Recent methods in the diagnosis and treatment of syphilis : the Wassermann serum reaction and Ehrlich's salvarsan / by Carl H. Browning and Ivy Mackenzie in collaboration with John Cruickshank [and others].

RECENT METHODS IN THE DIAGNOSIS AND TREATMENT OF SYPHILIS

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THE WASSERMANN SERUM REACTION AND EHRLICH'S SALVARSAN

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Authors' Preface

THE contents of this volume are essentially a record of original work. The results of a considerable part of ee researches, especially in Part I, have appeared in a different rm in various journals ; but many observations are published re for the first time. Towards the expenses of the research ork numerous grants have been received from the Carnegie rust, for which we have much pleasure in acknowledging our debtedness. It has been impossible to give a complete view of the very extensive literature on the matters under scussion ; but every effort has been made to omit no facts theoretical or practical importance. The references have een selected so as to enable those who are interested in any rticular subject to obtain access to the original sources, d as far as possible they have been brought up to date. addition to the index a full table of contents has been pre-red, and a list of incorporated papers has been added.

We owe a debt of gratitude to Professor Muir, whose teaching d encouragement have stimulated us to pursue the investiga-ons. We must also record our warmest thanks to Professor hrlich, to whom our understanding of the principles of chemo-erapy is due, and whose generous gifts of salvarsan and sseno-phenylglycin have enabled us to continue the work this subject.

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September, 1911.

Introduction

THE demonstration of the specific organism—the *spirochaete pallida*—in syphilitic lesions by Schaudinn and Hoffmann in 1905, the discovery of the serum reaction by Wassermann, Neisser and Bruck, in 1906, and the introduction of salvarsan (No. 606) as a therapeutic agent by Ehrlich in 1909, together constitute a chapter of events of unusual interest in the history of medicine. In the following pages the writers deal with the two last-mentioned subjects, and in a corresponding manner the book falls into two main divisions, the first dealing with the nature of the Wassermann reaction and the results obtained by its application, and the second giving an account of salvarsan and its therapeutic effects. The practical outcome of the new methods of diagnosis and treatment will naturally be the subject of greatest general interest to the members of the medical profession, but it is of the highest importance that the principle of the Wassermann reaction should be understood, especially as there has been a tendency to overlook difficulties and sources of fallacy in the search for a simple and rapid method of procedure. The basis of the discovery of the reaction may be said to be an attempt to discover anti-substances in the blood of syphilitic patients. That such anti-substances are developed as the result of the injection of many and diverse organic substances, and are also developed in the course of many natural infections, there being in the latter case a production of foreign molecules within the body by the activity of the virus, are facts now well known. The substances which thus give rise to anti-substances are known as *antigens*, and the serum diagnosis of disease depends on the discovery of the anti-substance by the use of the corresponding antigen. In typhoid fever, for example, an emulsion of typhoid bacilli (antigen) is used to detect the specific anti-substance, the agglutinin. In the case of many anti-substances,

e.g. antitoxins, agglutinins, precipitins, etc., the anti-substance along with the corresponding antigen produces the specific effect; but in the case of one class, viz. immune-bodies, the co-operation of another substance present in normal serum, *complement*, is necessary. If the red corpuscles of an animal are injected into another animal of different species, the serum of the latter acquires the power of dissolving the corpuscles in question, and this property is due to a specific immune-body acting along with the complement normally present. If such a haemolytic serum be heated at 55° C., it loses its haemolytic property, because the complement is destroyed, but it is restored on the addition of fresh normal serum (complement). Accordingly red corpuscles treated with the corresponding immune-body (haemolytic serum deprived of complement by heating) serve as a test for the presence of complement, and are usually spoken of as *sensitised corpuscles*; if complement is present they will undergo lysis, if it is absent they will remain intact. What is now generally known as the Bordet-Gengou reaction gives us a general method for testing for the presence of an immune-body. If an immune-body be added to its corresponding antigen, complement will be absorbed or fixed, and on the addition of sensitised corpuscles no lysis will occur. Such a test can be carried out successfully in the case of many infections. For example, typhoid bacilli along with a typhoid serum will lead to the fixation of complement, whereas this will not occur if a normal serum be substituted. Acting on this principle, Wassermann, Neisser, and Bruck, endeavoured to find anti-syphilitic immune-bodies in the blood of syphilitic patients. As cultures of the *spirochaete pallida* were not available, they made use of a watery extract of a syphilitic liver, rich in spirochaetes, as antigen, and on mixing this with a certain amount of serum from a syphilitic patient and then adding complement, they found, by the usual haemolytic test, that the complement was fixed or absorbed, whereas this did not occur when a normal serum was substituted for the syphilitic serum. Apparently then the presence of a syphilitic immune-body was demonstrated and the theoretical anticipations were realised. It was, however, shown by Marie and Levaditi that the same result was obtained when an alcoholic extract of a normal liver was used as antigen, and subsequent observations have shown that extracts of other normal organs, and also solutions of organic substances of known constitution, can be substituted for an

