial suspension.

(4) The results of field trials in man accord very well with the immunological expectations.

Von Sholly and Park (1921), using a mixed vaccine of the ordinary type, inoculated 1,327 persons and kept them under observation, together with 3,025 uninoculated controls, from September 1919 to May 1920; during this period 57.7 per cent. of the inoculated and 38.2 per cent. of the uninoculated developed colds. Jordan and Sharp (1921) observed 448 inoculated persons and 461 uninoculated controls from November 1919 to June 1920; 54.9 per cent. of the inoculated and 51.6 per cent. of the uninoculated developed colds. Ferguson, Davey and Topley (1927) observed 138 inoculated persons and 148 uninoculated controls from November 1924 to May 1925; 78.3 per cent. of the inoculated and 70.3 per cent. of the uninoculated developed colds. Brown (1932) observed 80 inoculated persons and 82 uninoculated controls from November 1929 to June 1930; the inoculated contracted 1.85 colds per person during this period, the uninoculated 2.17.

Vaccination against colds, with the reagents at present available, might be expected to fail on *a priori* grounds, and appears to do so in practice.

Active Immunization against Influenza.

(1) The case is analogous to that of the common cold though not identical with it. There are better grounds for a belief in the causative rôle of the influenza bacillus, or at least in its dominant importance as a secondary invader, than for accepting the claims of the common pathogens of the nose and throat

as causal agents of the common cold. But the evidence that has accumulated since 1918 has told against the influenza bacillus rather than in its favour; and it is probably true to say that the balance of bacteriological opinion is shifting definitely towards the view that epidemic influenza is caused by a filtrable virus. Even if we accepted Pfeiffer's bacillus as the primary ætiological agent, we should have very serious doubts as to the efficacy of a vaccine prepared from any stray sample of strains. The antigenic heterogeneity of the species, as recorded by many observers, is of the extremist kind (see Park, Williams and Cooper 1918, Valentine and Cooper 1919, Rivers and Kohn 1921, Yabe 1921, Anderson and Schultz 1921, Cooper *et al.* 1921, Povitsky and Denny 1921, Kristensen 1922, Knorr 1924 and others); though, when complement fixation instead of agglutination is employed for serological classification, we have a definite suggestion of a common antigenic component (Wollstein 1919, Rapoport 1919, Bieling and Weichbrodt 1920, McIntosh 1922, Kristensen 1922). Recent studies by Pittman (1931) suggest that the successful antigenic analysis of this species may depend on careful differentiation between smooth and rough strains.

(2) The vaccine employed consists of a killed suspension of influenza bacilli, sometimes with the addition of pneumococci and hæmolytic streptococci. The numbers present per c.c. of vaccine are usually stated.

(3) There are no data with regard to the active immunization of animals against the influenza bacillus that can be used as a basis for assessing its probable efficacy in man.

Some recent observations by Shope (1931*a*, *b*, 1932) on an infective disease of swine would appear, however, to have a direct bearing on this problem. Shope reports that this disease—swine influenza—is caused by a combined infection with a filtrable virus and a hæmophilic organism closely related to the influenza bacillus of man. The virus alone causes a trivial disease, which is, however, highly infectious. The hæmophilic bacillus alone is non-pathogenic. Immunization with the virus affords an effective active immunity against the severe disease caused by double infection with the virus and the hæmophilic bacillus. Immunization with the bacillus is entirely ineffective.

(4) The available records of field trials are so contradictory that no conclusion can be based on them. They reveal, in many cases, a curious neglect of simple statistical principles. In several instances mass inoculations have been carried out under the stimulus of an existing epidemic prevalence; and in assessing the results the necessity for weighting the incidence in the inoculated and uninoculated groups in accord with the period of exposure to risk has been overlooked.

Among the few trials that were carried out under conditions that render comparison possible are the following. Duval and Harris (1919) inoculated 3,072 persons with a vaccine prepared from the influenza bacillus. The vaccination was carried out at the height of the autumn epidemic of 1918, and no mention is made of any allowance for possible differences in the length of exposure between the inoculated and the 866 uninoculated controls. It is, however, stated that the controls refused inoculation, and that all inoculations were carried out within a period of 6 days, so that it would seem probable that the control and inoculated groups started on their trial experience at about the same time. The results are set out in Table XLIX.

The second trial (von Sholly and Park 1921) was started in October 1919 when influenza was not epidemic—a much more satisfactory test. The vaccine was a mixed bacterial

TABLE XLIX

	No.	No. Attacked.	Percentage Attacked.
Inoculated	3,072	102	3·3
Not inoculated	866	375	43·3

suspension containing pneumococci in addition to the influenza bacillus, but the doses of the latter organism differed little from those employed by Duval and Harris. A small outbreak of influenza occurred in January and February 1920, so that the inoculated and uninoculated were exposed to infection about 3 months after the former had received the vaccine. The results of this trial are shown in Table L.

TABLE L

	No.	No. Attacked.	Percentage Attacked.
Inoculated	1,327	232	17·5
Not inoculated.	3,025	494	16·3

The third trial is that conducted by Jordan and Sharp (1921). The type of vaccine used and the general conditions of the trial were essentially the same as in the experience recorded by von Sholly and Park, except that the incidence during the subsequent influenza epidemic was lower for both groups. The results are set out in Table LI.

TABLE LI

	No.	No. Attacked.	Percentage Attacked.
Inoculated	2,873	118	4·1
Not inoculated.	3,193	152	4·8

It will be noted that the results recorded by Duval and Harris diverge widely from those recorded by von Sholly and Park, and by Jordan and Sharp, while the two latter are in entire agreement in regard to the complete absence of protection.

Active immunization against influenza does not, at the moment, appear more hopeful than active immunization against the common cold.

Active Immunization against Tuberculosis.

It is obvious that immunization against tuberculosis is a practical problem of the first importance. It has, in truth, engaged the activities of a succession of workers in many countries for nearly 50 years. Any adequate consideration of our present views and of the data on which they are based would involve a discussion proportionate in length to those 50 years of ill-rewarded effort; and, at the end of it, our conclusion would have to be that an effective artificial immunity to tuberculosis is certainly extremely difficult to attain, and is perhaps unattainable.

Of the many methods of prophylactic immunization that have been attempted, the use of the attenuated strain of tubercle bacillus (B.C.G.) intro-

duced by Calmette (see Calmette 1924, Calmette and Guérin 1924, Calmette *et al.* 1924) has been tested on the largest scale. Judgment, as in all such cases, must be given in accordance with the statistical evidence produced. Those who desire a critical summary of the case as it stands to-day may be referred to Greenwood (1928), Rosenfeld (1928), Wolff (1930), or, on the more general and less statistical side, to Gloyne (1931).

ACTIVE IMMUNIZATION AGAINST VIRUS DISEASES

Active Immunization against Smallpox.

(1) In this classical example of prophylactic immunization, dating back almost a century before the bacteriological era, the immunizing reagent is living vaccinia virus. Jenner's belief that cow-pox was a form of smallpox, modified by passage through the cow, is supported, at least to the extent of clear evidence of a close immunological relationship, by numerous studies carried out within recent years (see p. 264). There is thus good reason for expecting that active immunization by this method will afford effective protection, particularly since we know that smallpox is one of those diseases that confers a high degree of immunity against subsequent infection.

(2) The calf-lymph employed for vaccination is tested for potency, but there is no exact standard. Its intradermal injection, by scarification or otherwise, induces an active lesion at the site of inoculation. The effective dose of antigen is, therefore, determined not so much by the amount injected as by the subsequent proliferation of the virus.

(3) There is ample evidence, based on animal experiments, that immunization with vaccinia virus confers a high degree of immunity to reinfection.

(4) and (5) Data indicating that vaccinated persons were far less frequently attacked than the unvaccinated and, when attacked, were less likely to die from the disease, were recorded in the early days of Jennerian vaccination, and all subsequent records have been in conformity with that conclusion (see Greenwood 1930). But it is not easy, at least in regard to attack rates, to provide figures that will be above statistical suspicion.

In recent years and in most civilized countries, the introduction of infant vaccination, yielding an immunity that certainly decreases with lapse of time, the revaccination at later dates of some persons but not of others, and the numerous vaccinations and revaccinations that are always performed during a local epidemic prevalence, have rendered it extremely difficult to divide those at risk into immunized and non-immunized groups. There is, however, no reason to dissent from the general consensus of medical opinion, that vaccination in infancy followed by revaccination at the age of entering school and again on leaving it confers an immunity lasting over many years at least, and that smallpox is practically never seen in a recently vaccinated or revaccinated person.

When we pass to differences in case fatality we are in better case. We can divide our smallpox cases into vaccinated and unvaccinated with a small margin of error. Table LII (see Report 1928) gives the case mortality at ages as experienced in the hospitals of the Metropolitan Asylums Board during the period 1901-04.

It will be noted that the difference in case mortality is at its highest in the lower age groups, diminishing rather rapidly after the age of 30, though the vaccinated show significantly lower figures at all ages. This is clearly an expression of waning immunity with age. Another fact emerges from the table which supports the view that vaccination

protects against infection as well as against death. The age distribution is very different in the vaccinated and the unvaccinated groups. Whereas the greatest number of admissions among the unvaccinated falls in the first decade, and the numbers steadily diminish as age advances, the maximum number of admissions among the vaccinated falls in the age group 21-30. Here again the obvious indication is that, in the absence of vaccination, the risk of infection is highest in childhood, while among the vaccinated this period is passed in comparative safety, the disease attacking most heavily those in whom the effect of infant vaccination is wearing off.

TABLE LII

Years.	Vaccinated.			Unvaccinated and Doubtful.		
	Admitted.	Died.	Percentage Died.	Admitted.	Died.	Percentage Died.
0-10 . . .	143	2	1.39	1,441	459	31.85
11-20 . . .	1,218	23	1.88	761	166	21.81
21-30 . . .	2,675	144	5.37	374	129	34.49
31-40 . . .	1,861	247	13.27	180	80	44.44
41-50 . . .	893	174	18.21	102	57	55.88
51-60 . . .	311	55	17.68	73	31	42.46

It would seem a reasonable assumption that, in a community in which every person was vaccinated in infancy and revaccinated twice or thrice thereafter at intervals of 5 to 10 years, smallpox would probably cease to exist, or would assume negligible proportions. But the practical question we have to ask ourselves when we turn from the individual to the herd is this. Given the administrative impossibility of compulsory vaccination and revaccination on this all-inclusive plan, what is the probable effect on the incidence of smallpox in the community as a whole of infant vaccination carried out on a practicable scale? The question is not unimportant, because vaccination is a procedure that is not entirely free from risk to life; and the recent experience of post-vaccinal encephalitis has made the risk loom larger than it did before (see Rep. 1928).

It is at the moment impossible to assess the probable advantage to the community as a whole of the vaccination of any given proportion of the infant population (Greenwood 1930). Some of the data which fall for consideration under this head are, in truth, extremely puzzling. The view generally held is that infant vaccination, even on an incomplete scale, affords a significant degree of protection to the community as a whole; that the risks attendant on vaccination are, in the aggregate, extremely slight—and this is most certainly the case; and that the particular risk of post-vaccinal encephalitis, small as it is, is greater after primary vaccinations performed in later childhood or at any subsequent period, than after vaccinations performed in infancy.

As regards the efficacy of vaccination and revaccination in checking the spread of an existing focus of infection, there is, of course, no doubt at all. The immediate vaccination or revaccination of all contacts, and of the largest

possible number of those living in the community at risk, has been found again and again to check the epidemic in its early stages.

PASSIVE ANTITOXIC IMMUNIZATION

Reference to Chapter XI will indicate quite clearly the principles that occasionally lead us to prefer passive to active immunization for prophylactic purposes; they depend on the differences in the time relations of the immunity induced. Active immunity is, as we have seen, induced relatively slowly, a matter of several days at the least, more often of several weeks. On the other hand, once induced, it lasts for months or years—sometimes apparently for the better part of a lifetime. Clearly this is the method of choice when the immediate risk is not particularly grave, and when the person to be immunized is likely to remain at risk for months or years.

In passive immunization, induced by the injection into a susceptible person of serum from an animal artificially immunized against a bacterium or its products, the protective effect is produced at once. On the other hand, the duration of the immunity is short; the concentration of antibodies falls steeply within a few days, and reaches a very low level within a few weeks. Clearly we shall use this method only when we know that a person has been, or is immediately likely to be, submitted to a serious risk of infection.

We may consider as illustrative examples three diseases in which passive immunization is frequently employed.

Passive Prophylactic Immunization against Diphtheria.

(1) Diphtheria antitoxin may be expected to confer an effective immunity so long as it remains in the blood and tissue fluids above a limiting concentration.

(2) It can be standardized with a high degree of accuracy.

(3) It has been shown to confer passive immunity on animals under experimental conditions.

(4) and (5) Passive immunization has been employed to meet particular emergencies, for instance the accidental admission to a children's ward of a child incubating diphtheria and the consequent exposure to infection of the other children in the ward when the disease develops. Under such conditions the administration of 500–1,000 units of antitoxin to each of the children at risk, the injection being given by the subcutaneous or intramuscular route, has been found an effective method of stopping the spread of the disease. The introduction of the Schick test has enabled us to differentiate between susceptibles and immunes, and only the positive reactors need be given antitoxin (see for instance, Nabarro and Signy 1931). Even under such conditions as these active immunization has the advantage of conferring a lasting immunity, though it presents greater administrative difficulties.

Passive Prophylactic Immunization against Scarlet Fever.

The same general considerations apply as in the case of diphtheria. But since an active immunity is more slowly attained, and appears to be less cer-

tainly effective and perhaps of shorter duration, passive immunization is commonly practised in such emergencies as the accidental introduction of a case of scarlet fever to a children's ward.

Passive Prophylactic Immunization against Tetanus.

(1) Tetanus antitoxin may be expected to induce effective immunity against tetanus, so long as it remains in the blood and tissue fluids above a limiting concentration.

(2) It can be standardized with a high degree of accuracy.

(3) It has been shown to confer passive immunity in animals under experimental conditions.

It may be noted (MacConkey and Homer 1917) that the passive immunity conferred on guinea-pigs by the subcutaneous injection of $3\frac{1}{2}$ units of antitoxin disappears in about a fortnight. Increasing the dose up to 40 units fails to prolong this period. With very large doses (250-3,600 units) the animals remain immune for about a month.

(4) and (5) This disease presents an example of a situation in which passive prophylactic immunization is clearly indicated. We know that a person who has suffered a severe wound with extensive damage to the tissues and considerable soiling has incurred an appreciable risk of tetanus. We know that there will be a considerable interval, several days or even weeks, before the toxæmic stage of the disease manifests itself. We know that, if this particular risk can be surmounted, the patient will not be exposed to a similar risk in the future, unless another accident of the same kind happens to him. We have every

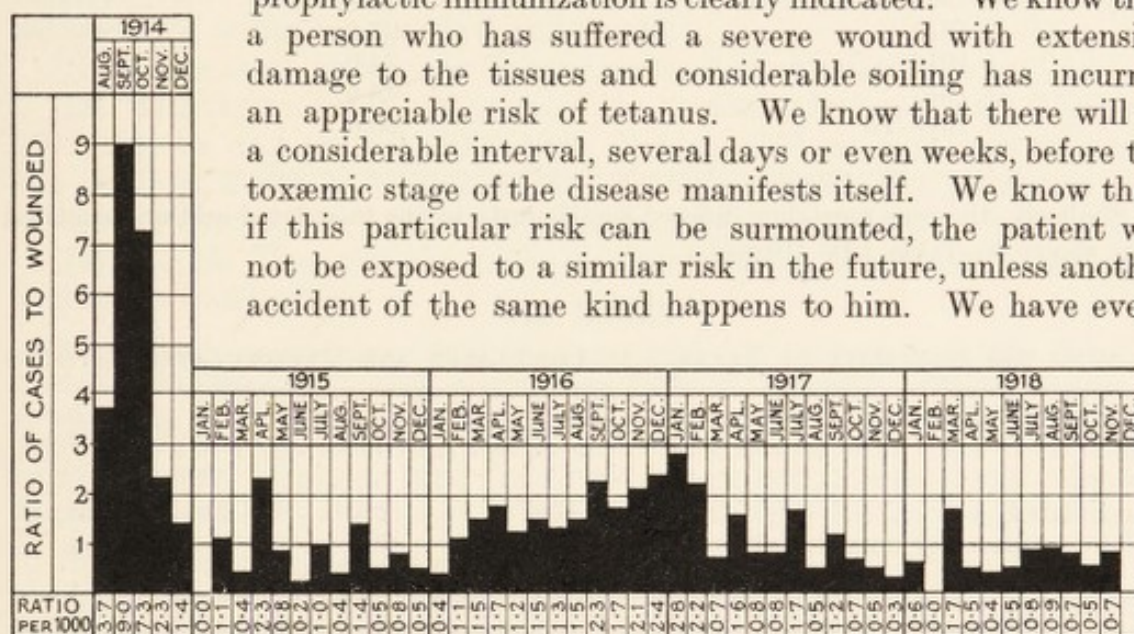


FIG. 37.