extract of a syphilitic organ with equal efficacy. It is somewhat unfortunate that to any combination of substances which act in this way the term "antigen" has been applied, but no harm will be done so long as it is clearly understood that the term is now used only in a figurative sense. The general conclusion may be said to be that a syphilitic serum along with solutions of, especially, lipoidal substances has the peculiar property of fixing a large amount of complement.

It will thus be seen that in the first stage of a Wassermann reaction a mixture is made of organ extract (antigen), the suspected syphilitic serum, and complement (normal guinea-pig's serum), the mixture being then placed in the incubator at 37° C.; in the second stage sensitised corpuscles are added in order to ascertain whether or not the complement has disappeared. Details as to the amounts, modes of preparation of each of the ingredients, etc., will be found in the text. Here, however, certain points with regard to complement may be referred to. It is to be noted that any organic extract used as antigen may of itself fix or absorb a certain amount of complement, a non-syphilitic serum may do the same, and a mixture of the two may fix still more, though the amounts may be relatively small. Accordingly the test comes to be a quantitative, not a qualitative, one, and the peculiarity of the syphilitic serum is simply the large amount of complement which it fixes when mixed with the antigen. The estimation of a positive or negative result thus depends on the amount of complement fixed. Now the only means which we have of estimating the amount of complement in a fresh serum is to find the haemolytic dose, i.e. to find the smallest quantity of serum which is just sufficient to produce complete lysis of the test amount of sensitised corpuscles; and tested in this way it is found that different samples of serum vary widely as regards the amount of complement. It is thus essential to accuracy that the haemolytic dose be estimated; the quantity of complement used as the criterion in the reaction will then be expressed not in terms of the amount of fresh serum, but in terms of haemolytic doses. In large institutions where numerous tests have to be carried out at one time, the fallacy referred to is avoided by using the mixed or "pooled" serums of a number of guinea-pigs, but of course in ordinary circumstances such a procedure is not practicable. The determination of the amount of complement fixed which is to be accepted as evidence of a positive reaction is a matter of great importance;

if this is placed too high a number of syphilitic cases will escape detection, if too low there is a risk of non-syphilitic cases being accepted as giving a positive reaction. It is manifest, however, that whatever standard may be adopted, it will be an advantage to know not merely whether the test amount of complement has been fixed, but also the actual amount of complement fixed. This can readily be ascertained by using a series of tubes to which different amounts of complement are added; such a method requires a little more labour, but this is amply repaid by the information yielded. The property of the serum on which the Wassermann reaction depends neither appears nor disappears suddenly in syphilitic affections. There will accordingly always be a certain proportion of cases which are almost positive, as well as those which are clearly so, and these cannot be recognised by any "one-tube method." Proceeding on these principles, the authors have, in the case of each test made, estimated the dose of complement and then ascertained the number of doses fixed.

A great many different organ-extracts, solutions or suspensions of lipoidal substances, etc., are found to act efficiently as antigens in the Wassermann reaction, though with varying efficiency. It is necessary not only that the antigen should deviate complement well along with a syphilitic serum, but also that it should give the minimum deviation with non-syphilitic serums, that different samples should be comparatively uniform in composition, and that it should be free from various incidental faults referred to later. Much work has been done in this direction, and various antigens are recommended by different observers. The authors have made important observations in connection with this subject, and have introduced the use of an emulsion of lecithin and cholesterin as antigen, the former being prepared from the liver of the ox. The basis of this method is the fact observed by them, that a syphilitic serum plus the lecithin-cholesterin emulsion leads to the fixation of a much larger amount of complement than does a syphilitic serum plus an emulsion of lecithin alone, whereas this does not occur with a non-syphilitic serum. The claims in favour both of the reliability and delicacy of the method will be found to be fully set forth in the text.