The ratio per 1,000 of cases of Tetanus to wounded. (After Bruce)

reason to expect that if we can introduce into his tissue fluids an adequate amount of antitoxin before the toxin elaborated locally reaches the sensitive nerve cells, and maintain it in adequate concentration as long as the local production of toxin continues, we shall greatly increase the odds against his contracting the disease.

The extensive experience gained in the Great War was in general accord with expectations though fully controlled figures were obviously impossible to obtain (see Bruce 1919, 1920).

The incidence of tetanus in the British Armies in France, per 1,000 wounded men per month, is shown in Fig. 37 for the whole period 1914-18.

The sudden fall in November 1914 can, in all probability, be ascribed to the prophylactic use of antitetanic serum, which was introduced about the middle of the preceding month (Bruce 1920). That the method did not serve to eliminate tetanus as one of the major

risks of war is clearly shown by the remainder of the secular graph. Bruce considers that this apparent failure can be in part accounted for by differences in diagnosis between 1914 and 1915. Local tetanus, which was recorded in the latter years of the War, was largely overlooked during its initial stages. Also the exigencies of warfare and transport prevented every wounded man receiving the serum, particularly the repeated doses (500 units subcutaneously every week or fortnight until healing) that are advisable in severe and lacerated wounds.

There was evidence that, where serum did not prevent tetanus, it modified it. Table LIII shows that the incubation of the disease lengthened throughout the War as the prophylactic injections were more universally and rigorously given.

TABLE LIII

SHOWING THE AVERAGE INCUBATION PERIOD OF TETANUS IN DIFFERENT YEARS OF THE WAR.

Year.	Length of Incubation Period in Days.
1914	11.8
1915	27.3
1916	34.0
1917	48.0
1918-19	50.0

Similarly, the case mortality differed significantly in the inoculated and uninoculated as is shown in Table LIV (Bruce).

TABLE LIV

SHOWING THE MORTALITY OF TETANUS IN INOCULATED AND UNINOCULATED PATIENTS

	No. of Cases.	Died.	Case Mortality.
Inoculated	899	203	22.6 per cent.
Non-inoculated or unrecorded . .	559	298	53.3 per cent.

PASSIVE ANTIBACTERIAL IMMUNIZATION

There is, at the moment, no instance in which prophylactic immunization with an antibacterial, as opposed to an antitoxic, serum has been tested on any adequate scale, nor is there any reason to suppose that it would be successful, save under quite exceptional conditions. As we have seen, antibacterial immunization is in general less effective than antitoxic immunization: in part because the presence of specific antibodies is only one of the factors upon which the mechanism of antibacterial immunity depends; in part because a degree of immunity that ensures an effective clearing of the blood stream, and hence protects against an acute bacteraemic infection, does not necessarily ensure a complete freeing of the tissues from the bacterial invader.

PASSIVE ANTIVIRAL IMMUNIZATION

Numerous examples of the protective action of antiviral sera will be found in Chapter XVI. Present-day methods of measles prophylaxis afford an

excellent example of the way in which this protective action may be used to control a virus infection in man, and illustrate several important principles that have a more general application.

The Use of Convalescent Serum in Measles Prophylaxis.

(1) Nicolle and Conseil (1918) proposed the use of serum, obtained from cases of measles during convalescence, as a prophylactic for susceptible children who had been exposed to infection, and recorded a small series of highly encouraging results obtained by this method. The reagent, therefore, consists of human serum—plasma or whole blood has also been employed—obtained under conditions that, from analogy, should ensure the presence of a high concentration of protective antibodies.

The blood is drawn off after the febrile period has passed; and the patient from whom it has been drawn is examined by all available means to exclude the presence of syphilis, tuberculosis or malaria, and should be observed for some days subsequent to bleeding to ensure that he is not in the invasive stage of some secondary or adventitious infection. If these conditions are satisfied the serum is separated and stored.

(2) There is at present no known method of standardization.

(3) With the probable exception of the monkey (Anderson and Goldberger 1911, Nicolle and Conseil 1911, 1920, Purdy 1925) no experimental animal has yet been shown to be susceptible to measles virus. The protective value of convalescent serum has been established by observations on man.

(4) and (5) The evidence available under this head is now considerable, and quite conclusive.

(See Richardson and Connor 1919, Degkwitz 1920, 1921, 1922, McNeal 1922, Debré and Ravina 1923, Méry *et al.* 1923, von Torday 1923*a, b*, Rolleston 1923–24, Weaver and Crooks 1924, Zingher 1924, Debré *et al.* 1925, Copeman 1925, Toomey 1926, Debré *et al.* 1926, Haas and Blum 1926, Park and Freeman 1926, Benson and Lawrie 1927, Karelitz and Levin 1927, Kingsbury 1927, Gunn 1928, Benson 1929, Morales and Mandry 1930, Barenberg *et al.* 1930, Debré *et al.* 1930, Nabarro and Signy 1931, Gunn 1932.)

It may be summarized as follows:

Convalescent measles serum, injected intramuscularly in a dose of about 5 c.c. into a child of 3 years of age or under within 4 days after exposure to infection, will, in the great majority of cases, prevent any illness developing. In older children the dose should be increased, allowing about 2 c.c. of serum for each year of age, until a dose of 10 c.c. is reached with children over 5. When the time of injection is delayed until the 5th to 7th day these doses should be increased to secure complete protection (about 10 c.c. for a child of 3 or under, about 15–20 c.c. for older children). After the 7th day of the incubation period protection is very uncertain even with larger doses. If serum is given on the 8th day or later the disease almost always develops however large the dose. When these conditions with regard to dosage and the time of infection are fulfilled complete protection is almost always afforded.

Thus, among the large series of observations that have been recorded, Degkwitz (1922) reports 97 per cent. successes in some 1,000 cases, von Torday (1923*b*) 97 per cent. successes in 2,000 cases, Park and Freeman (1926) 92 per cent. successes in 243 cases, Nabarro and Signy (1931) 98 per cent. successes in 625 cases, and Gunn (1932) 94.2 per cent. successes in 206 cases.

The immunity thus conferred is purely passive and appears not to last more than 2 to 4 weeks. There are many records of children who have been exposed to infection 2 weeks or more after receiving the prophylactic injection and have developed the disease.

We have thus a typical illustration of the value of passive immunization in cases where a known risk has been incurred at a known time, and of the decisive importance of time relations in passive immunity.

It has been noted that the injection of serum on or after the 8th day of disease seldom, if ever, prevents an attack; but the attack that develops after a delayed injection, or after a small dose of serum given at an earlier stage, is not of the usual kind. If the serum is given on or before the 9th day of incubation the subsequent attack is in most cases exceptionally mild. The catarrhal symptoms are absent or minimal. Koplik's spots do not usually develop. The temperature seldom rises above 100·4° F. on 1 or 2 days. In place of the usual copious rash there is a small discrete eruption scattered over the face and body (see Copeman 1925). A child who has passed through this modified and trivial attack has acquired an active and lasting immunity. (Cf. the methods of mixed active and passive immunization of animals described in Chapter XVI.) Obviously such a result is to be preferred to a complete but transient protection unless there are reasons for shielding the child even from this modified attack.

With home contacts it is usual to be guided by the age and condition of the child. With children of 2 or under, in whom the disease is frequently severe, it is customary when possible to give complete protection. With older children in good health a preferable course is to give smaller doses of serum, or to delay the injection, so that a lasting immunity is purchased at a trivial cost. With a child of any age that is in poor health complete protection should, of course, be given.

In institutions where many children have been exposed to risk all susceptible contacts are usually afforded complete protection in order to prevent an epidemic of the disease.

The difficulty of obtaining an adequate supply of serum would obviously be lessened if we could substitute for the serum from a convalescent case the serum of any adult who had passed through an attack of measles in earlier life. This method has been given an extensive trial (see Debré *et al.* 1925, 1926, Copeman 1925, Karelitz and Levin 1927, Barenberg *et al.* 1930, Morales and Mandry 1930). Such adult sera have a very definite protective value, but relatively large doses must be given—about four times the dose of convalescent serum—and the results obtained appear to be less uniform.

Thus, in a particular series of cases, Debré *et al.* (1926) record 83·5 per cent. complete protection and 12·5 per cent. attenuation with convalescent serum as against 75 per cent. protection and 20 per cent. attenuation with adult serum. Morales and Mandry record 85 per cent. protection with convalescent serum in a dose of 4–6 c.c., as against 40·6 per cent. protection with adult serum in a dose of 10 c.c., rising to 54 per cent. protection with a dose of 20 c.c., and 82·4 to 85·7 per cent. protection with doses of 30 to 40 c.c.

It may be noted that serum of any kind given after the 9th day of the incubation period appears to have no effect on the course of the disease.

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CHAPTER XX

THE PRACTICAL APPLICATION OF IMMUNITY IN TREATMENT

WHEN we turn from the prevention to the cure of infection we are faced with a new set of immunological circumstances ; and it will be convenient to consider the general implications of these changed conditions before discussing the evidence available with regard to any particular disease.

The change in time relations is the decisive factor. In prophylaxis we usually—not always, as witness the cases of tetanus and measles—intervene before infection occurs. We have in most cases ample time at our disposal. Moreover, we have the advantage of being first in the field. A degree of immunity that will avail little in the presence of an established infection may well be effective in preventing its establishment *ab initio*. We know, for instance, that a relatively trivial amount of antitoxin in the circulating blood, combined with the power of producing more antitoxin in response to the stimulus of infection, provides an effective and lasting immunity against diphtheria, and that an equally effective but transient immunity can be passively induced in a susceptible person by the injection of a relatively small dose of antitoxin (500–1,000 units). When we come to the treatment of an established case of diphtheria the dose of antitoxin required is of quite a different order. In treatment we shall not, in general, be greatly concerned with how long our induced immunity lasts. The risk we are concerned with is a present not a future one. We shall, however, be very greatly concerned with the time taken in the induction of immunity, for there is usually no time to spare.

It follows that while active immunization is, as we have seen, usually the method of choice in prophylaxis, passive immunization will usually be the method of choice for therapeutic purposes.

We may clear the ground for a discussion of the latter procedure by a brief reference to the function of active immunization—vaccine therapy—as a method of treating an established infection.

ACTIVE IMMUNIZATION IN TREATMENT

The only way of dealing with this question in such a book as this is by a frank admission of our inability to answer it satisfactorily. Those who desire further information may be referred to a recent monograph by Dudgeon (1927). It is extraordinarily difficult to assess the value of the claims that have been put forward on behalf of this method of treatment as applied to an endless

variety of infections ; and quotations from a copious but largely unconvincing literature would serve no useful purpose. The data recorded are not, in general, of a kind that permit the application of statistical analysis ; and, when the only evidence available consists of a vast collection of clinical records, each of which must be considered on its own merits, we can only await the final crystallization of opinion among competent clinicians. We may, in the meantime, note a few points that seem to arise fairly clearly from the experimental study of infection and resistance.

So far as the essential basis of vaccine therapy is to be regarded as the specific stimulation of antibody formation, its usefulness would seem to be limited to those cases in which the stimulus provided by the existing infection is inadequate to provoke an optimal response, while the host's tissues are in a position to respond effectively if an adequate stimulus is applied. We should, then, be inclined on *a priori* grounds to suspect that vaccine therapy would prove of little service in acute and generalized infections and that the injection into the tissues of any considerable amount of bacterial antigen might do harm rather than good (see Chapter IX). The views expressed by many clinical observers seem to confirm this suspicion. In localized infections we should have to consider whether the absorption of bacterial antigens from the active focus of infection was likely to be so slight that the additional antigenic material introduced with our vaccine might be supposed to convert an ineffective into an effective stimulus. We should have no quantitative data. We could not even guess intelligently. If, by empirical trial, we convinced ourselves that the vaccine exerted a significant curative action, we might offer this assumption of an added stimulus as an explanation ; but that would be rationalizing, not reasoning.

In prolonged and particularly relapsing infections—such for instance as recurrent staphylococcal boils—the position is rather different. Treatment here shades into prophylaxis. A patient may, perhaps, be protected against a relapse, even when we cannot influence the infective focus actually in existence ; again we are assuming that our vaccine supplies a more effective stimulus than the infection itself. We may note, in passing, that our present knowledge with regard to antigenic heterogeneity supports the view that an autogenous vaccine—one derived from the actual infecting organism—should be preferred to a “stock” vaccine, except in those cases in which it is certain that the immunologically significant antigenic component is present in the latter.

Another rationalization of vaccine therapy that retains the element of specificity is based on an analogy with the phenomenon of desensitization in acute anaphylaxis. So far as this appeal is valid it involves the view that the infected host will benefit from the neutralization of antibody by antigen—a view that accords ill with our knowledge of the bacterial “aggressins.”

If we abandon the assumption of specificity we are on different ground (see, for instance, Wright 1931). We shall now regard our vaccine as a means of eliciting non-specific reactions of the type discussed in Chapter XIII, and we shall select the particular vaccine we employ, not because it bears any

antigenic relation to the infecting organism, but because we believe it to be an effective agent for inducing this non-specific response—we might, for instance, prefer a solution of an albumose or of a nucleo-protein to any bacterial suspension.

It does not seem unreasonable to appeal for a little more of the scientific spirit in approaching such problems as these. At the moment it seems—to some of us at least—that the practice of vaccine therapy rests on a very inadequate basis of experimental evidence. If it can be justified on a basis of clinical experience it will obviously take its place with other empirical remedies. But the evidence required for clinical justification can hardly fail to give us hints as to the nature of the mechanisms involved. Those who believe that bacterial vaccines are worth a further trial as therapeutic agents would do well to differentiate between the possible assumptions as to their mode of action, and so to plan their trials that there shall be adequate controls not only of the ability of the vaccine to exert some significant therapeutic effect, but of the possible factors concerned.

PASSIVE IMMUNIZATION IN TREATMENT

Here the evidence in the case is of quite a different kind. We know that, under favourable conditions, specific serum therapy will exert a beneficial effect on experimental infections in animals; and this evidence is, as we shall see, confirmed in many instances by the results of trial in man. A clear understanding of the immunological reactions concerned is, however, essential for the successful exploitation of this method of treatment; and we may cite a few illustrative examples, considering them under the same headings as in the preceding chapter.

Passive Antitoxic Immunization in Treatment

The Treatment of Diphtheria with Antitoxin.

(1) *The Nature of the Serum Employed.*

The serum employed is derived from a horse immunized by repeated injections of diphtheria toxin until the antitoxin titre reaches a high level—800 units or more per c.c. Sometimes a concentrated antitoxin is employed having a still higher titre.

(2) *Standardization.*

The antitoxin can be standardized with a high degree of accuracy (see p. 381).

(3) *Evidence from Animal Experiments.*

There is ample experimental evidence that antitoxin will neutralize toxin *in vivo*; but there is also evidence (see the observations of Glenny and Hopkins, p. 110) that the amount of antitoxin required to exert a significant protective effect rises steeply as time passes after toxin gains access to the tissues. The curative action of antitoxin on the host as a whole is probably a prophylactic action in regard to the individual cells. The antitoxin appears to

exert its main effect by intercepting the toxin on its way from the primary focus of infection to the cells that are susceptible to its action.

(4) *The Results of Trial in Man.*

It might be expected that after some 40 years it would be easy to produce irrefutable statistical evidence of the beneficial effects of the antitoxic treatment of diphtheria in man. The evidence does, in truth, seem decisive to most of us; that it is still possible to bring forward contrary arguments that are not altogether specious is due to the fact that once a strong presumptive case has been made out in favour of a particular therapeutic measure it is not justifiable to continue the period of trial at a risk of human lives.

Of the classical studies from the early days of antitoxin treatment, the only one that affords an entirely satisfactory basis for comparison is that of Fibiger (1898). For a period of one year all cases of diphtheria admitted to hospital were divided into two groups by separating those admitted on alternate days. All cases admitted on one day were given antitoxin, all those admitted on the next day were treated without antitoxin, and so on throughout the period of trial. The results were as follows: of 239 cases treated with antitoxin 8 died, a mortality of 3.5 per cent.; of 245 cases treated without antitoxin 30 died, a mortality of 12.25 per cent. Applying the formula given on p. 15, the difference between the case mortality in the two groups is 8.75 per cent. and the standard deviation of this difference is 2.445 per cent., so that the odds against the difference being due to random sampling are several thousands to one.

Other results recorded during the early and middle 'nineties—comparisons between the case-fatality rates in hospitals in which antitoxin was given and others in which it was not, or between the experience in one hospital before and after the introduction of antitoxin—all pointed in the same direction; and the period of trial ended with the adoption of antitoxin as a routine method of treatment.

If we survey the course of events since that time we find that they strongly support the view that the use of antitoxin has resulted in a significant lowering of the case-fatality rate; but they would hardly be accepted as demonstrative proof in the absence of the earlier and controlled trials.