In the fifty pages or so devoted to the clinical application of the test, the reader will find a wealth of interesting facts, taken from the experience of the writers or gathered from the extensive literature of the subject. From what has been stated

above, it will be seen that while the discovery of the serum reaction was the result of testing a theory, and was thus arrived at by induction and experimentation, its *rationale* is something quite different from what it was supposed to be. Accordingly, while in the case of bacterial infections the serum reactions can be explained according to the general principles of immunity phenomena, this is not so in the case of syphilis. Countless observations show, as the writers state, that the presence of a positive reaction, under the conditions laid down, may be taken in this country as proof that a syphilitic infection has occurred. But it cannot be said that the reaction is quite specific for syphilis (as it has been found in a considerable proportion of cases of leprosy and framboesia), nor is it possible to give at present any explanation of the relation between the peculiar property of the serum and the infecting organisms. The value of the test thus rests on an empirical basis. As syphilis is so protean in its manifestations, so protracted in its course, so variable in its activity, passing often into a condition of latency, the degree and regularity with which the reaction is given might be expected to vary, and this is found to be the case. Abundant data are given, and the general facts established show that there is in the various stages of the disease a certain relationship between the serum reaction and the wideness of distribution and activity of the spirochaetes, though to this there may be exceptions. When we come to the so-called parasyphilitic affections, we have to deal with exceedingly interesting though perplexing results. It is found that in general paralysis of the insane a positive reaction is nearly always present, though it may have been absent before the onset of the symptoms of this disease. In other words, there is a relationship between the Wassermann reaction and the processes underlying the symptoms and structural changes in general paralysis. An important question thus arises—on what does this relationship depend ? Can it be on the presence and activity of spirochaetes ? Unfortunately no one has yet demonstrated these organisms in general paralysis, though, on the other hand, we cannot conclude that they are absent. The fact that fresh inoculation with syphilis is not known to occur in general paralytics is also in favour of the syphilitic virus still being present. Manifestly if the organisms are not present, the interpretation of the serum reaction becomes more difficult and obscure than ever.

Some other points of interest may be referred to. In cases

where a woman has borne a syphilitic child without herself showing symptoms of the disease, it has been found that the serum gives a positive reaction in a large proportion of instances, a fact which shows clearly that her reacting mechanism has been affected by the syphilitic virus, and suggests strongly that infection of the maternal tissues has really occurred. The presence of a positive reaction in many cases of latent syphilis and the disappearance of this under treatment (*vide infra*) are also facts whose correct interpretation is of great practical importance. Moreover, the presence of a positive reaction in various morbid conditions not generally regarded as syphilitic, would indicate that syphilis is even more far-reaching in its effects than was formerly supposed; this is well illustrated, for example, in cases of congenital mental deficiency, imbecility, and Little's disease. In fact, it may be said that the various clinical phenomena of the disease have now been investigated in relation to the Wassermann reaction, and fresh light has been thrown on many of them. An important section is that devoted to the properties of the cerebro-spinal fluid in para-syphilitic affections. The presence or absence of a Wassermann reaction given by the fluid is compared with its protein content and with its cytological characters, and the conclusion is arrived at that the first constitutes the most reliable method of diagnosis. Whilst the substances concerned in the deviation of complement are precipitable with the globulins, there is no fixed relationship between the amount of the latter and the Wassermann reaction. There will also be found a full discussion on the significance of the reaction given by the cerebro-spinal fluid as compared with that given by the serum.

The second half of the work is devoted to an account of salvarsan and its therapeutical applications. The discovery of the drug by Ehrlich was no happy accident, but was the result of a systematic examination of a long series of substances, carried out with practically unequalled experimental and chemical resources. His endeavour was to find an agent possessed of the most active spirillicidal (spirochaetecidal) properties, and at the same time exerting the minimum toxic effect on the tissues of the host. The action of the various substances investigated was tested on spirillar diseases and on syphilitic infection in rabbits, and the remarkable therapeutic effect, with comparatively slight toxic action, of salvarsan was thus established. The extension of the investigation to syphilis in the human subject gave no less striking results, and the

drug was then placed in the hands of clinicians in various parts of the world. There has been practical unanimity as to the remarkable effect produced by it on syphilitic manifestations, and the testimony of the writers as the result of their experience is: "We have not encountered a single case with the more acute manifestations of infection, such as lesions of the skin, mucous membranes, bones and eyes, which has not promptly yielded to treatment provided that absorption of the drug occurred." In estimating, however, the probable value of the drug in human syphilis, it is important to keep in view the morbid anatomy of the disease and the distribution of the spirochaetes in the body. Were syphilis a blood infection like relapsing fever, we could predict with practical certainty the complete sterilisation of the tissues and consequent cure of the disease by means of salvarsan, so marked are its spirillicidal properties. But this is not the case. Syphilis is essentially a tissue disease, though the spirochaetes are also carried by the blood stream; the organisms have a special preference for dense connective tissue structures, and imbedded in these or at the margin of caseous lesions they may remain alive, though inactive, for years. If these points be kept in view, the difficulty of bringing the drug efficiently into relation with the spirochaetes will be readily appreciated, especially when it is borne in mind that probably the drug, as indicated by the arsenic excretion in the urine, has disappeared from the blood within three or four days after intravenous injection. Accordingly, due caution must be used in speaking of permanent cure; as the writers rightly remark, several years must elapse before the real value of salvarsan in this respect can be estimated. At present we can only provisionally judge of probable cure, on the one hand by the disappearance of the signs and symptoms of the disease, and on the other hand by the permanent establishment of a negative Wassermann reaction. This latter is of no small importance, as cases have been observed where the former requirement has been fulfilled along with a positive serum reaction, and where such patients have proved a source of infection. So far, however, as can be judged by the tests mentioned, a cure has been effected by salvarsan in a large proportion of cases. Important testimony that the drug does actually cure is also supplied by the fact that several patients satisfactorily treated by it have acquired a fresh attack of the disease by natural infection, there being no evidence that this ever happens when the disease is present in a latent condition.

The effect in such parasyphilitic affections as general paralysis of the insane, even though only in the direction of ameliorating the symptoms, is very striking, and strongly suggests that in these conditions the syphilitic virus is still present and capable of being neutralised, though of course the permanent lesions cannot be overcome. The general conclusion is thus reached that in this drug we have by far the most active anti-syphilitic or anti-spirochaetal remedy yet discovered.

Granted, however, that salvarsan has the remarkable properties claimed for it, a scarcely less important requirement is that it should be non-toxic to the human tissues in the doses necessary for successful treatment. This is an aspect of the subject which is treated of at length and in a dispassionate manner. The necrotic and sometimes septic lesions following subcutaneous and intramuscular injections are referred to, though these are now of comparatively little importance since the intravenous method of injection was introduced. Seeing that optic nerve atrophy may follow the continued administration of atoxyl and other arsenic compounds, the possibility of salvarsan possessing a neurotoxic action has to be carefully inquired into. A considerable number of cases have been recorded in which symptoms depending on lesions of cranial nerves have developed after the administration of the drug; these cases are critically examined, and the conclusion is come to that the lesions in question are really syphilitic manifestations. In other words, they are not due to the action of the drug, but to the persistence in the sheaths of the nerves of spirochaetes which have escaped its action. When we consider the very manifold syphilitic affections of the nervous system, it is manifest that the greatest caution must be exercised in judging the significance of such occurrences. In view, however, of the very extensive administration of the drug, on the one hand, and the relatively small number of such complications as well as their nature, on the other, the conclusion arrived at, namely, that salvarsan is practically without neurotoxic action, seems to be fully justified. Various disturbances of the urinary and alimentary systems, nephritis, jaundice, etc., following the administration of the drug, are also duly considered.

Special attention is properly devoted to the fatalities which have been reported as occurring after the administration of salvarsan. Nothing but harm can result from overlooking such occurrences or minimising their significance. The only proper method is, as the authors have done, to gather together all the

fatalities recorded, to give the chief facts with regard to each, and then to estimate critically their import. Twenty-six cases in all are thus reported, and the conclusion reached is that in a great proportion of these the occurrence of death could not justifiably be ascribed to the use of the drug. In forming a judgment on this subject, it is only right to bear in mind that in salvarsan we have a drug but recently introduced, and one to be administered according to a prescribed method, and that already many thousands of patients have been treated by it, many of whom have doubtless been suffering from grave syphilitic lesions of important organs. In fact, according to the law of probabilities alone, the occurrence of death in a certain number of cases within a comparatively short time after the injection would not be surprising. The facts must be allowed to speak for themselves, but at present the conclusion can hardly be avoided, that if due attention be paid to the contra-indications already clearly laid down and to the instructions as to the method of use, the administration of salvarsan is relatively a very safe procedure.