There is, for instance, the decline in the case-fatality rate as shown by the returns of the hospitals of the Metropolitan Asylums Board (see Table LV).

Antitoxin came into general use about 1895, and the figures suggest that it had produced its full effect on the case-fatality rate about 10 years later; since when this rate has been kept down to a figure of about one-third of that prevailing in pre-antitoxin days. But, if we are disposed to be critical, we shall note that the downward trend had shown itself before the introduction of the antitoxin, and that secular trends in case fatality are known to occur apart altogether from the introduction of new therapeutic measures—as in the classical example of scarlet fever.

There is another factor which would add to our doubt. At about the same time as antitoxin was coming into general use the basis of diagnosis of diphtheria was changing. The introduction of bacteriological methods made it possible to recognize as diphtheria many cases that would earlier have been regarded as non-diphtheritic anginas. For this reason the total of recorded cases would tend to be increased by the inclusion of many mild infections, and the recorded case mortality would automatically fall.

We may probably accept the fall in the case fatality of diphtheria since the early 'nineties as due, in large part at least, to the introduction of antitoxin; but our confidence in the uniform efficacy of this form of treatment has been a little shaken by the happenings of recent years. In Germany and elsewhere there has been an increase in the frequency of severe and fatal cases that is not easy to explain. Deicher and Agulnik (1927), for example, give figures for a Berlin hospital and for one of the districts of that city that are set out in Table LVI.

TABLE LV

Year.	Case Mortality Per cent.	Year.	Case Mortality Per cent.
1889	40.7	1908	9.7
1890	33.5	1909	9.4
1891	30.6	1910	7.8
1892	29.3	1911	8.4
1893	30.4	1912	6.2
1894	29.3	1913	6.2
1895	22.8	1914	7.9
		1915	8.4
1896	21.2	1916	6.8
1897	17.7	1917	6.7
1898	15.4	1918	7.7
1899	13.9	1919	9.3
1900	12.3	1920	8.6
1901	11.1	1921	8.8
1902	11.0	1922	8.7
1903	9.7	1923	6.8
1904	10.0	1924	7.0
1905	8.3	1925	5.0
1906	8.8	1926	4.9
1907	9.6	1927	4.0

TABLE LVI

RUDOLF VIRCHOW HOSPITAL.

Year	1923	1924	1925	1926
Total Cases	132	140	114	172
Deaths	8	7	10	30
Mortality per cent.	6.1	5.0	8.7	17.4

ALT BERLIN.

Year.	1923	1924	1925	1926
Total Cases	1,068	1,016	1,109	1,421
Mortality per cent.	7.58	5.9	8.1	11.1

Whatever the cause of this rise in case fatality, it has not been the failure to administer antitoxin in doses that would be generally regarded as adequate. The average dose in the Berlin cases is recorded as 20,000 units intravenously and 20,000 units intramuscularly, frequently followed on succeeding days by doses of 10,000-20,000 units.

A possible explanation of the increased frequency of severe and fatal cases in particular areas has recently been offered by Anderson, Happold, McLeod and Thomson (1931) who, while studying a large sample of strains of the diphtheria bacillus isolated in Leeds, noted a correlation between the type of the infecting bacillus and the severity of the disease. They divided their bacilli into types on the basis of the character of the colony formed on a special tellurite medium, the ability or inability to ferment starch and glycogen, the type of growth in broth, and certain subsidiary characters. They dis-

tinguished two main types *gravis* and *mitis*, so named because the first was associated with severe, the second with mild cases. There were a minority of strains that were classified as intermediate. From later studies (see Anderson *et al.* 1933) it would seem that these "intermediate" strains form a separate and distinguishable type, and that they correspond rather to the *gravis* than to the *mitis* strains in regard to the severity of the infections which they produce. The results of other workers, so far as they have yet been recorded, have not confirmed those obtained in Leeds (see Parish, Whatley and O'Brien 1932*a, b*, Wright and Rankin 1932). The question as to whether the association observed in Leeds is significant or accidental must await the collection of further evidence on a wider scale.

If the association between the *gravis* and intermediate types and severe and fatal cases is found to be a real one, the bacterial characters that determine it have yet to be discovered. The *mitis* strains are fully toxigenic; on the basis of the data so far recorded they appear to be more toxigenic than the *gravis* strains. There is, apparently, no significant difference in the pathogenicity of the two types for the guinea-pig. The Leeds workers have recorded a relatively higher pathogenicity of *gravis* strains for the rabbit, evidenced mainly by a higher mortality after the intravenous injection of small doses, and a more rapid death with all doses and routes of infection studied. Here, again, the evidence as a whole is divergent. There is, on the present evidence, no reason to suppose that the toxin produced by the *gravis* strains differs in any way from that elaborated by the *mitis* strains, or that antitoxin fails to neutralize it. It may be that the more massive doses of antitoxin that are possible with the sera now on the market will be found effective even in the severer type of case.

Somewhat better evidence that a large part of the observed fall in case fatality since the middle 'nineties may justly be ascribed to the use of antitoxin is afforded by the figures for the mortality rates in cases of laryngeal diphtheria (excluding those cases treated by tracheotomy), since this gives a series in which the clinical signs are sufficient to establish a diagnosis, and in which the infection may be regarded as uniformly serious. In 1894 the mortality in this class of case was 62 per cent.; after 1895 it fell, at first rapidly then less steeply, until in 1910 it was 11.2 per cent. (see Monograph 1923).

A system of dosage in common use is that advocated by Park (1921) (see Table LVII); but there has been a tendency to increase the amount of antitoxin given in the severer type of case as the result of the experience of recent years, and this tendency seems likely to continue. It will be noted that the serum should be given intravenously or intramuscularly, never subcutaneously. Our object is to attain a high concentration of antitoxin in the blood at the

TABLE LVII

UNITS OF ANTITOXIN TO BE ADMINISTERED TO CASES OF VARYING GRADES OF SEVERITY.
(AFTER PARK.)

Age or Weight of Patient.	Mild Cases.	Early Moderate.	Late Moderate and Early Severe.	Severe and Malignant.
Under 2 years.				
10-30 lb. weight .	2,000-3,000	3,000-5,000	5,000-10,000	7,500-10,000
2-15 years.				
30-90 lb. weight .	3,000-4,000	4,000-10,000	10,000-15,000	10,000-20,000
Adults.				
90 lb. or over . .	3,000-5,000	5,000-10,000	10,000-20,000	20,000-50,000
Route of administration	Intramuscular	Intravenous	Intravenous	Intravenous

earliest possible moment, and subcutaneous injection will defeat this end (see p. 185).

Bie (1922) has contributed a good critical review of the evidence in favour of the antitoxin treatment of diphtheria. Much of it he finds unsatisfactory; but he notes that the use in his own hospital of greatly increased dosage (92,000–170,000 units) during the years 1920–21 was followed by a marked drop in case fatality, to about one-half that of the average of the few preceding years.

It is sometimes taught that the administration of antitoxin is without effect after the 5th or 6th day of disease, a dictum based mainly on the figures recorded by Faber (1904), which show a progressive increase in case mortality from 7.1 per cent. in hospital patients receiving antitoxin on the 1st day of disease, to 17.0 per cent., 21.3 per cent. and 19.9 per cent. among those treated on the 6th, 7th or later days. It has, however, been pointed out that to conclude from such data that antitoxin is useless after the 6th day of disease is to display a blind faith in figures that is the antithesis of sound statistical reasoning. These particular figures tell the fate of patients admitted to hospital on the 1st, 2nd, 3rd days of disease and so on, not the fate of a random sample of patients suffering from diphtheria and all diagnosed on the 1st day of illness but receiving antitoxin after different intervals. Cases admitted to hospital on the 5th day or later will *ipso facto* include a large number of severe infections; since the delay in admission will in most cases be due either to delayed diagnosis, often resulting from failure to obtain a medical opinion, or to an unwillingness to resort to hospital, and mild cases falling within this class will recover at home, and will hence not be included in hospital figures. That delay in the administration of antitoxin lessens the chance of recovery there is no doubt; but Faber's figures afford no justification for withholding antitoxin merely because the disease is in an advanced stage.

The Treatment of Tetanus with Antitoxin.

(1) *The Nature of the Serum Employed.*

The serum is obtained from a horse that has been immunized by repeated injections of tetanus toxoid followed by toxin until a high titre antitoxin is obtained.

(2) *Standardization.*

The antitoxin titre of the serum can be accurately standardized (see p. 385).

(3) *Evidence from Animal Experiments.*

There is ample evidence that the antitoxin will neutralize the toxin *in vivo*. As in the case of diphtheria there is evidence that the curative is far higher than the prophylactic dose.

Thus (see von Behring 1892, Kitasato 1892) an antitoxic serum was found to protect mice when given before, at the same time as, or 24 hours after the insertion of a splinter of wood soaked in a suspension of tetanus spores; but whereas 0.001 c.c. of serum gave protection when injected 15 hours before the tetanus spores, 0.1 c.c. was required if the serum and spores were injected at the same time, and 0.4 c.c. when the serum was given 24 hours later. Other early observers (Vaillard 1891, Tizzoni and Cattani 1891) were unable to convince themselves that antitoxin possessed any curative, as opposed to protective power.

Observations of particular importance from our present point of view were recorded by Sherrington (1917). Using monkeys as his experimental animals—chiefly *Macacus rhesus* and *Callithrix*—he injected 8 M.L.D. of tetanus toxin into the outer head of the gastrocnemius muscle. From 47 to 78 hours later, when the early symptoms of tetanus had appeared, he injected 2,000 units of antitoxin per kilo of monkey, giving the serum

by different routes to separate batches. All the control animals receiving no toxin died; the mortality among the inoculated monkeys is shown in Table LVIII.

TABLE LVIII

Serum given by	Deaths.	Recoveries.	Percentage Mortality.
Subcutaneous route	23	2	92
Intramuscular route	22	3	88
Intravenous route	18	7	72
Lumbar intrathecal route	11	14	44
Bulbar intrathecal route	7	13	35
Cerebral subdural route	10	0	100

It would appear from these results that antitoxin has a considerable therapeutic value, provided that it is injected intrathecally in sufficient amount. It may be noted that there was in these experiments no active infection. The antitoxin was required to neutralize a limited and relatively small dose of toxin. Also, the dose of antitoxin was very large—2,000 units of antitoxin per kilo of monkey would correspond to about 120,000 units for an average man.

(4) and (5) *The Results of Trial in Man.*

Tetanus differs from diphtheria in that there is no diagnostic local lesion preceding the development of the toxæmic symptoms. The tetanic muscular contractions, localized or general, that are the first detectable sign of disease indicate that the toxin has already gained access to the motor nerve cells. It is possible in many cases, as we have seen above, to intervene earlier in administering antitoxin to any person who has suffered an extensive and badly soiled wound; but this is prophylaxis rather than treatment. We are here concerned with the treatment of an established case; and we are unable, owing to the absence of premonitory symptoms, to intervene as early as we should wish.

Clinical experience in man has, in truth, given disappointing results (see Bruce 1915, 1916, 1917*a, b, c, d*, 1919, Dean 1917, Leishman and Smallman 1917). Whether larger doses than those hitherto employed would be more effective it is impossible to say. Using a potent serum, containing 800 units per c.c., it would be necessary to inject 150 c.c. at one time to equal the monkey dosage employed by Sherrington, and this amount could not be given intrathecally. The order of dose commonly employed in human cases has been from 1,000 to 30,000 units given daily by several different routes.

In such a disease as tetanus all available therapeutic measures must clearly be employed, and among them antitoxin; but, unless the larger doses that should clearly be tried are found to be more effective than those used in the past, the treatment of this disease can hardly claim to be one of the successes of applied immunity.

The Treatment of Scarlet Fever with Antitoxin.

(1) *The Nature of the Serum Employed.*

The serum usually employed is derived from a horse that has been immunized against the erythrogenic toxin of the hæmolytic streptococcus. It would

appear (see p. 334) that such sera also contain appreciable amounts of anti-hæmolysin; but it has usually been assumed that their action is wholly or mainly due to the antitoxin that neutralizes the rash-producing toxin.

(2) *Standardization.*

This antitoxin can be standardized approximately, though not nearly so accurately as diphtheria and tetanus antitoxin (see p. 386). No attempt is made to standardize the anti-hæmolysin.

(3) *Evidence from Animal Experiments.*

The results that have been obtained in animals are outlined on p. 386. They throw little light on the probable efficacy of the serum as a curative agent.

(4) and (5) *The Results of Trial in Man.*

It seems reasonable to suppose that a specific antitoxic serum will exert an effect on those manifestations of scarlet fever that are directly toxic in origin. We might expect a saving of life in severe toxæmic cases, a diminution in the rash and a more rapid subsidence of the fever in the milder type of infection. There would seem no *a priori* reason to expect any effect on those complications, such as otitis and cervical adenitis, that are caused by the invasive spread of the hæmolytic streptococci.

Figures in regard to death or recovery are not easy to obtain because the prevalent type of scarlet fever is very mild and the case fatality very low. Such figures as are available support the view that there is a significant saving of life in severe toxæmic cases when these occur.

Toyada and others (1929) record 8 deaths in 33 serum-treated cases of severe scarlet fever against 15 deaths in 33 untreated cases of the same order of severity; and, so far as data with regard to death or recovery are forthcoming, other records are in general conformity with these observations.

In regard to the diminution in severity of the rash, the subsidence of fever, and the general alleviation of toxic symptoms, the consensus of opinion clearly supports the view that antiscarlatinal serum has a beneficial effect (see Benson and Maciver 1926, Harries 1927, Discussion 1927, Kinloch and others 1927, Husler 1927, Lenthe 1927, Eley 1928, Scott 1928, Bröcker 1928, Friedemann and Deicher 1928, Toyada and others 1929, Veldee and others 1931). It should be noted that Veldee and his collaborators, almost alone among those who have reported on this point, failed to demonstrate any significant effect on the temperature although there was a striking effect on the rash.

The data recorded by several of the observers referred to above are in accord with the view that antiscarlatinal serum has little effect on the frequency of complications; but two important exceptions must be noted.

Harries (1927) reports on 100 cases treated with antitoxin and 100 untreated controls. The cases for treatment were selected by alternate admissions in order to obtain a random sample. Among the control groups there were 15 cases with complications, among the treated group 7; and this difference was almost entirely accounted for by the different frequency of otitis media—9 cases in the control group, 2 in the treated. Veldee and others (1931) record a careful study of 112 serum-treated patients and 84 controls, the allocation of the patients to the serum and no-serum groups again being by alternation of admission. The difference in the frequency of complications was striking, that in the control group being higher in each case—cervical adenitis, 19.5 per cent. as against 0.4 per cent.: otitis media, all types, 17.1 per cent. as against 7.3 per cent.: suppurative otitis, 7.3 per cent. as against 3.6 per cent., and so on. Those complications that are probably due to toxæmic rather than invasive processes showed the same wide differences—nephritis, 12.2 per cent.

in the controls as against 0.9 per cent. in the treated: arthritis, 6.1 per cent. as against 0.0 per cent.

Summing up, we may say that there is clear evidence that antitoxic serum has a significant therapeutic effect on the toxic manifestations of scarlet fever, and suggestive evidence that it may—contrary to our *a priori* expectations—lessen the frequency of those complications that are due to the invasive action of the streptococci.

It would be natural to proceed to a consideration of the curative effect of antitoxic and antibacterial sera on other forms of streptococcal infection—puerperal fever, other kinds of streptococcal septicæmia, erysipelas and so on—but, in truth, the time is not yet ripe for any fruitful discussion of this larger problem. Such data as are available are contradictory; and they are very incomplete. In most cases we cannot tell what antibodies have been present in the sera administered, or how they have been related to the antigenic components of the infecting organism.

Passive Antibacterial Immunization in Treatment

The Treatment of Lobar Pneumonia with Antipneumococcal Serum.

(1) *The Nature of the Serum Employed.*

The serum employed is obtained from a horse that has been immunized by the repeated injection of whole cultures of pneumococci of one or more of the serological types. In the discussion that follows we are assuming that such curative action as is exerted by an antipneumococcal serum depends on the presence of antibodies acting on the pneumococcal cells. On this assumption our expectation would be that such a serum would be type-specific in its action. We should expect a Type I serum to have some curative effect on Type I pneumonia, but not on Type II pneumonia and so on.

(2) *Standardization.*

In the past the standardization of antipneumococcal serum has been extremely unsatisfactory. Recent developments in the method of assay have provided a far better basis of comparison, and there seems a reasonable hope that, in the near future, the content of these sera in the significant antibodies will be measurable with some degree of exactness (see p. 388).

(3) *Evidence from Animal Experiments.*

We have quoted numerous experiments that establish the protective action of a type-specific antiserum as tested in the rabbit or mouse, and a few that indicate a curative action when the serum is injected shortly after the establishment of an experimental bacteraemia (see pp. 125–128). In either case it has been shown that the serum increases the efficiency of the normal clearing mechanism and suppresses, or cuts short, the bacteraemic phase.

Of particular interest, from our present point of view, are the following observations.

Cecil and Blake (1920) studied the effect of serum treatment on experimental lobar pneumonia in monkeys, induced by the intratracheal inoculation of virulent Type I pneumococci. They found that the intravenous injection of the specific antiserum exerted a prompt therapeutic effect, freeing the circulating blood from pneumococci, shortening the infection and greatly reducing its severity. Of 5 treated monkeys all recovered while the controls died. Curphey and Baruch (1932) infected rabbits by the intradermal injection of virulent Type I pneumococci, and studied the effect of the sub-

sequent injection of a Type I antipneumococcal serum by the intravenous or intramuscular route. With the intravenous injection of 400 units 24 hours after infection, 1 of 13 animals survived; with 800 units 12 of 32 animals survived; with 2,500 units 14 of 19 animals survived. Injection of serum by the intramuscular route gave slightly less satisfactory results. With shorter intervals between infection and the administration of serum smaller doses sufficed to ensure the survival of a considerable proportion of the animals.

(4) *The Results of Trial in Man.*

This method of treatment has since 1915 received an extensive clinical trial in man, particularly in America. It is, however, only within the last few years that its value has been established with an approach to certainty, and a serious attempt been made to assess its effect in terms of a percentage reduction in mortality. Even now we can only offer approximate estimates, and some few observations are hard to fit into the general picture. The history of the serum treatment of pneumonia affords an illuminating example of the neglect of a useful therapeutic measure that may follow a failure to plan early trials in such a way as to allow significant but undramatic effects to be measured with some approach to accuracy.

The earliest considerable series of cases are those recorded by Avery and his colleagues (Avery *et al.* 1917). In 107 cases of serum-treated pneumonia there were 8 deaths, a mortality of 7.5 per cent. There were no controls; but a favourable conclusion with regard to the influence of serum on mortality was based on the contrast between the observed case-fatality rate and the rates of 25–30 per cent. recorded by these workers before they commenced the use of serum, and by other observers elsewhere. This method of comparison is, however, not valid. The variability of the case-mortality rates in different outbreaks of pneumonia is well known; and the records of serum-treated cases show a similar variability, though, for the most part, over a lower range. Thus Wadsworth (1924), commenting on a series of collected figures, notes case-fatality rates in serum-treated patients varying from 6.0 to 18.0 per cent., and reference to the tabulated results that follow will show higher figures. Clearly the only satisfactory way of assessing the effect of serum is to compare treated with untreated cases in the same place and at the same time. Within recent years a considerable series of trials have been carried out along these lines. It may be noted that they refer, in each case, to patients treated with concentrated polyvalent antipneumococcal sera containing antibodies for Types I, II and III, the Type III antibody being present in relatively low concentration.

In a series recorded by Cecil (1926) the data were collected as follows. During the winters of 1920, 1921 and 1922 six medical wards were set aside as "antibody wards," all cases of pneumonia admitted to these wards were treated with Huntoon's polyvalent antibody solution, while all cases of pneumonia admitted to six other wards were treated on ordinary medical lines without serum therapy. The type of infecting pneumococcus was determined in each case. The results are recorded in Table LIX.

It will be noted that there is a clear suggestion that Type I and Type II pneumonias were benefited by the serum, while Type III pneumonias were not. Only in the case of Type I pneumonia does the difference between the treated and untreated groups as compared with its standard deviation attain a value that would be conventionally regarded as statistically significant. The suggestion that some therapeutic effect was exerted on pneumonias due to infection with strains belonging to the antigenically heterogeneous Group IV—now split by Cooper and her colleagues into some 29 differentiated types—is not in accord with our expectations; but the difference in this case falls short of statistical significance.

A very valuable record is that provided by Park, Bullowa and Rosenblüth (1928). The serum employed was Felton's concentrated polyvalent antipneumococcal serum, and

random sampling was ensured, as far as possible, by giving serum to alternate cases in order of admission. The relevant figures are set out in Table LX.

TABLE LIX

Type of Pneumococcus.	Antibody Wards.			Control Wards.			Difference.	Diff. : S.D. Diff.
	Cases.	Deaths.	Deaths per cent.	Cases.	Deaths.	Deaths per cent.		
I	158	21	13.3	162	36	22.2	8.9 ± 4.27	2.09
II	83	23	27.7	67	27	40.3	12.6 ± 7.74	1.63
III	73	29	39.7	60	24	40.0	0.3 ± 8.5	—
Group IV .	110	18	16.4	121	29	24.0	7.6 ± 5.2	1.46

TABLE LX

Type.	Hospital.	Treated.		Untreated.		Difference Percentage.	Diff. : S.D. Diff.
		Cases.	Deaths per cent.	Cases.	Deaths per cent.		
I	Combined ¹	266	19	249	33	14 ± 3.8	3.7
	Bellevue	144	19	132	33	14 ± 5.3	2.6
	Harlem	114	20	109	34	14 ± 5.8	2.4
II	Combined	176	35	165	45	10 ± 5.3	1.9
	Bellevue	107	39	95	53	14 ± 6.9	2.0
	Harlem	61	30	63	32	2 ± 8.3	0.2
III	Combined	82	33	92	29	-4 ± 7.0	0.6
	Bellevue	32	38	52	27	-11 ± 10.6	1.0
	Harlem	47	32	38	32	0 ± 10.2	—
Group IV	Combined	313	24	324	26	2 ± 3.4	0.6
	Bellevue	131	30	128	40	10 ± 5.2	1.9
	Harlem	171	20	190	15	-5 ± 4.0	1.2

Taking the combined experience it will be noted that the concentrated serum exerted a significantly beneficial effect on Type I pneumonia. In Type II pneumonia the effect is more doubtful. The combined experience clearly suggests a beneficial effect; but this is due almost entirely to the results obtained in Bellevue Hospital. In Harlem Hospital over the same period of time serum-treated Type II pneumonias showed no advantage, a fact that emphasizes the danger of putting trust in small numbers or in single trials. The Type III cases received no benefit from the serum. The Group IV cases were unaffected as judged by the combined experience. In Bellevue they seemed to receive some benefit. In Harlem they fared worse than the controls. We need not then attach much importance to the apparent therapeutic effect in Group IV cases noted by Cecil.

More recently Cecil and Plummer (1930) have recorded a series of Type I pneumonias treated with Felton's serum between 1924 and 1929. Of 239 treated cases 20.1 per cent. died; of 234 untreated cases 31.2 per cent. died, giving a difference of 11.1 ± 4.01 per cent. The experience of the same observers with Type II pneumonia is curious (see Cecil and Plummer 1932). During the seasons 1924-25, 1926-27 and 1927-28 (the records for 1925-26 were incomplete) the serum-treated cases did better than the controls, the case-mortalities during these 3 years being 33.3 per cent. as against 48.3 per cent., 41.1

¹ The combined figures include a small series of cases treated at the New York Hospital.

per cent. as against 48.4 per cent. and 38.4 per cent. as against 53.5 per cent. During the two following seasons the position was reversed: in 1928-29 the serum-treated patients showed a case mortality of 46.8 per cent. as against 39.3 per cent. in the untreated: in 1929-30 a case mortality of 39.3 per cent. as against 24.5 per cent. Over the whole period of trial 252 treated patients showed a case mortality of 40.5 per cent., 253 untreated patients showed a case mortality of 45.8 per cent. During the season 1930-31 the trial was continued on the alternate case method, limiting the treated and control groups to cases of Type II pneumonia admitted within 72 hours of onset. Of 21 treated cases 3 died; of 20 control cases 13 died.

Taking our evidence as a whole, we seem justified in concluding that the use of an antipneumococcal serum with a high content of Type I antibody will save the lives of about one-third of those Type I cases that would otherwise have died—a result well worth achieving. With the sera now available the benefit in Type II pneumonia is less striking; but when sera with a higher content of Type II antibody can be obtained better results may reasonably be expected. Against Type III pneumonia the sera at present obtainable are of little avail, a fact not to be wondered at in view of their low content of Type III antibody.

Against other types we should not expect, nor do we find, that the sera prepared against Types I, II and III pneumococci exert any significant effect. Antisera against these types may presently be available. Cooper and others (1932) report that relatively potent sera have already been prepared against 14 of the new types recently differentiated. We may note that the assumption on which we started—that antipneumococcal sera owe their therapeutic effect to the antibodies acting on the type-specific polysaccharide antigens—is in accord with the results of clinical trial; though the possible action of other antibodies acting on other antigens with the same type distribution is not, of course, excluded. If non-type-specific antibodies are concerned, acting indifferently on all types of pneumococci or their products, their effect is not obvious in the clinical records available.

If we intend to use an antipneumococcal serum we must determine the type of infecting pneumococcus at the earliest possible moment; though we may if we choose administer a dose of a polyvalent serum at once, discontinuing it if the infecting pneumococcus is found not to belong to Type I, II or III. The method of typing usually employed is to inject a specimen of sputum into the peritoneum of a mouse, to kill the animal after 6 to 8 hours, and to carry out agglutination and precipitation tests with the sediment and supernatant fluid of the peritoneal washings. This method can be made more rapid by using slide agglutination followed by staining (Sabin 1929), and by noting certain morphological changes in the pneumococci—mainly an apparent swelling of the capsule—as well as their aggregation into clumps. A useful indication can sometimes be obtained by applying this slide test directly to emulsions of sputum (Armstrong 1932); but this should be subject to confirmation by the mouse test (Logan and Smeall 1932).

As regards the dose of serum and the number and frequency of the injections that should be administered, there are good reasons for believing that these factors are of primary importance. We might suppose from our knowledge of passive protection in the rabbit or mouse that a comparatively small dose of serum would suffice to render the pneumococci sensitive to phagocytosis. But the experiments of Curphey and Baruch show that, with antibacterial as with antitoxic sera, the effective curative dose is a high multiple of the

protective dose. It is highly probable that this is due to the fact that the specific antigen, or hapten, accumulates in the blood in considerable quantities during a pneumococcal infection and exerts an aggressive effect, combining with the corresponding antibody and so diverting it from the living pneumococci (see Chapter IX).

Thus, Park and Cooper (1928) note that, when an antipneumococcal serum is injected into a normal person, the specific antibody disappears relatively slowly from the circulating blood; so that one-quarter to one-half of the amount administered is still present 24 hours after an intravenous injection. In severe cases of pneumonia the course of events is quite different; the specific antibody is rapidly neutralized. If a polyvalent serum is administered it will be found that the antibodies corresponding to types other than that responsible for the infection can be detected in the circulating blood for relatively long periods, as in normal persons. In order to estimate the amount of antibody that might be required to neutralize the circulating antigen, Park and Cooper titrated samples of blood from cases of severe pneumonia against the corresponding antipneumococcal sera. In two Type I cases they found that 1 c.c. of the patient's blood neutralized one unit of antibody, and in one Type I case 1 c.c. neutralized 5 units of antibody. In two Type II cases 1 c.c. of blood neutralized 50 units of antibody. This means that several thousand units of antibody would be required to neutralize the circulating antigen.

Park and Cooper recommend that every pneumonia patient should, on admission to hospital, receive 10,000 units of Type I and Type II antibody administered intravenously. The administration of the same amount of Type III antibody would be desirable but is at present impossible. So long as the temperature remains high, the injections should be continued every 8 to 12 hours. When the type of the infecting pneumococcus has been determined a monovalent serum may be substituted for the polyvalent preparation. If the infection is found to be due to a type of pneumococcus against which no specific serum is available, the injections should be discontinued.

The Treatment of Meningococcal Meningitis with Antimeningococcal Serum.

(1) The Nature of the Serum Employed.

The serum employed is obtained from a horse immunized by repeated injections of large doses of meningococci including strains of each of the recognized serological types. We know that such sera contain specific sensitizing antibodies, demonstrable by agglutination or by complement-fixation reactions against each of the types used in immunization. Gordon (1920) believes that they contain anti-endotoxins, and that these are the constituents that have the principal curative value.

No *a priori* guess can be made with regard to the probable curative value of such sera. If we assume that the sensitizing antibodies are the significant factor, we must remember that we are dealing with an infection in a situation where the normal clearing mechanism may well be inoperative; or, at best, will be working with a greatly reduced efficiency. We shall call to mind that animals that have been successfully protected against the immediate results of an experimental pneumococcal infection may later succumb to a pneumococcal meningitis. If we accept the anti-endotoxin view, we have no data that will allow us to estimate the probable efficacy of such an antibody in inhibiting, or neutralizing the effects of, an extensive infection of the meninges.

(2) *Standardization.*

There is at present no satisfactory method of standardizing antimeningococcal sera (see p. 390).

(3) *Evidence from Animal Experiments.*

We know that an antimeningococcal serum will protect a mouse against the intraperitoneal injection of a massive dose of meningococci, or of a meningococcal extract; but beyond telling us that such a serum has some protective action under these particular conditions this knowledge is hardly relevant to the question immediately at issue.

Of far greater significance are the results obtained by Flexner (1907) in the serum treatment of experimental meningitis in monkeys.

Von Lingelsheim (1905) had shown that a fatal meningitis could be produced by the intraspinal injection of meningococci in these animals and his findings were confirmed by Flexner (1907*a*) and by McDonald (1908); though it should be noted that the results were irregular, and suggest a wide variation in resistance among different monkeys of the same species. Flexner (1907*b*) found that the simultaneous intraspinal injection of meningococci and an antimeningococcal serum was not followed by death or severe illness, although the meningococci persisted for a time in the cerebrospinal fluid. The injection of serum 6 hours after the injection of meningococci saved the lives of some of the monkeys although they already showed characteristic symptoms. It should be noted that the development of the meningeal syndrome in the monkey may be extremely rapid.

(4) *The Results of Trial in Man.*

The records of serum treatment in man are somewhat confusing and difficult to interpret.

Jochmann (1906) was the first to attempt this form of treatment, but the earliest extensive records are those of Flexner and his colleagues (Flexner and Jobling 1908*a, b*, Flexner 1912, 1913). The results obtained in a large series of cases were collected and analysed by Flexner (1913). Excluding a small number of cases dying within 24 hours of receiving the serum (the exact number excluded is not stated), of a total of 1,294 patients receiving serum 400 died, a mortality of 30.9 per cent. The only available comparison is with the then-prevalent case-fatality rates in untreated cases: in Great Britain 70-80 per cent., in Germany 42-67 per cent., in France 75 per cent., in Italy 55.6 per cent., in Palestine over 80 per cent., in Greece 58.6 per cent., and in the Transvaal 74 per cent. These figures suggest that the serum produced a significant decrease in mortality, but as no deductions have been made from the rates in untreated cases to counterbalance the hopeless cases excluded from the treated series, the exact difference in case fatality cannot be stated; and, as we have seen, this method of comparison is never a satisfactory one.

In 1,211 of Flexner's cases the available records allowed an assessment of the effect of serum treatment in relation to the period of disease at which the treatment was commenced. The relevant figures are set out in Table LXI.

TABLE LXI

Period of Injection.	No. of Cases.	Recovered.	Died.	Per cent. Recovered.	Per cent. Died.
1-3 days	199	163	36	81.9	18.1
4th-7th day	346	252	94	72.8	27.2
Later than 7th day	666	423	243	63.5	36.5
Totals	1,211	838	373	69.2	30.8

The earlier the serum was given the lower was the case fatality, and this may perhaps be regarded as supporting the view that the serum itself exerted a beneficial effect; but, here again, there is no comparison with untreated cases coming under observation at the same periods of disease, and we are left in doubt as to how much weight we should attach to these findings.

Dopter (1921) analyses 882 cases treated during 1909-11 in various parts of France. The number of deaths was 129, giving a case-fatality rate of 14.5 per cent. He makes various deductions and estimates a "corrected" rate of 11.7 per cent.; but clearly it is the crude rate that must be compared with the prevailing case fatality in untreated cases, which is given as about 65 per cent. Netter and Debré (1911) record a small series of cases in which the first 33 cases were not treated with serum while the subsequent 100 cases received serum intraspinally. They record a case-fatality rate of 48.5 per cent. for the untreated cases, of 28.0 per cent. for the treated. "Correcting" these rates they give 37.0 per cent. for the untreated and 10.9 per cent. for the treated. This suggests the difficulties and possible fallacies in using rates subject to arbitrary deductions. The crude rate for the untreated has been reduced by less than a quarter—in its corrected form it will be seen that it gives a figure approximating to Flexner's rate for treated cases—while the crude rate for the treated has been reduced by much more than a half.