This work goes forth as an attempt on the part of unprejudiced observers to test the value of the Wassermann reaction and of Ehrlich's method of treatment of syphilis. It would be out of place to say anything in the way of commendation, but we believe that no impartial reader will fail to appreciate the labour expended by the writers in carrying out the researches recorded and in attempting to arrive at an independent judgment on the questions at issue.

R. M.

List of Papers Incorporated

1. I. Mackenzie : "The serum diagnosis of syphilis."—*Journ. of Path. and Bact.*, vol. xiii., 1909, p. 313.

2. C. H. Browning and I. Mackenzie : "Modifications of serum and organ-extract due to physical agencies, and their effect on the Wassermann syphilis reaction."—*Journ. of Path. and Bact.*, vol. xiii., 1909, p. 325.

3. C. H. Browning and I. Mackenzie : "On the complement-containing serum as a variable factor in the Wassermann reaction."—*Zeitschr. f. Immunitätsforsch.*, Bd. ii., 1909, p. 459.

4. C. H. Browning and I. Mackenzie : "The biological syphilis reaction; its significance and method of application."—*Lancet*, vol. i., 1909, p. 1521.

5. C. H. Browning and I. Mackenzie : "On the Wassermann reaction and especially its significance in relation to general paralysis."—*Journ. of Ment. Science*, vol. lv., 1909, p. 437.

6. C. H. Browning, J. Cruickshank and I. Mackenzie : "Constituents concerned in the Wassermann syphilis reaction, with special reference to lecithin and cholesterin."—*Journ. of Path. and Bact.*, vol. xiv., 1910, p. 484.

7. I. Mackenzie : "Individual properties of complement and organ-extract."—*Brit. Med. Journ.*, vol. ii., 1910, p. 1435.

8. C. H. Browning : "Lecithin and cholesterin as reagents for the detection of syphilitic sera."—*Brit. Med. Journ.*, vol ii., 1910, p. 1439.

9. C. H. Browning and I. Mackenzie : "On syphilis ; recent methods of diagnosis and treatment."—*Glasg. Med. Journ.*, vol. lxxiv., 1910, p. 325.

10. C. H. Browning and I. Mackenzie : "The Wassermann reaction in rabbits infected with the trypanosomes of nagana, and the effect of treatment with arseno-phenylglycin (Ehrlich)."—*Journ. of Path. and Bact.*, vol. xv., 1910, p. 182.

11. H. Morton : "Biological examination of the cerebro-spinal fluid in cases of mental disease."—*Journ. of Ment. Science*, vol. lvii., 1911, p. 1.

12. W. Gilmour: "The Wassermann reaction: a more reliable technique."—*Journ. of Ment. Science,* vol. lvii., 1911, p. 28.

13. C. H. Browning, J. Cruickshank and W. Gilmour: "The action of lecithin from different sources in the Wassermann reaction."—(Abstract) *Journ. of Path. and Bact.,* vol. xv., 1911, p. 361.

14. C. G. A. Chislett: "Syphilis and congenital mental deficiency."—*Journ. of Ment. Science,* vol. lvii., 1911, p. 499.

15. C. H. Browning and J. Cruickshank: "The action of cholesterin and its derivatives with lecithin as syphilitic antigen and as haemolysin with cobra-venom."—*Journ. Path. and Bact.,* vol. xvi., 1911, p. 225.

16. C. H. Browning and I. Mackenzie: "The treatment of syphilis by salvarsan."—*Brit. Med. Journ.,* vol. ii., 1911, p. 654.

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PART II

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PART I

THE DIAGNOSIS OF SYPHILIS BY THE SERUM REACTION OF WASSERMANN, NEISSER, AND BRUCK.

CHAPTER I

HAEMOLYSIS AND COMPLEMENT-DEVIATION IN GENERAL

THE PHENOMENA OF HAEMOLYSIS: PROPERTIES OF IMMUNE BODY AND COMPLEMENT—DEVIATION OF COMPLEMENT: NON-SPECIFIC AND SPECIFIC DEVIATION—THE BIOLOGICAL SYPHILIS-REACTION OF WASSERMANN, NEISSER AND BRUCK.

THE biological syphilis reaction of Wassermann, Neisser and Bruck depends on the phenomenon known as deviation of complement, the complement referred to being that which is active in causing haemolysis. Accordingly, it will be well in the first place to discuss the various factors on which haemolysis depends. Only those facts which are of immediate importance from the point of view of the syphilis-reaction will be treated of here.*

The Phenomena of Haemolysis

The fundamental haemolytic experiments are as follows:

(1) A suspension of ox's red blood-corpuscles in isotonic (0·85 per cent.) NaCl-solution suffers no change in the presence of the fresh serum of a normal rabbit at 37° C.

(2) If instead of normal rabbit's serum we employ the serum of an immunised rabbit, i.e. one which has received several subcutaneous or intraperitoneal injections of washed ox-blood, then we find that in the mixture of fresh immune serum and ox-corpuscles at 37° C. diffusion of haemoglobin occurs from the latter (*haemolysis*), so that the previously opaque suspension of blood-corpuscles becomes transparent.

(3) When the immune serum is heated for an hour at 55° to 57° C. it loses the power of causing haemolysis.

* For investigations into the action of haemolytic sera the reader is referred to Muir's *Studies on Immunity*, Oxford, 1909.

(4) The addition of fresh serum from a normal rabbit—which by itself has no effect on the ox-corpuscles (*v.* 1)—restores the haemolytic power of the immune serum ; so that in the mixture blood-corpuscles + heated immune serum + fresh normal serum, haemolysis occurs.

From these experiments we see that for the occurrence of haemolysis it is necessary to have the co-operation of two substances, (*a*) a substance which makes its appearance in rabbit's serum as the result of immunising with blood of the species under consideration (in this case ox-blood), the *immune body* (IB.), and (*b*) a substance present in fresh normal serum which is readily destroyed at 55° to 57° C., *complement* (C.). Neither immune body nor complement by itself has any lytic effect on the blood-corpuscles.

The *Immune Body* is specific. Thus, an immune serum obtained by injecting blood of one species does not effect lysis of the blood of another species in the presence of complement. In the case of closely allied species, however, there is some effect ; the immune body got by injecting a rabbit with ox-corpuscles will cause lysis of sheep's blood in the presence of complement. As has been mentioned, the immune body is as a rule thermostable, since it resists prolonged heating at 55° to 57° C. The immune body has usually a marked affinity for the homologous blood-corpuscles, so that treatment of the immune serum with the homologous blood will deprive the serum of its action. This union of immune body occurs at 0° C., as well as at room-temperature or 37° C. If the blood after contact with the immune serum is repeatedly washed by centrifugalising with NaCl-solution and is then re-suspended, it is found that it undergoes lysis in the presence of complement at 37° C. Thus the blood-corpuscles whose general physical properties may not have been altered at all by the immune serum, have been *sensitised* to the action of complement.

The term *Complement* designates, so far as we are concerned, merely a property of fresh serum ; whether it is a substance at all is thus far unknown. In order to obtain complementing action it is not necessary to use the serum of the same species of animal as that which furnished the immune body. This statement holds good with certain restrictions. Thus, normal guinea-pig's serum is most frequently used to complement the immune body from the rabbit for sheep's or ox's blood. The reason is that the guinea-pig's serum is active in very

small amounts, ten times as much rabbit's serum as guinea-pig's being required, on the average, to cause haemolysis of a certain amount of ox-blood in the presence of immune body. As Muir and Browning have shown, this is due to the fact that the complement molecules in a given volume of guinea-pig's serum are more numerous than in the same volume of rabbit's serum. In contrast to immune body, complement has almost no affinity for the red blood-corpuscles by themselves. The complementing power of most sera disappears rapidly on heating at 55° to 57° C., exposure to this temperature for one hour being sufficient to deprive guinea-pig's or rabbit's serum entirely of its complementing power. Even at room-temperature the complement deteriorates apart from bacterial contamination, and exposure to bright light is harmful. Complement acts most energetically at 37° C. ; at room-temperature the action is slower and at 0° C. it usually ceases altogether.