Outbreaks of cerebrospinal fever were of relatively frequent occurrence during the War period, and very numerous records are available of the effect of serum treatment. A useful summary of these, and of some earlier and some later reports, is given by Wadsworth (1931). No purpose would be served by recording these in detail, but a few points may be noted. Among 37 recorded series of serum-treated cases the case fatality ranged from 14.5 to 62.5 per cent. The mean case fatality appears to have been in the neighbourhood of 36.8 per cent. In only a few instances is a direct comparison given between the case-fatality rate in specified numbers of treated and untreated cases; where it is, the difference is in each instance decidedly in favour of the treated. The figures actually recorded for case-fatality rates in untreated cases (whether for small control series or for general returns) vary from 48.5 to 84.6 per cent. The mean figure as estimated by Wadsworth (1931) is 74.3 per cent.

Summing up, there would seem to be suggestive, but not conclusive, evidence that the use of antimeningococcal serum will, on the average, reduce the case fatality of cerebrospinal fever by about one-half. There have, however, been several instances in which it has seemed to have little if any effect. We shall probably never get a decisive answer in the form of a direct comparison between treated and untreated patients. There is already a presumptive case in favour of serum treatment, and cerebrospinal fever is a disease in which any remedy that holds a reasonable hope of cure will naturally be used in every case. It is very possible that the discordant results that have been obtained are in part due to differences in the efficacy of the serum employed. Before we can hope for optimal results we must determine the real nature of the antibodies that exert a curative effect, and develop a satisfactory method of standardization.

With regard to dosage, in an acute case in an adult it is a usual practice to give an initial injection of 20-30 c.c. of serum, after having withdrawn rather more than the equivalent amount of cerebrospinal fluid. If the fluid is under considerable pressure it may be allowed to run until it is coming at the rate of one drop per second. The serum should be given at least once a day during the first 4 or 5 days, twice daily in severe cases. Since the meningeal infection is frequently, perhaps always, accompanied by a bacteraemia, it has been suggested that the serum should be given intravenously as well as intrathecally.

Herrick (1918) treated 137 cases by intrathecal injection with a mortality of 34.3 per cent. In 128 cases treated by combined intrathecal and intravenous injection the mortality was 14.5 per cent. The larger the amount of serum injected, the lower was the mortality. He advises giving a total dosage of about 100 c.c. intrathecally and 400-600 c.c. intravenously.

Passive Antiviral Immunization in Treatment

The Treatment of Poliomyelitis with Convalescent or Adult Human Serum.

(1) *The Nature of the Serum Employed.*

The serum employed by most observers has been obtained from patients convalescent from the disease. Such serum is known to contain neutralizing antibodies for poliomyelitis virus.

It has been shown by various observers (see Aycock and Kramer 1930*a, b, c*, Shaughnessy *et al.* 1930, Fairbrother and Brown 1930, Faber 1931, Kramer 1932, Brodie 1932) that a large proportion of normal adults have similar neutralizing antibodies in their blood, and there is some evidence that the distribution and concentration of such antibodies is related to the distribution and frequency of the disease, suggesting that the antibodies are produced in response to sub-clinical or latent infection. If adult sera are selected by testing their neutralizing power in the monkey, there is no reason to suppose that their curative power will be inferior to that of convalescent serum. Such evidence as is available suggests that they have approximately the same value (Brodie 1932). Faber (1931) points out that by testing the neutralizing power of sera of blood donors, retained by a hospital for call when a blood transfusion is required, it is possible to have available a considerable supply of serum with a known antibody content.

Attempts have been made to produce effective neutralizing sera by the immunization of horses (see Weyer, Park and Banzhaf 1929, 1931, Fairbrother and Morgan 1930). Some of the sera so produced have shown definite neutralizing power; but the results obtained have been irregular.

The records summarized in the following sections refer to results obtained with convalescent serum.

(2) *Standardization.*

The convalescent serum is not standardized. The only animal available for neutralization tests is the monkey. Apart from the question of expense, the titration of relatively small samples of pooled serum would involve a serious wastage of a supply that is, from the nature of the case, very limited.

(3) *Evidence from Animal Experiments.*

The evidence under this head has established an *a priori* case for the trial of serum treatment in man.

Römer and Joseph (1910) found that the serum of monkeys convalescent from experimental infection contained neutralizing antibodies; and Flexner and Lewis (1910*a*) and Netter and Levaditi (1910) showed that similar antibodies were present in the blood of convalescent human cases. Moreover, Flexner and Lewis (1910*b*) found that an intrathecal injection of convalescent monkey or human serum would prevent paralysis in the monkey if given as late as 24 hours after the injection of the virus.

(4) and (5) *The Results of Trial in Man.*

Numerous trials in man have in fact been made.

(See Netter *et al.* 1911, Netter 1915, 1928, Amoss and Chesney 1917, Draper 1917, Peabody 1917, Ulrich 1917, Zingher 1917, Shaw *et al.* 1925, Aycock and Luther 1928, Shaw and Thelander 1928, 1930, Aycock *et al.* 1929, Ayer 1929, McEachern *et al.* 1929,

McKenzie 1929, Lomer and Sherriff 1930, McKay and Hardman 1931, Macnamara 1931, Macnamara and Morgan 1932, Kramer *et al.* 1932, Park 1932 and a good review and summary by Neal 1932.)

The evidence is confusing but instructive. Taking all records up to 1932, it would be a fair summary to say that there was a definite balance of opinion among the clinicians conducting the trials that the intraspinal injection of serum during the early, preparalytic stage of the disease lessened the frequency and severity of paralysis. Great emphasis has been laid by many observers (see for instance Macnamara 1931, Macnamara and Morgan 1932) on the importance of the time factor, and it has been urged that the adequate exploitation of this method of treatment demands facilities for the diagnosis of every possible case during the preparalytic stage and for the immediate administration of serum. In many of the series recorded during this period there were, however, no control observations. In a few series the records contain details of treated and untreated cases, in some showing an apparent advantage to the treated patients, in others either no advantage or a higher incidence of paralysis than among the controls. It was pointed out by some of the more cautious observers (see Peabody 1917) that it was unsafe to draw conclusions from the frequency of paralysis in patients treated in the preparalytic stage without knowing the frequency of paralysis in cases diagnosed in this stage, but not treated. And it is quite clear (see Aycock and Luther 1928) that some at least of the groups taken as untreated controls were not really comparable with the treated series.

Two recent studies, designed to test the validity of the current views on this question, have given results that clearly throw the burden of proof on those who regard serum treatment as the one method that offers a hope of preventing the serious consequences of this disease.

Kramer *et al.* (1932) record an experience in which 82 cases of early poliomyelitis—all diagnosed within three days of onset by clinical signs combined with an examination of the cerebrospinal fluid, and all in the preparalytic stage—were divided into two approximately equal groups, those in one group receiving serum, those in the other being observed as controls. Two patients died, both in the treated group. In the remaining cases the average paralysis—recorded on a basis of its extent in each patient—was 12.1 per cent. in the treated group, 5.9 per cent. in the untreated; it is noted that this difference was due to 5 severely paralysed cases in the treated group. The proportion of patients developing paralysis of some degree did not differ significantly in the treated and untreated groups.

Still more impressive are the observations recorded by Park (1932). These were carried out in New York during the summer of 1931. The cases were diagnosed in the preparalytic stage of the disease, and the treated cases received rather large doses of convalescent serum (40–100 c.c.) administered by various routes singly or in combination, in most cases either intrathecally or intramuscularly. The results in the treated and untreated groups are set out in Table LXII.

Perhaps the most significant and striking feature of these figures is the large proportion of untreated cases that develop no muscular weakness or paralysis. It would appear that the illusion of benefit from serum therapy—an illusion, at least, if these figures are confirmed by subsequent observers—has been created by the efforts that have been made to diagnose all cases in the earliest stages and so reap the maximal advantage from serum therapy.

In this way a large number of mild or abortive cases that would not otherwise have been diagnosed have been included in the treated group; and, where no control group diagnosed at an equally early stage has been observed, the high proportion of recoveries without paralysis or weakness has been mistakenly ascribed to the effect of the serum.

TABLE LXII

SHOWING THE CONDITION OF TREATED AND UNTREATED CASES OF POLIOMYELITIS AT THE END OF 3 WEEKS. (After Park 1932.)

	No. of Cases.	Died. %	Paralysis. %	Weakness. %	No Paralysis or Weakness. %
Treated	519	3.8	19.6	7.7	68.8
Controls	408	0.9	11.0	14.2	73.7

Our summing-up must, then, be tentatively against the value of convalescent serum. The fairest verdict would perhaps be "non-proven"; but, since the most recent and careful observations have given such decisively negative results, and since serum therapy is clearly not to be encouraged if it has no value, we should echo the hope expressed by Neal (1932), that, if the serum treatment of poliomyelitis is attempted, equal numbers of comparable treated and untreated cases will be carefully observed and recorded.

CHEMOTHERAPY IN THE TREATMENT OF BACTERIAL INFECTIONS

This is a convenient juncture for a reference, perforce cursory and inadequate, to the results that have been obtained in attempts to apply to the treatment of bacterial infections measures similar to those that have had such dramatic effects in many protozoal and spirochætal diseases. There seems no valid reason why we should not some day discover chemical substances that will exert specific bactericidal effects without injuring the tissues of the host; but our efforts in this direction have, up to the present, been sadly disappointing.

The demonstration of Morgenroth and Levy (1911) of the specific bactericidal action of ethylhydrocupreine (optoquine) on the pneumococcus is, perhaps, the nearest approach to successful antibacterial chemotherapy that has yet been attained. Unfortunately this drug has an appreciable toxicity for man and animals; and, among the toxic symptoms that sometimes follow its administration in man, are disturbances of vision that may amount to temporary or, very rarely, permanent blindness. The curative effect of optoquine in cases of pneumococcal infection has not, apparently, been sufficiently uniform or striking to encourage its use in face of the risks involved.

There are indications that certain mercury compounds such as mercurochrome 220 (see Fleming 1931) and certain arsenical compounds such as salvarsan and novarsenobillon (see Colebrook 1928) render blood bactericidal to certain organisms, particularly to hæmolytic streptococci. But the therapeutic effects of such substances can be judged only by empirical trial, and no adequate figures are as yet available.

The action of these mercury and arsenic compounds, so far as it is not due to a direct bactericidal action on the infecting organism, is perhaps analogous to that of a variety of metallic compounds that have been reported to cause a temporary increase in the

antibody content of the blood, or in its bactericidal power, *i.e.*, to produce their effect by a non-specific stimulation of the normal defence mechanism of the host. The gold salt, sanocrysin, that has been extensively used in the treatment of tuberculosis, would appear to belong to this category, though Möllgard (1924) originally regarded it as acting directly on the tubercle bacillus. The experimental evidence in regard to reactions of this type has been discussed in Chapter XIII. The clinical records are copious, but indecisive.

In regard to superficial bacterial infections the case is somewhat different. We are concerned with antiseptics rather than chemotherapy, using these terms with their ordinary connotation and admitting that the distinction cannot be logically defended. The number of antiseptics that have been employed is legion; and it would be altogether beyond the scope of this book to discuss their merits or their mode of action.

For a more adequate discussion of the factors involved in chemotherapy and antiseptics reference may be made to Browning (1930, 1931) and to Fleming (1919, 1931).

THE THERAPEUTIC ACTION OF THE BACTERIOPHAGE

We have had no occasion in this book to discuss the phenomenon of transmissible bacterial lysis described independently by Twort (1915) and by d'Herelle (1917). Whether we believe the lytic agent to be a living virus or some active substance elaborated by the bacteria themselves, it is clear that its behaviour *in vitro* suggests that it might have a protective or curative action *in vivo*. An agent that, while harmless to the tissues of the host, would lyse bacteria and in so doing reproduce itself, so that its concentration would increase progressively as lysis proceeded, might well be regarded as exceeding all reasonable hopes. For this ideal to be realized the bacteriophage, as the lytic agent was called by d'Herelle, must behave in the tissues as it does in the test-tube. D'Herelle (1921, 1926) has been emphatic in his claims that it does so, and that it provides a reagent of the utmost value in the prevention and cure of infective disease. But here there is a conflict of evidence.

If we take the evidence in its natural order, beginning with experiments on animals, the presumptive case against the bacteriophage as an effective therapeutic agent looks very strong indeed. There are a few observations which suggest that it may have some slight protective value if injected into an animal simultaneously with such organisms as the colon bacillus or the typhoid bacillus (Wollman 1925, Arnold and Weiss 1926, Walker 1929). But the great majority of observers, and especially those who have worked with bacteria that are natural pathogens of the rodents of the laboratory, have recorded consistently negative results.

Thus bacteriophage filtrates, active against *Bact. aertrycke* or *Bact. gaertneri* in the test-tube, have no curative action on mouse typhoid (Topley and Wilson 1925, Topley *et al.* 1925, Levy 1925, Wollman 1925, Richet and Hauduroy 1925, Bronfenbrenner and Korb 1925-26, Ebert and Peretz 1929). Similarly, homologous bacteriophages have shown little or no curative action on salmonella infections in the fowl (Pyle 1926), on experimental plague in rats (Compton 1928, 1930, Doorenbos 1929), on hæmolytic streptococcal infection in the rabbit (Clark and Clark 1927) or in the guinea-pig (Colvin 1932), or against anthrax in the mouse (Cowles and Hale 1931). Nor is there any evidence that the presence of an active bacteriophage in the intestine of an experimental animal affords any protection against natural contact infection (Topley and Wilson 1925, Topley *et al.* 1925).

It is curious that an agent so active in the test-tube should prove so apparently inert in the living body. The explanation is certainly not that

resistant strains of bacteria are developed in the tissues and then multiply and cause the host's death. Many of the authors referred to above have noted that the bacteria isolated from the dead animals were fully sensitive to the bacteriophage with which those animals were treated. Nor is the bacteriophage destroyed in the tissues. It is, for instance, quite common to recover an active bacteriophage, and a bacterium that is fully sensitive to it, from the tissues of an animal that has died from the infection.

It seems more likely that the process of bacteriophage lysis is inhibited by the cells and fluids of the body. It is well known that it is inhibited *in vitro* by such substances as gelatin or agar (Bail 1922, Doerr and Berger 1922, Otto and Munter 1923, Bronfenbrenner and Korb 1925) and this effect would appear to be due to the adsorption of the bacteriophage on to the colloidal particles of the medium. Zdansky (1924) has suggested that the colloids of the body fluids will probably produce a similar inhibitory effect; and several of those who have failed to demonstrate any curative action *in vivo* have been inclined to attribute their failure to this cause.

When we turn to therapeutic or prophylactic trials in the naturally occurring diseases of man we can only record a bewildering divergence of opinion—the literature is too vast and for the most part too nebulous to make quotation profitable. Certainly there are no records on which a statistician would give a verdict in favour of this form of treatment.

D'Herelle and others have insisted on the importance of using a bacteriophage of maximum activity; or in certain cases on using bacteriophage filtrates that are active against many strains and variants of the causative organism (see Asheshov, *et al.* 1930, Morrison 1932, Gratia 1930–31). Andrewes and Elford (1932), while expressing no views as to the possible behaviour of the bacteriophage *in vivo*, have demonstrated *in vitro* a direct killing effect on bacteria that is not dependent on the occurrence of lysis, but is strictly dependent on the activity of the bacteriophage filtrates; weakly active filtrates are entirely devoid of this action.

It is not easy to believe that all the negative results that have been recorded in experimental animals have been due to the use of bacteriophage of low activity—in several cases the activity *in vitro* has been specifically recorded as maximal. At the same time we cannot exclude the possibility that, by some means or other, we may some day be able to exploit the bacteriophage in treatment or prophylaxis.

It seems fair, in view of the overwhelmingly adverse evidence afforded by experiments on animals, to conclude that the burden of proof is quite clearly on those who maintain that bacteriophage therapy, or prevention, is of value in naturally occurring infections. It would not be justifiable to conclude that the evidence so far obtained renders further inquiry useless, though the prospects hardly seem hopeful.

THE USE OF OTHER BIOLOGICAL AGENTS IN TREATMENT

We may close this section with a brief account of some recent observations that, though they have as yet been confined to experimental animals, seem to open a new chapter in the treatment of bacterial infections.

Dubos and Avery (1931) record the isolation of a bacterium which, when grown in a synthetic medium containing the specific polysaccharide of Type III pneumococcus as the source of carbon, elaborates an enzyme that specifically

attacks this happen, and that can be obtained apart from the organism that produces it by filtration of a partly autolysed culture. This enzyme has no bactericidal action on Type III pneumococci, but it attacks the polysaccharide hapten as it is produced by the growing bacterial cells. When mice are injected with the specific enzyme together with a fatal dose of virulent Type III pneumococci they are effectively protected (Avery and Dubos 1931). This protection is sharply type-specific; it is quite ineffective against Type I and Type II pneumococci. In addition to its protective effect the enzyme exerts a curative action in mice if injected during the earlier stages of infection. Goodner, Dubos and Avery (1932) have studied the curative action of this enzyme on Type III infections in the rabbit, induced by the intradermal injection of a virulent strain. This type of infection, which starts as a spreading local lesion and leads, in untreated controls, to an acute bacteraemia fatal on about the 3rd or 4th day, offers obvious advantages for studies of this kind as compared with the acutely fatal peritoneal infection in the mouse. The enzyme, injected intravenously in adequate dosage 24 hours after the intradermal injection of pneumococci, was found to have a striking curative effect. In one series of tests, for instance, one of 19 treated animals died as compared with 36 of 38 untreated controls. Goodner and Dubos (1932) have studied the quantitative relationship between the degree of bacteraemia 24 hours after infection and the curative dose of enzyme. Of 10 rabbits showing 3-10 pneumococci per c.c. of blood and receiving 2.5-100 "units" of enzyme all survived. Of 11 rabbits showing 103-630 cocci per c.c. of blood 5 that received 4-10 units died, 6 that received 20-100 units recovered. Of 9 rabbits showing over 13,000 cocci per c.c. of blood and receiving 5-100 units of enzyme all died. We have still to learn how natural infection in man will respond to this form of treatment.

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CHAPTER XXI

THE STANDARDIZATION OF IMMUNOLOGICAL REAGENTS

IN applying immunological methods to the treatment, prevention or diagnosis of disease one of the first problems that faces us is the standardization of our reagents. None of the active substances with which we are here concerned has yet been isolated in a chemically pure state, so that we cannot measure them by weight or by volume. We must, of necessity, have recourse to some biological method of standardization. In such methods we adopt as our unit a specified quantity of a particular preparation, that we have arbitrarily agreed to regard as our standard. The exact definition of the standard unit—provided that it gives a measure of the activity with which we are concerned, and can be quantitatively determined within a small margin of error—is altogether immaterial. So long as we can measure the activity of our preparations in standard units of some kind, empirical trial will teach us how many units we should employ for any particular purpose.

One consequence of having to rely on biological methods of standardization is that we have no universally applicable units, such as those given by any system of weights and measures. For every new reagent we must adopt a new unit that applies to that reagent alone. This involves in each case an agreed understanding between those who produce the reagents and those who use them, and, for efficient working, some method of official testing and control. In the past one of our difficulties has been the use of different standards and different methods of testing in different countries, another has been the absence of any organized body charged with the duty of exploring this field and promoting research into possible methods of standardizing reagents that are as yet unstandardized. An advance of the first importance was made in 1921 when the Health Organization of the League of Nations set up a Permanent Commission on Standardization of Sera, Serological Reactions and Biological Products. This Commission has already done important work in obtaining international agreement in regard to the standardization of several immunological reagents, and is gradually extending its field of activity. An excellent summary of its work along these lines up to 1928 is given by Prausnitz (1929).

It is of interest to consider briefly the methods that are at present employed in the standardization of some of the immunological reagents that are in common use; partly because those who use such reagents should have some idea as to how their potency is measured, partly because the methods employed

serve to illustrate many of the immunological principles that we have considered in earlier chapters.

THE STANDARDIZATION OF ANTITOXIC SERA

We are here dealing with immunological standardization in its simplest form. We are concerned with the direct neutralization of a toxic product that can readily be separated from the bacterial cells; and the law of combination in multiple proportions holds good—if x c.c. of antitoxic serum will neutralize y c.c. of toxic filtrate, then nx c.c. of antitoxic serum will neutralize ny c.c. of toxic filtrate, over a wide range.

The Standardization of Diphtheria Antitoxin.—The classical method of standardizing diphtheria antitoxin was devised by Ehrlich (1897). The unit of antitoxin was defined by him as the smallest amount that would completely neutralize 100 M.L.D. of toxin. The instability of a toxic filtrate, and the fact that its M.L.D. varies independently of its combining power as the result of a progressive change of toxin to toxoid, rendered it a highly inconvenient standard of reference; and Ehrlich soon adopted a particular antitoxic serum as his actual standard, measuring the potency of any other serum by determining first the amount of a suitable toxin equivalent to one unit of the standard serum, and then the amount of the serum under test equivalent to that amount of toxin. The standard antitoxic serum maintains its potency unchanged over long periods when dried and preserved *in vacuo* in the presence of phosphorus pentoxide, and thus provides a satisfactory arbitrary unit of measurement—just as the equally arbitrary standard yard or standard metre provides a satisfactory standard for the measurement of length. There is, in truth, an obvious advantage in measuring the potency of any reagent by comparing it with a standard reagent of the same kind; and this principle has now been very generally adopted for official purposes.

The correct definition of a unit of antitoxin is not, therefore, 'the least amount that completely neutralizes 100 M.L.D. of toxin'—that definition held only for the particular filtrate employed by Ehrlich in his original studies. *One unit of antitoxin (I.A.U.) is contained in that amount of any antitoxic serum that has the same total combining capacity, for toxin or toxoid, as one unit of the standard antitoxin, i.e. as one unit of the antitoxin originally employed by Ehrlich.*

Standard antitoxin is now preserved and issued by selected Institutes in several countries, and is tested from time to time under the auspices of the Biological Standardization Commission of the League of Nations (see Report 1923). The International Unit is thus available for standardization purposes whenever required.

As regards the actual method of comparison employed, three methods are available. Two of these depend on *in vivo* tests, the third on an *in vitro* titration.

The first is Ehrlich's classical method of injecting mixtures of toxin and antitoxin subcutaneously into guinea-pigs; and this method is still the official one in many countries (see Report 1923, Prausnitz 1929). It has been found convenient to employ the end-point of toxæmic death rather than the end-point of complete neutralization—the $L+$ rather than the Lo dose of toxin (see p. 79). The first procedure is, then, to determine the smallest amount of a suitable toxic filtrate that, when mixed with one unit of standard antitoxin and injected into a 250 gm. guinea-pig, will, on the average, kill the animal by

the 4th day. In practice batches of guinea-pigs are injected with each mixture prepared and the mixture producing a 50 per cent. mortality, or thereabouts, is regarded as containing the L+ dose of toxin. The procedure is now reversed. The amount of toxin is held constant at the L+ dose, and the amount of the serum to be tested is varied, again using an adequate number of guinea-pigs for each dose in the range within which the L+ mixture is expected to fall. The amount of serum in the mixture that gives a 50 per cent. mortality within 4 days contains one unit of antitoxin. The number of guinea-pigs that must be inoculated with any mixture in the neighbourhood of the L+ mixture in order to give an estimate of the unit value of an antitoxic serum within a specified margin of sera has been considered on p. 25. In practice a rough preliminary titration is made, and the exact value of the serum is then assessed using larger numbers of guinea-pigs.

The second method depends upon the observation of Römer (1909) that the intradermal injection into a guinea-pig of 1/250–1/500 M.L.D. of toxin is followed by a localized swelling and erythema. With slightly larger doses, this erythematous reaction is followed by definite necrosis. The neutralization of toxin by antitoxin prevents this reaction. The great advantage of this method is that it allows the toxicity of several different mixtures to be tested on a single guinea-pig, and thus not only economizes animals but eliminates much of the difficulty due to differences in susceptibility between one guinea-pig and another. The successive steps in the process of standardization do not differ in any essential from those followed in the Ehrlich method. Varying amounts of a suitable toxin are first mixed with one unit of the standard antitoxin and 0.2 c.c. of each mixture is injected into the depilated skin of a guinea-pig. The amount of toxin in the mixture that gives a minimal skin reaction is noted. This amount of toxin has been defined by Glenny and Allen (1921) as the Lr dose. A series of mixtures is now prepared in which the Lr dose of toxin is mixed with varying amounts of the serum under test, and 0.2 c.c. of each of these mixtures is injected intradermally into another guinea-pig. The amount of serum in that mixture that gives a minimal skin reaction contains one unit of antitoxin. In practice it is customary to use fractions of I.A.U. and corresponding fractions of the Lr dose of toxin, in order to avoid the administration of lethal doses of toxin in the mixtures containing toxin in excess.

The third method depends on the observation of Ramon (1922) that a satisfactory measure of the combining power of an antitoxic serum can be obtained by mixing falling amounts of the serum with a constant amount of toxin and noting the ratio of one reagent to the other in the tube that first shows flocculation. This is the method of optimal proportions (see p. 66). Glenny and Okell (1924) (see also Glenny and Wallace 1925) have suggested that the amount of toxin corresponding to one unit of antitoxin in the mixture that shows optimal flocculation when tested by the Ramon method should be called the Lf dose. The procedure with this *in vitro* test is essentially the same as with either of the *in vivo* methods. The Lf dose of a suitable toxic filtrate is determined by titration against the standard antitoxin. The amount of the serum under test that gives optimal flocculation with the Lf dose of toxin is determined by a second titration. This amount of serum contains one unit of antitoxin.

Table LXIII (see Glenny 1925) sets out the units (or named doses) employed in testing diphtheria toxin and antitoxin. The meaning to be attached to the Schick dose is considered later.

The comparison of large numbers of different antitoxic sera—natural or concentrated—by these different methods has brought to light facts of considerable theoretical and practical importance. The L+ dose of toxin is always appreciably larger than the Lo dose. The Lr dose of toxin always approximates closely to the Lo dose, as would be expected since a very small excess of unneutralized toxin will elicit the Römer reaction. The Lf dose is, in general, slightly less than the Lr dose. Glenny (1925) notes the following relation between the various doses of an average toxic filtrate: L+ dose = 0.21 c.c., Lo dose = 0.18 c.c., Lr dose = 0.175 c.c., Lf dose = 0.155 c.c. The ratio of the Lf dose to any of the *in vivo* doses is not constant for all toxic filtrates. Antitoxin gives

TABLE LXIII
(After Glenny 1925.)

Character measured.	Unit or Dose.		Result of Inoculation in Guinea-pig.
	Toxin.	Antitoxin.	
Toxicity . . .	M.L.D.	—	Death on 4th day.
" . . .	M.R.D.	—	Minimal skin reaction.
Combining power	L + dose	1 A.U.	Mixture causes death on 4th day.
" "	Lo "	1 A.U.	Mixture causes minimal oedema.
" "	Lr "	1 A.U.	Mixture causes minimal skin reaction.
" "	Lf "	1 A.U.	(Mixture gives optimal proportions for flocculation.)
" "	Schick "	0.001 A.U.	

flocculation with both toxin and toxoid while the determination of the L+, Lo and Lr doses depends on the presence of unneutralized toxin, and is hence affected by differences in the proportion of toxin to toxoid. Moreover, as was shown by Glenny, Pope and Waddington (1925), not only does the Lf/Lr ratio vary from one toxic filtrate to another, when these are tested against the same serum, but the Lf/Lr ratio of a single toxic filtrate varies when it is tested against different antitoxic sera. It follows that, when sera are compared with one another by *in vivo* and *in vitro* methods, their apparent relative potency may vary according to the method of comparison employed; and, with such sera, the ratio $\frac{\text{in vitro value}}{\text{in vivo value}}$ will vary from one serum to another.

Glenny and his colleagues note that they have obtained *in vitro/in vivo* ratios varying from 0.4 to 2.0 with different antitoxic sera, and that, in general, if the Ehrlich value is considerably higher than the Ramon value—i.e., if the *in vitro/in vivo* ratio is low, the serum will be found to give rapid flocculation; while, if the Ramon value is higher than the Ehrlich value, the serum will be found to give very slow flocculation and the toxin-antitoxin complex will show considerable dissociation on simple dilution. In their later papers, Glenny and his colleagues use the inverse ratio—*in vivo/in vitro*—so that a serum with a ratio greater than unity has a greater protective action than its flocculation value would lead one to expect.

Such findings obviously raise again a controversy that was at its height some 20 years ago—Does the antitoxin content of a serum as determined in terms of Ehrlich units give a satisfactory measure of the curative power of that serum? Many clinicians and experimental workers have, from time to time, cited observations that seemed to suggest that this was not always the case. In particular, Kraus and his colleagues (see Kraus and Schwoner 1908, Kraus and Baecker 1913) recorded a series of experiments on rabbits and guinea-pigs that failed to show any close correlation between the antitoxin content of different sera, as determined by Ehrlich's method, and their curative power in these animals. They suggested that the quality as well as the quantity of antitoxin plays an important part in determining its curative effect and that one quality concerned—which they named *avidity*—determines the rate at which the neutralization of toxin occurs. This notion of avidity has been rendered far more precise by recent observations recorded by Madsen and Schmidt and by Glenny and his colleagues: and there seems little doubt that principles of considerable practical and theoretical importance are involved.

Madsen and Schmidt (1929) found that, with some antitoxic sera, toxin-antitoxin mixtures that were neutral as judged by subcutaneous injection in the guinea-pig might produce a fatal toxæmia when injected intravenously into rabbits. If such toxin-anti-

toxin mixtures were allowed to stand for some time before injection the protective value of the serum as judged by intravenous injection in the rabbit approximated to the value determined by the Ehrlich method in the guinea-pig. Moreover, those sera that gave a lower protective value by the rabbit-intravenous than by the guinea-pig-subcutaneous method were relatively ineffective as curative agents when injected after an earlier injection of toxin. They found that such sera—sera of poor avidity—always showed slow flocculation in the Ramon test, though sera that flocculated slowly were not always of poor avidity. Thus rapid flocculation appears to connote high avidity, but slow flocculation may or may not indicate poor avidity.

Glenny and Barr (1932a) note that it is possible, by treatment with various physical or chemical agents, to change the speed of flocculation of an antitoxic serum without altering its *in vivo/in vitro* ratio or its curative value. They suggest that "avidity" is important because it ensures firmness of union between toxin and antitoxin rather than because it increases the rate of flocculation, and they cite interesting experimental findings in support of this view. They examined several antitoxic sera, and fractions prepared from these sera by salting out with different concentrations of ammonium sulphate, determining in each case the time of flocculation, the *in vivo/in vitro* ratio, the curative action in rabbits, and the effect of dilution on mixtures of toxin and antitoxin. For the latter purpose they determined a value which they call the *dilution ratio*: it is the ratio of the amount of antitoxin necessary to form a neutral mixture with one Lr dose of toxin in a total volume of 2 c.c. to the amount necessary to form a neutral mixture with the same amount of toxin in a total volume of 200 c.c., neutrality being determined by the inoculation of 0.2 c.c. of each mixture tested into the skin of a guinea-pig. With a mixture that dissociates easily more antitoxin will be required to neutralize the Lr dose of toxin when the dilution of the reagents is greater, *i.e.*, the dilution ratio will be less than unity. With a mixture giving firm union the dilution ratio will tend to be rather greater than unity, since the only effect of dilution will be to decrease the amount of free toxin in 0.2 c.c. of an under-neutralized mixture. The results showed a high correlation between the dilution ratio, the *in vivo/in vitro* ratio and the curative action in rabbits, but a less close correlation between the rate of optimal flocculation and any of the other factors, though there was a general tendency for slowly flocculating sera to show a low dilution ratio and a low *in vivo/in vitro* ratio, and to be relatively ineffective as curative agents in rabbits. In confirmation of the view that dissociation of the toxin-anti-toxin complex on simple dilution is an important factor in the relative ineffectiveness of non-avid sera, Glenny and Barr (1932a, b) and Glenny and others (1932) note that, with sera of this kind, it is possible to prepare mixtures such that rabbits will survive the intravenous injection of 10 c.c. but not of amounts varying from 0.001 c.c. to 0.5 c.c., the twenty-fold to ten-thousand-fold greater dilution of the mixture in the rabbits' circulation in the case of the smaller injections apparently permitting the dissociation of a lethal dose of toxin.

The avidity of antitoxic sera appears to be determined, at least in part, by the type of serum protein with which the antitoxin is associated. Thus (Barr and Glenny 1931a, b, Glenny and Barr 1932a, Glenny *et al.* 1932) if successive globulin fractions are precipitated with increasing amounts of ammonium sulphate, the earlier fractions show a higher *in vivo/in vitro* ratio, a higher dilution ratio and a greater curative power in rabbits than the original serum, while the avidity as judged by these tests decreases with each successive fraction precipitated.

Non-avid sera do not form a high proportion of the antidiphtheria sera obtained in routine serum production, and Glenny and his colleagues note that they are usually derived from horses that have proved difficult to immunize. With other antitoxic sera (see Glenny *et al.* 1932) it would seem that they may occur more frequently.

These findings do not, of course, lessen the importance of determining the antitoxin content of a serum in terms of standard units, or of producing sera with the highest possible antitoxin content. We clearly want to know how many units we are giving, and to

give as many units as we can. They do suggest that the protective or curative value of a serum is not always completely described by stating the number of units it contains; and it may prove desirable in the future to adopt some standard of avidity in addition to our standard of content.