Certain normal sera may possess a lytic action on a particular species of blood, e.g. fresh guinea-pig's serum frequently causes lysis of ox-blood, and in the case of some guinea-pigs this action is marked. Such normal lytic action is also due to the co-operation of small amounts of a natural immune body with the serum-complement. The serum may be freed from its lytic power by taking advantage of the fact that at 0° C. the natural immune body is anchored by the blood-corpuscles, but at that temperature the complement does not combine ; so that after treating the serum with blood-sediment at 0° C. and then centrifugalising, all the complement is found in the supernatant fluid, while the immune body has been bound to the sediment.

From the practical as well as the theoretical point of view, an important property of immune body and of complement is that they react quantitatively along with the blood-corpuscles. If a certain amount of guinea-pig's complement, say 0·01 c.c., is necessary to produce just complete lysis of 1 c.c. of a 5 per cent. dilution of ox-blood, along with immune body, then 0·02 c.c. of complement will be necessary to lyse 2 c.c. of the same dilution of ox-blood, and so on. The same holds good for the immune body. The immune body and the complement can thus be titrated to determine their haemolytic values.* In such titration-experiments the standard

* Fractions of a dose of immune body or of complement do not necessarily produce a corresponding amount of haemolysis. ¶

amount of blood-suspension employed is 1 c.c. of a 5 per cent. dilution of the full blood. It is found that the amount of either immune body or complement which is necessary to produce complete lysis depends to a certain extent on the amount of the other component which is present. Thus, the amount of immune body required is a minimum when an excess of complement is present, and vice versa. In general, with ox-blood, guinea-pig's complement and immune body from the rabbit, the *minimum haemolytic dose of immune body* (*M.H.D. of IB.*) is the amount necessary to cause just complete lysis of 1 c.c. of 5 per cent. blood-suspension in one hour at 37° C. in the presence of four or five doses of complement. Similarly, the *minimum haemolytic dose of complement* (*M.H.D. of C.*) is the amount necessary to cause just complete lysis of 1 c.c. of 5 per cent. ox-blood in one hour at 37° C. in the presence of four or five doses of immune body.

It follows from what has been said regarding immune body and complement, that a mixture of red blood-corpuscles along with one of these substances constitutes a test for the other. Accordingly, *if red blood-corpuscles sensitised with the corresponding immune body undergo lysis in a particular fluid, while unsensitised corpuscles do not, we can conclude that free complement was present in the fluid.* In the process of haemolysis complement is used up, and as Muir has shown, much more complement may be used up than is necessary for the production of lysis. Thus, if two doses of complement are added to one cubic centimetre of blood-suspension, sensitised by the addition of four or five doses of immune body and then after incubation at 37° C. for a suitable time (1½ hours), when haemolysis has occurred, a further quantity of one cubic centimetre of sensitised blood-suspension is added, no lysis of the latter occurs; whereas if the two cubic centimetres of sensitised blood had been added to the complement all at once the whole would have undergone complete lysis. That is, the first portion of blood in the process of haemolysis has absorbed all the complement and has rendered it unavailable for the haemolysis of the second portion. Immune body is, on the other hand, recoverable to a certain extent after haemolysis (Muir).

Deviation of Complement

Haemolytic complement can be rendered unavailable for producing haemolysis of sensitised blood-corpuscles (fixed,

absorbed, deviated) in many other ways, however. Thus, formed elements such as tissue-cells, bacteria, also organic and inorganic particles may fix complement, and chemicals may inhibit its action (e.g. concentrated NaCl-solution) or destroy it (e.g. acids). Such action on complement may be termed *non-specific*. The amount of complement absorbed by a given suspension of bacteria may be quite inconsiderable; but, when the homologous immune serum is added to the suspension, the amount of complement now absorbed is very many times increased. This is known as *specific absorption* of complement; it is the manifestation of the reaction between the bacteria and the homologous antibodies (immunity-reaction). In the same way bacterial extracts and solutions of proteid substances absorb complement in the presence of the homologous antisera. The biological significance of the process of complement-fixation for the living organism need not be discussed here (*v.* Muir's *Studies on Immunity*).