The Standardization of Tetanus Antitoxin.—The standardization of tetanus antitoxin has only recently been placed on an agreed international basis (see Report 1923, Prausnitz 1929). Prior to 1926 the method of standardization varied from country to country. In Germany toxin-antitoxin mixtures were injected subcutaneously into mice, both tetanic symptoms and death being noted in assessing the results. In America toxin-antitoxin mixtures were injected subcutaneously into guinea-pigs, the end-point of titration being taken as death within 4 days. In France decreasing quantities of serum were injected into guinea-pigs, and a single lethal dose of toxin was injected 10–20 hours later. The definition of the unit also differed. In America, to take one example, it was defined as ten times the least quantity of antitoxin that, when mixed with the official test dose of toxin (100 M.L.D.), allowed to stand for 1 hour at room temperature, and injected subcutaneously into a 350 gm. guinea-pig, preserved the life of the animal for 4 days (see Rosenau and Anderson, 1908). As with diphtheria antitoxin, or any other antitoxic serum, such definitions have little interest, except as giving a rough indication of the order of neutralizing power that a unit represents. The standard is not toxin but antitoxin, and standardization is carried out by comparing an unknown serum with the official standard by the same method of indirect titration as is employed in the case of diphtheria antitoxin. The problem before the Permanent Standardization Committee of the Health Organization of the League was the comparison of the different national units then in use, and the definition of an agreed International Unit. The relation of one national unit to another, taking the means of the values recorded by different observers, was found to be as follows: 1 German Unit = approximately 66 American Units = approximately 3,750 French Units. It was agreed that the International Unit should be equivalent to half the existing American Unit.

An interesting point that emerged during trials by Madsen, Walbum and others (see Report 1923, Prausnitz 1929) was that different results were obtained according as the Lo or $L+$ mixture was employed as the end-point of titration—the mixture that marked the limit between signs of tetanus and no tetanus, or the mixture that marked the limit between death or survival at the 4th day. Repeated comparisons between the German and American Units gave the constant result 1 German Unit = 70 American Units when the $L+$ dose of toxin was employed, while with the Lo dose the result was 1 German Unit = 76–80 American Units. Glenny and his colleagues (1932) point out that such a result might be expected if the German and American Standard sera differed in avidity. With a less avid serum there is a greater tendency to dissociation within the tissues, hence more than the equivalent amount of antitoxin must be added to pass from the mixture that contains one free lethal dose to that containing no free antitoxin. They note that non-avid sera appear to occur more frequently among samples of tetanus antitoxin than among samples of diphtheria antitoxin.

The opinion expressed by Prausnitz (1929), based on the observations of Madsen and Walbum, is that the most accurate results are obtained by using the mouse as the test animal and the Lo dose of toxin as the end-point.

No intradermal test is available for the standardization, and no flocculation test has yet been found to give satisfactory results.

The Standardization of Scarlatinal Antitoxin.—Here, as elsewhere, the standard of reference is an arbitrarily selected sample of serum—that employed in the United States. It happens (see Dyer 1928) that the unit is defined as ten times the amount of antitoxin that, in the skin neutralization test, will neutralize the “test-dose,” of toxin; while the “test-dose” is defined as five “skin test-doses,” and a “skin test-dose” as the least amount of toxin that, in a majority of susceptible persons, gives rise to a local skin reaction of no less than 10 mm. in diameter. But there is nothing exact or binding about this definition. The Standard American serum was adopted by the Standardization Committee of the League in 1928, not as an agreed International Standard and Unit, but as a provisional standard for further study.

The difficulty of standardization is due to the fact that there is, at the moment, no completely satisfactory test animal other than man, and the supply of susceptible Dick-positive persons available for testing is clearly limited. Reports have from time to time been published in which one or another animal has been stated to give skin reactions comparable with those obtained in man; but none of these claims has yet been substantiated. Until some satisfactory method of standardization in laboratory animals is devised, and generally accepted, the adequate assay of scarlatinal antitoxin will remain a very difficult matter.

The most hopeful approach to an animal test at present available is that devised by Parish and Okell (1927). Rabbits are injected intravenously with varying doses of the serum under test, and, 4 to 6 hours later, with 5 c.c. of a young broth culture of a virulent strain of a hæmolytic streptococcus. The effect of the antitoxin is to prevent the acute septicæmic death that occurs in controls. Most of the rabbits that have received the antitoxin die eventually of a subacute streptococcal infection associated with arthritis, pericarditis, etc.; but death seldom occurs before the 6th day, and this is taken as the end-point.

This method has been compared with the skin-neutralization test in man by O'Brien, Okell and Parish (1929). Sera, the relative strengths of which were unknown to the observer, were compared by each method. By the skin test in man it was possible, under favourable conditions, to place four dilutions of serum 1/40, 1/80, 1/160 and 1/320 in their correct order, *i.e.*, a serum would not often be returned as having half, or twice, its real antitoxic value. By the rabbit method, using 8 rabbits for each comparative test at any one dosage—4 for each of the two sera under comparison—it was usually possible to distinguish one serum from another having half or twice its value. By neither method can results be obtained of the same order of accuracy as with diphtheria or tetanus antitoxin, but, on present evidence, the rabbit test seems little if at all inferior to the human test, though its theoretical basis seems more dubious.

A flocculation test has been attempted (Dyer 1925, Ramon *et al.* 1928), but the method has not given satisfactory results in the hands of most workers (see O'Brien, Okell and Parish 1929, Prausnitz 1929).

Before passing on to the standardization of antibacterial sera, it should perhaps be added that certain other antitoxic sera, such as anti-dysentery (anti-Shiga) serum, and anti-welchii (anti-perfringens) serum are now being satisfactorily standardized in terms of agreed international units.

THE STANDARDIZATION OF ANTIBACTERIAL SERA

This is an intrinsically more difficult problem. The curative action of an antibacterial serum is not determined completely by the union of sensitizing

antibody with the invading bacterial cells ; it depends on the efficient working of secondary cellular or humoral reactions, to which sensitization is an essential preliminary.

The most obvious method of estimating the potency of an antibacterial serum is by protective or curative tests on some suitable laboratory animal. The main difficulty that meets us here results from the statistical considerations discussed in Chapter II. Taking, as we commonly do, death or survival of 50 per cent. of the test animals injected with any one dose as marking the end-point of our titrations, we are faced with the necessity of using relatively large groups of animals for each dose of serum tested if we desire to determine our end-point with any approach to accuracy. Larger groups are necessary than in the case of antitoxic sera, because the slope of the 'characteristic' describing the relation between increase in dose of living bacteria and increase in death rate is less steep than that describing the relation between increase in dose of a bacterial toxin and increase in death rate. Since the decrease in lethal effect due to decreasing the dose of bacteria, or partially neutralizing a given dose by a specific antiserum, is smaller, the number of animals required to detect or to measure this decrease will be greater (see p. 22).

Using such a method, and taking the necessary statistical precautions, we can, in theory, keep our dose of serum constant, varying our dose of living bacteria ; or we can keep our dose of living bacteria constant, varying our dose of serum. For reasons that will be considered later, the latter is the more satisfactory procedure. In either case we shall require some arbitrary standard—our measuring rod—in terms of which to express our results. A living bacterial culture is an even more unhandy reagent for this purpose than a bacterial toxin, since it is much more difficult to keep constant. The obvious standard, and one that is being generally adopted where possible, is a particular specimen of antiserum that has been adequately tested and to which a value has been assigned in terms of some arbitrary unit. Any serum to be tested is then compared with this standard serum, and is labelled with a value expressed in terms of the arbitrary unit, on which the therapeutic or preventive dose is based.

More formidable difficulties are introduced when the bacteria with which we are concerned fail to produce an acutely invasive infection in any available test animal. In such cases the lethal dose of bacteria is usually very large, and the fatal result is brought about by a variety of factors, the exact nature of which is little understood. The relative potency of two sera as judged by our animal tests may then have little relation to their relative curative or protective efficacy in man, in whom the type of infection may be quite different.

Whether the animal test is satisfactory or not, it will be an obvious advantage to substitute, where this is possible, some *in vitro* method of measuring antibody content. To do this we must, of course, first determine on which particular antibody, or antibodies, the protective or curative effect depends.

The points at issue will be made clearer by considering the methods at

present employed in standardizing a few of the antibacterial sera in common use.

The Standardization of Antipneumococcal Serum.—The standardization of this serum is based on the experimental observation that the immunity conferred is, at least in the main, type-specific. A serum is thus prepared and standardized against a Type I, a Type II or a Type III pneumococcus, and so on; or a polyvalent serum may be prepared and standardized against a few selected types.

In the original attempts to measure the potency of such a serum (see Neufeld and Händel 1909, Neufeld and Schnitzer 1927–28) titrations were made in mice both with a constant dose of serum and a varying dose of pneumococci, and with a constant dose of pneumococci and a varying dose of serum. These and later experiments (see Felton 1930a) have made it clear that the relation between the number of pneumococci injected and the dose of serum necessary to protect against them is not of a simple linear kind, *i.e.*, if x c.c. of serum will protect against y pneumococci it does not follow that nx c.c. of serum will protect against ny pneumococci. Felton's experiments suggested that, under optimal conditions, this linear relationship held over a limited range; but the results obtained by Smith (1932) indicate that it is very unsafe to assume that if one serum in a particular dose gives a certain degree of protection against n pneumococci, and another serum in the same dose gives the same degree of protection against $2n$ pneumococci, the second serum is twice as potent as the first. He found, for instance, that with a constant dose of a particular serum and varying doses of pneumococci, the death rate in a group of 50 mice receiving 12,500 cocci was greater than that in another group of 50 mice receiving 125,000 pneumococci, while in another experiment a hundredfold increase in the number of pneumococci, keeping the dose of serum constant, failed to cause any significant increase in the percentage mortality.

It happened, however, for reasons that are not altogether clear, that the method of varying the dose of pneumococci was at first adopted in testing antipneumococcal sera (see Prausnitz 1929). Sera were not titrated in terms of units, but a limit of potency was laid down which had to be attained before a serum was regarded as satisfactory for issue. In Germany this limit was protection by 0.2 c.c. of serum against 0.1 c.c. of a culture of such virulence that 0.000,001 c.c. was fatal for untreated mice. In America it was protection by 0.2 c.c. of serum against 0.1 c.c. of a culture of such virulence that 0.000,000.1 c.c. was fatal for untreated mice. A method that does not assign a value in terms of units to each batch of serum issued is clearly unsatisfactory from the clinical point of view, and within recent years attempts have been made to improve the method of standardization, though no international agreement has yet been reached.

Felton (1925, 1928, 1930a, b, 1931) has carried out extensive studies on this problem, adopting the method of testing varying doses of the serum to be standardized against a constant dose of pneumococci and defining the unit of serum as the least amount that will protect mice against 1,000,000 lethal doses of a virulent pneumococcal culture. His studies, and those of Parish (1930), Trevan (1930), Falk *et al.* (1931) and Smith (1932), make it clear that the unit value of a serum cannot be accurately determined by direct titration against any particular pneumococcal culture. Smith, for instance, in repeated titrations of a particular serum against a particular strain of pneumococcus found that the amount of serum necessary to protect 50 per cent. of mice against the test-dose of culture employed varied between 0.000.9 c.c. and 0.003.4 c.c. Felton (1930a) would ascribe such divergent results, particularly when different strains of pneumococci are employed, to independent variation of virulence and of capacity to unite with antibody.

This difficulty may in any case be avoided by comparing an unknown serum with a standard serum, and assigning a value to the former, not in terms of the particular dose of pneumococci against which it protects, but in terms of the arbitrary unit value

assigned to the standard preparation. Any variation in the culture will presumably affect the neutralizing power of the standard and the unknown serum to the same extent; and it is probably a fair assumption that, if n c.c. of the standard and $2n$ c.c. of the unknown serum each give a mortality of 50 per cent. when injected into an adequate sample of mice together with the same dose of the same pneumococcal culture, the unknown serum contains half as many units of protective antibody as the standard.

The titration curves recorded by Parish (1930), Trevan (1930) and Smith (1932) make it clear that this method allows an assay within a reasonable degree of accuracy provided that an adequate sample of mice is injected with each dose. Actually (see p. 26) the standardization of an antipneumococcal serum to within a margin of error of ± 30 per cent. demands the use of 40 mice for each dose of the unknown serum tested, and another 40 mice for each dose of the standard serum with which it is to be compared.

It may be added that the common practice is now to inject the serum and culture simultaneously into the peritoneum and to use 0.0025–0.1 c.c. of culture as the test-dose, according to its virulence and bacterial content. The virulence should be maximal, so that 10 pneumococci or less will kill untreated mice, and the inoculum should correspond to 500,000–5,000,000 lethal doses.

An important saving in mice would obviously be effected if we could substitute an *in vitro* for an *in vivo* test. The type-specificity of the protective action clearly suggests that the effective antibody is that which corresponds to the type-specific polysaccharide; but, equally clearly, we must establish this identity by direct experiment before we can accept an *in vitro* estimation of this antibody as affording an adequate measure of protective value.

Certain of the earlier observers (see for instance Wadsworth 1917) reported a complete and puzzling lack of correlation between agglutinin titre and protective power. It seems likely that some particularly unfavourable conditions must have affected such results as these; since Falk *et al.* (1931) and Felton (1931) record a close parallelism between agglutinin titre and protective power in mice. Felton, testing 39 sera by both methods, reports a correlation coefficient of $+0.80$; but his protocols show a few markedly divergent results.

Since the isolation of the type-specific polysaccharide hapten of Heidelberger and Avery (1923) attempts have naturally been made to correlate precipitin content with protective power, and these have, on the whole, given better correlation than the agglutinin estimations (see Sobotka and Friedlander 1928, Friedlander *et al.* 1928, Zozaya *et al.* 1930, Heidelberger *et al.* 1930, Falk *et al.* 1931, and Felton 1931). None of these comparative titrations can, however, be regarded as an adequate test, since the method of optimal proportions was not followed in determining the precipitin content of the serum.

More recently Smith (1932) has carried out a series of careful comparative titrations along these lines. Using the optimal-proportion method of Dean and Webb (1926) in the precipitin estimations, and injecting adequate samples of mice (40 for each dose of serum) in the protection tests, he has compared several Type I antipneumococcal sera with a particular serum taken as a standard. His results have been extremely encouraging. With four unconcentrated sera the protective power, measured in terms of the standard serum, corresponded almost exactly to the figure obtained by comparing the precipitin content. Three concentrated sera did not, however, give such close agreement. In each case the protective power was greater than would have been indicated by the precipitin content, and in one instance the discrepancy was considerable. If this difficulty can be surmounted, it seems likely that the precipitin test will afford an accurate and relatively simple method of standardization.

In this connection we may recall the observations of Enders (1930) and of Wadsworth and Brown (1931), which taken in conjunction with the findings of Schiemann and Casper (1927) and of Schiemann (1929) indicate that pneumococci possess, in addition to the

polysaccharide hapten isolated by Heidelberger and Avery, another type-specific antigenic component which, unlike the purified hapten, is able to stimulate antibody formation and to induce active immunity. The exact chemical nature of this bacterial component, and how, if at all, it is related to the type-specific antigen still more recently described by Felton (1932), are questions to which we have as yet no answer. It is possible that such divergences as have been observed between the *in vivo* and *in vitro* assay of anti-pneumococcal sera may, in part at least, be due to the presence of more than one protective antibody. This possibility has been clearly envisaged by Enders (1932) who notes that antipneumococcal sera that contain precipitin for his type-specific antigen, but no precipitin for the polysaccharide hapten of Heidelberger and Avery, may give type-specific agglutination to high titre. Assuming, as is most probable, that the antipolysaccharide precipitin is the most important protective antibody, there would be little correlation between the agglutinin titre of such a serum and its protective value. Assuming further, as is quite possible, that the antibody acting on Enders' antigenic component is also of some protective value, the protective power of a serum might not be completely determined by measuring its precipitin content against the purified polysaccharide hapten. This would only mean that we should have to standardize our sera for two components, instead of for one.

When we have an adequate knowledge of the chemical nature of the different bacterial antigens and haptens, and of the immunological importance of the antibodies acting upon each, it is probable that we shall in fact produce our antisera with the object of attaining particular concentrations of particular antibodies, and that we shall standardize them by *in vitro* methods of this kind.

The Standardization of Antimeningococcal Serum.—In no animal except the monkey is it possible to produce a meningococcal infection that bears any close relationship to cerebrospinal meningitis in man.

Recent observations by Zdrodowski and Voronine (1932) suggest that it may be possible to induce a meningeal infection of a kind by injecting large doses of meningococci into the subarachnoid space of young rabbits; but these observations have not yet been confirmed.

So far as the ordinary laboratory animals are concerned, it is possible to kill them by the intraperitoneal injection of massive doses of meningococci, but the infection is not of the characteristic invasive type.

The most sensitive animal is the mouse, and Murray (1929) puts the lethal dose for a 20 gm. mouse at 0.5–8 mg. of living virulent meningococci, that is, at an average dose of thousands of millions. The lethal dose of dead meningococci is greater than that of live meningococci of the same strain, but not inordinately greater; and if the meningococci are lysed by some appropriate means before injection the lethal inoculum is of the same order as in the case of the living organisms. It seems clear that some toxic effect, not dependent on the multiplication of meningococci within the tissues, is a dominant factor in experimental infection in the mouse. We may, if we choose, ascribe it to the so-called endotoxins, but we shall be wise to remember that these behave very differently from the filtrable toxins, especially in regard to their neutralization by antitoxin (see p. 37).

If we desire to standardize an antimeningococcal serum we can, if we think fit, use the mouse-protection test in spite of its obvious disadvantages; and there are those who believe that it affords the best available test of therapeutic potency (see Hitchens and Robinson 1916, Matsunami and Kolmer 1928, Gordon 1920, Murray 1929). Those who hold this view, and particularly Gordon, do so because they believe that the toxin-neutralizing power of a serum is an important, perhaps the most important, factor in its therapeutic effect. Whether all serological types of meningococci elaborate the same toxic products

is not yet clear. There are suggestions that anti-endotoxic sera are most effective against the serological type of meningococcus against which the serum was prepared, but whether this is due to differences in the antitoxic effect or to other factors cannot be decided.

Those who believe that the therapeutic action of antimeningococcal serum in man is mainly antibacterial, sensitizing the bacteria to phagocytosis and perhaps to lysis within the tissues, tend to view the mouse-protection test with less favour; since the dominance of the toxic effect in that animal may obscure the protective action of bacterial sensitization. Moreover, those who regard the antibacterial effect as dominant would expect it to be type-specific, and would therefore demand that the serum employed should contain antibodies for the infecting type of meningococcus. This demand is not so onerous as in the case of the pneumococcus, since the prevalent types of meningococci fall within four antigenic categories, and of these I and III and II and IV are closely related. It is not then a difficult matter to prepare sera that contain a high titre of antibodies acting on each of the main types; and this is what is done in practice.

The presence of these antibodies may be demonstrated by agglutination, or by complement fixation, or by an opsonic reaction; but there seems at the moment no sound reason for preferring one *in vitro* reaction to another, and agglutination is the test that is most conveniently and commonly employed (see Wadsworth *et al.* 1919, Wadsworth 1921, 1931, Wadsworth and Kirkbride 1926).

There is no standard serum, no official unit. An antimeningococcal serum, in the sense at present employed, is the serum of a horse that has been immunized against the four common types of meningococci and tested for agglutinins against these types. Where official testing is carried out it prescribes a limiting agglutinin titre below which the serum must not fall.

All this, of course, is highly unsatisfactory. What we need is an extensive empirical trial in which different sera, each of which has been carefully standardized by all the methods available, are tested on a sufficient number of human cases, so that we can determine which method of standardization gives us the best measure of the therapeutic effect in man. The records contain many expressions of opinion based on small series of cases; but, in view of the difficulty that has been experienced in arriving at a final decision as to whether antimeningococcal serum has any significant therapeutic value, it is clear that only a well-planned inquiry carried out on the largest scale and with the closest co-operation among all the workers concerned is likely to enable us to arrange several different samples of serum in the order of their therapeutic efficacy.

The Standardization of Antistreptococcal Serum.—The term "antistreptococcal serum" is usually employed to denote the serum derived from a horse that has received repeated injections of several different strains of streptococci. It thus differs, in its mode of preparation and in its assumed antibody content, from the antitoxic antiscarlatinal serum that we have considered above. It is probable that such sera frequently contain antitoxin acting on the erythrogenic toxin of the hæmolytic streptococcus, and also a varying amount of anti-hæmolysin; but no specific attempt is made to induce the formation of these antibodies, or to determine their presence or amount in the final product.

In so far as such sera have any antibacterial effect it is presumably type-specific, and in view of the antigenic heterogeneity of streptococci it will be a fortunate chance if any particular sample of antistreptococcal serum happens to contain specific sensitizing antibodies for the type of streptococcus with which any particular patient is infected.

Standardization is obviously impossible.

THE STANDARDIZATION OF CERTAIN DIAGNOSTIC OR PROPHYLACTIC REAGENTS

With the reagents used in immunity reactions in the test-tube we are not here concerned; their standardization has been referred to briefly in Chapter V. The reagents that fall for discussion here are those that provoke a diagnostic reaction, local or general, in man or animals.

The Standardization of Schick Toxin.—The Schick dose of toxin needs to be clearly defined. A person who will react negatively to a particular dose of toxin may react positively to a larger one. We shall not be able to assign the same significance to all records of Schick testing, unless we know that an uniform dose has been employed. Obviously, too, we shall keep our dose as small as possible, since we shall wish to avoid serious reactions in our fully susceptible subjects. The Schick dose was originally defined on an empirical basis as 1/50th of the guinea-pig M.L.D.; but, as Glenney (1925) has pointed out, we are concerned in the Schick test not only with toxicity but with combining power. We want to know how much toxin the subject's tissue fluids can neutralize. The M.L.D. is not a satisfactory unit for this purpose since it is not a measure of combining power; but an adequate degree of toxicity must be ensured, since the dermal reaction depends on the presence of free toxin.

The subject was considered by the Biological Standardization Commission of the League of Nations in 1931 (see Report 1931), and two alternative definitions of the Schick dose were adopted. One reads as follows:

"The Schick test-dose, for injection in a volume of 0.2 c.c., shall be that quantity of toxin which, when mixed with 1/750th part or more of an international unit of antitoxin and injected intracutaneously into a normal guinea-pig, causes no local reaction; but, when mixed with 1/1,250th part or less of an international unit and similarly injected, causes a marked reaction of the type of a 'positive' Schick reaction; *provided that* the toxin is such that 1/25th of the Schick test-dose as above determined, without admixture with antitoxin, when injected intracutaneously into a normal guinea-pig, causes a definite local reaction of the type of a 'positive' Schick reaction; but that 1/50th of the Schick test-dose, similarly injected without admixture, causes no local reaction of this type."

In the alternative definition the main clause defines the Schick dose in terms of the M.L.D., and the proviso deals with combining power. Both conditions must be fulfilled under the Regulations (1931) made in this country in accord with the Therapeutic Substances Act (1925).

The Standardization of Erythrogenic Streptococcal Toxin for use in the Dick Test.—Reference has already been made to the difficulties in the way of standardization of scarlatinal toxin and antitoxin. There is as yet no possibility of defining the skin test-dose of toxin otherwise than as the smallest

amount of filtrate that is, on the average, sufficient to produce an erythematous reaction, 1 cm. in diameter, in the skin of susceptible human beings within 24 hours (see Dyer 1928). This is inherently unsatisfactory, partly because the assumption that any particular sample of human beings are fully susceptible is hardly a safe one, partly because suitable subjects are not easily obtained. The adequate standardization of this reagent awaits the appearance of a satisfactory test animal.

The Standardization of Tuberculin.—Here we are dealing with an active substance against which there is no known specific neutralizing antibody. An antiserum can be produced that gives specific precipitation with tuberculin, but the reacting antigenic substance is not the active principle on which the tuberculin test depends—it is probably a polysaccharide hapten derived from the tubercle bacillus—and the results of the precipitin test bear no relation to the reactions obtained in tuberculous guinea-pigs (see Dreyer and Vollum 1924, Okell and others 1926). We must, then, measure tuberculin in terms of its toxic effect in tuberculous guinea-pigs. Tuberculin is fortunately a relatively stable substance, so that we can adopt one particular preparation as a standard and, by comparing all other preparations with this and adjusting them by dilution, we can ensure the issue of tuberculin of approximately standard activity. We can then express our dose as a volume or dilution, with the assurance that 0.2 c.c. of a 1:1,000 dilution of tuberculin will always represent approximately the same amount of active substance.

The Biological Standardization Commission of the League have adopted the preparation of tuberculin stored at the Statens Serum Institut, Copenhagen, as an international standard, recommending that all samples of tuberculin should be compared with this by appropriate tests in tuberculous guinea-pigs.

As to the method of comparison to be employed, the classical method, still used in Germany and elsewhere, is the subcutaneous injection of tuberculin into guinea-pigs that are suffering from an obvious tuberculous infection (usually towards the end of the 4th week after the injection of living tubercle bacilli). The minimal lethal dose—i.e., the smallest dose that will kill tuberculous guinea-pigs within 24 hours with the typical signs of acute tuberculin shock—is determined for the standard preparation and for the preparation under test (see Otto and Hetsch 1921, Prausnitz 1929). It need hardly be said that this method demands the sacrifice of large numbers of guinea-pigs if results of reasonable accuracy are to be obtained.

Another method is to test the allergic activity of tuberculin by intracutaneous injection in the tuberculous guinea-pig (see Eagleton and Baxter 1923, Lewis and Aronsen 1923, Calmette and de Potter 1926, Okell *et al.* 1926, Okell and Parish 1928). This method has the obvious advantage of all skin tests, that comparison between one preparation and another can be carried out on the same animal, thus eliminating one of our more important uncontrollable variables. It enables a comparison between two preparations of tuberculin to be made, within any specified limits of accuracy, with the expenditure of fewer guinea-pigs than would be required for the lethal subcutaneous test. Thus, Trevan (see Okell and Parish 1928) has calculated that, in order to distinguish from one another two tuberculin preparations having activities of 1.0 and 1.4, it would be necessary, in the subcutaneous test, to use 40 guinea-pigs for each sample of tuberculin, given that the minimal lethal dose of each was approximately known. With much smaller numbers of guinea-pigs—4 to 6 for each comparison of two samples, instead of 80—Okell and Parish were able to attain this degree of accuracy with the intracutaneous test. It would

appear (see Okell and others 1926) that the values assigned by this test afford a satisfactory measure of the activity of different preparations in man and animals, at least when the differences in potency as tested on the guinea-pig are wide.

The Standardization of Diphtheria Toxoid, or other Similar Prophylactic.—The diphtheria prophylactic now most generally used is probably formolized toxoid (anatoxin), though several other preparations are under trial.

The flocculation test of Ramon (1922) provides an obvious method of determining the amount of antigenically active material in any diphtheria prophylactic; since anti-toxin combines either with toxin or with toxoid (see Ramon 1924); and Glenny, Pope and Waddington (1925) found that the immunizing value of a modified toxin was closely correlated with its Lf value. Since, however, methods are available by which the immunizing value of any preparation can be directly determined in laboratory animals it is clearly desirable to correlate tests of this kind with the flocculation test before accepting the latter as a final measure of prophylactic efficacy. The Biological Standardization Commission of the League have planned an extensive inquiry into this problem which is not yet completed (see Report 1931).

The relevant regulations in force in this country, under the Therapeutic Substances Act, are as follows:

“Diphtheria Prophylactic shall be submitted to the following tests:

(a) *Tests to determine that the specific toxicity of the toxin used in its preparation has been so reduced that it does not exceed the prescribed maximum.*—Five human doses of the Diphtheria Prophylactic under test shall be injected into each of 5 normal guinea-pigs each weighing 250–350 grammes. This injection must not cause the death of any of the guinea-pigs within 6 days following the injection. If all the guinea-pigs injected survive for 6 days but any of them die within 30 days following the injection from the specific toxæmia, one human dose of the Diphtheria Prophylactic under test shall be injected into each of 5 normal guinea-pigs, each weighing 250–350 grammes. This injection must not cause the death of any of the guinea-pigs within 30 days following the injection.

If a batch of Diphtheria Prophylactic is shown by either of these tests to have a greater toxicity than the maximum hereby indicated, it shall not be issued unless and until the toxicity has been so reduced by further treatment that it does not exceed that maximum.

(b) *Test for potency as an immunizing antigen.*—A quantity of Diphtheria Prophylactic not exceeding five human doses shall be injected on one occasion into each of 10 normal guinea-pigs; or, alternatively, a quantity of Diphtheria Prophylactic not exceeding one-tenth of a human dose shall be injected into each of 10 normal guinea-pigs on each of two occasions, separated by an interval of not more than 4 weeks. The guinea-pigs shall be tested for immunity to diphtheria toxin, if they have received the single injection hereinbefore prescribed, at a date not later than 6 weeks after injection, and if they have received the two injections hereinbefore prescribed, at a date not later than 3 weeks after the second injection.

The test for immunity may be made by either of the two following methods:

(i) by intracutaneous injection into each guinea-pig of one test-dose of Schick Toxin. If more than 2 out of the 10 guinea-pigs exhibit a positive Schick reaction, the batch of Diphtheria Prophylactic shall be treated as insufficiently potent, and shall not be issued; or

(ii) by subcutaneous injection into each guinea-pig of five minimal lethal doses of diphtheria toxin. If more than 2 out of the 10 guinea-pigs die as the result of this injection the batch of Diphtheria Prophylactic shall be treated as insufficiently potent, and shall not be issued.”

This, it will be noted, is the prescription of limits of toxicity and antigenic efficiency, not standardization in terms of units. The latter may follow.

The Biological Standardization Commission (Report 1931) has recommended that the possibility be explored of obtaining for international acceptance a standard preparation of diphtheria prophylactic, preferably consisting of a formol toxoid (anatoxin), and of defining in terms of this standard a unit of immunizing potency for international acceptance. Methods of standardization will, no doubt, be recommended when the Commission's inquiry has been completed.

THE STANDARDIZATION OF BACTERIAL VACCINES

As we have just seen, the standardization of the antigenic agent employed to produce active antitoxic immunity against diphtheria is based on a direct determination of immunizing potency—an obviously desirable method until we can be sure that some alternative *in vitro* test will give us an equally accurate measure of the activity we wish to exploit.

In the case of bacterial vaccines it is the custom, at the moment, to standardize them simply in terms of content. The present position in this country can again be most easily made clear by quoting the relevant clauses from the Regulations issued under the Therapeutic Substances Act.

"The proper name of any vaccine shall be the name of the micro-organism from which it is made, followed by the word 'vaccine' unless the relative Schedule otherwise provides or if there is no provision in the relative Schedule some other name is approved by the licensing authority: Provided that in the case of the under-mentioned preparations the proper name of the vaccine may be as follows:

- Anti-typhoid vaccine;
- Anti-typhoid-paratyphoid vaccine (T.A.B.);
- Anti-typhoid-paratyphoid-cholera vaccine (T.A.B.C.);
- Anti-plague vaccine;
- Anti-dysentery vaccine;
- Whooping-cough vaccine.

Cultures used in the preparation of vaccines must, before being manipulated into a vaccine, be thoroughly tested for identity by the generally accepted tests applicable to the particular micro-organism. The permanent records which the licensee is required to keep shall include a record of the origin, properties and characteristics of the cultures."

"Vaccines may be issued either singly or combined in any proportion in the same container. In the case of combinations of vaccines a name for the combined vaccine may be submitted by the licensee to the licensing authority, and, if approved, may be used as the proper name of the vaccine.

The label on the container shall indicate the composition of the vaccine by reference either:

- (a) to the number of micro-organisms per c.c.; or
- (b) to the weight of dried substance of micro-organisms per c.c.; or
- (c) to the number of micro-organisms or weight of dried substance of micro-organisms used in preparing 1 c.c. of the finished product."

It would, perhaps, be difficult to insist on more than this at the immediate stage of our knowledge; but when we know a little more with regard to the antigenically active components of the common pathogenic bacteria we shall be in a position to aim much higher. Already we know quite enough to be sure that the specification of the number of bacteria per c.c. of any vaccine is an inadequate measure of its immunizing power. Assuming that the bac-

terium in the vaccine is the cause of the infection against which we desire to induce immunity, assuming also that the bacterium in question is not divisible into different serological types, or, alternatively, that all the serological types are present in the vaccine, there is still room for a wide variation in antigenic efficiency.

We know that effective antibacterial immunity depends on the production of antibodies acting on particular antigenic components—in the typhoid-paratyphoid group, for instance, mainly on the sensitizing antibodies corresponding to the polysaccharide somatic haptens of the smooth virulent form. A strain that has lost these haptens is useless, or almost useless, as an immunizing agent. A strain of intermediate type, in which these haptens are poorly represented, is probably a relatively poor immunizing agent.

Quite recently Grinnell (1932) has examined twelve cultures of the "Rawlins" strain of typhoid bacillus—a classical laboratory strain that is widely used for the preparation of typhoid vaccine—and has compared their antigenic potency with that of several recently isolated strains. Although the Rawlins cultures were not rough in the ordinarily accepted sense, several of them showed intermediate characters as judged by the ordinary cultural tests. Their virulence for mice was definitely lower than that of the recently isolated completely smooth strains. Mice were immunized by the injection of killed cultures of the Rawlins strains or of the smooth strains and were subsequently tested for resistance. In one experiment 28 of 32 mice immunized with Rawlins strains and 1 of 31 mice immunized with the completely smooth strains died as the result of the test inoculation. In a second experiment the deaths were 55 of 60 mice immunized with the Rawlins strains and 2 of 37 mice immunized with the smooth strains. Perry, Findlay and Bensted (1933) have recently recorded very similar observations.

Similarly, Gardner and Leslie (1932) have found that many strains of the whooping-cough bacillus, used in the preparation of vaccine, have lost their smooth somatic antigen in whole or in part and are in consequence inefficient immunizing agents.

Obviously we need some test of immunizing potency—a direct animal test in the first place where that is available, an appropriate *in vitro* test as soon as we are quite sure that it will give us a true measure of our active immunizing agent, or agents.

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