The term *antigen* is applied to a substance (red blood-corpuscle, bacterium, proteid, etc.) which can give rise to an antibody when injected into a suitable animal. According to Ehrlich, such an antigen possesses chemical groups (*receptors*) which are the active agents in stimulating the production of the anti-substance in the body of the immunised animal. It is these receptors which *in vitro* combine with the antibody. The specificity of the antigen is due to the fact that it possesses receptors peculiar to itself. The antibody to which it gives rise will thus combine only with receptors which are similar in constitution to those which caused its production (*Ehrlich's Side Chain Theory*).

These facts form the basis of the species-identification of proteids (Neisser-Sachs). Thus, if a particular proteid, e.g. human serum, is suspected of being present in a solution the procedure is to add anti-human serum and complement; after a period of incubation sensitised blood is added. If the corpuscles undergo lysis then the complement has not been absorbed, that is to say, the anti-human serum is not the homologous antibody to the serum-proteid present: on the other hand, if the test-corpuscles remain intact, then human serum-proteid has been present, which along with the corresponding antiserum has absorbed the complement, so that it was not available for the haemolysis of the subsequently added sensitised red blood-corpuscles (Bordet-Gengou effect). The complement is said to have been *deviated*. The proteid

solution and the antiserum may each by itself exert a slight inhibitory effect on the complement; but the inhibitory effect of the combination is very much greater than the sum of the inhibitory effects of each separately.

It must be remembered that tests such as that described above are not qualitative, but strictly *quantitative*, and that it is only by carrying out the experiments under quantitative conditions that a conclusive result can be obtained. Thus in the above example it can be said generally that in whatever proportion antihuman serum is mixed with a non-homologous serum, deviation of complement will fail to occur; but on the other hand, it is only when the antibody is mixed with the homologous serum in particular proportions that deviation of complement will occur. The term "*zone-phenomenon*" is applied to the inhibition of complement-fixation which may occur when one of the reacting bodies (antigen or anti-serum) is present in excess.

It has been found that antigens can be extracted from bacteria by macerating the latter in a watery solution. Thus if the spleen from a case of enteric fever, which contains typhoid bacilli, is extracted with salt solution, typhoid antigens pass into the fluid, so that on centrifugalising a solution is obtained which along with anti-typhoid serum produces fixation of complement. It is obvious that the extract contains in addition to the typhoid receptors, a variety of tissue-products. The important fact, however, is that the anti-typhoid serum leads to the detection of the specific typhoid antigen in the mixture, owing to the fact that deviation of complement occurs.

The course of events in a complement-deviation experiment may be graphically represented thus :—

Antigen + Antibody + C ⋮ + O + IB ⋮ = no lysis.

(the circle indicates the red blood-corpuscles, and the dotted line a period of incubation at 37° C.).

The Biological Syphilis Reaction of Wassermann, Neisser and Bruck

Proceeding on the analogy of the extraction of typhoid antigens from the spleen in enteric fever, Wassermann, Neisser, and Bruck[1] argued that the watery extract of an organ containing the spirochaete pallida along with the serum of a syphilitic subject which might be expected to contain antibodies

to the spirochaetes, ought to deviate complement. They prepared a watery extract of the liver of a syphilitic foetus which contained a large number of spirochaetes, and they found that as a matter of fact this extract along with syphilitic serum deviated complement, whereas along with normal sera absorption of complement did not occur. The specificity of the reaction appeared to be further supported by the fact that a similarly prepared extract of a normal liver did not deviate complement with syphilitic serum under the same conditions. Accordingly, it was argued that the essential factor in the extract of the syphilitic liver was the presence of spirochaete-receptors. It was found, however, that the extraction of normal tissues for a prolonged period or in an alkaline watery solution yielded an efficient "antigen" (*v.* Bruck [2]).

A great advance was made both from the theoretical and the practical aspect, when it was discovered (Porges and Meier [3]; Landsteiner, Müller and Pötzl [4]; Levaditi and Yamanouchi [5]) that alcoholic extracts of normal as well as of syphilitic tissues had the property of deviating complement in the presence of syphilitic serum. Accordingly, it appeared that the reaction did not correspond in the ordinary biological sense to that between syphilitic antigen and antibody. A watery alcoholic tissue-extract obviously contains a very complicated mixture of substances, both organic and inorganic, consequently the nature of the reacting bodies in the extract remained undetermined. The fact that the "antigenic" property is possessed by alcoholic extracts led to the supposition that the active agents are of lipoid nature, and a large number of lipoids and allied compounds have been investigated. These are referred to in more detail later (*v.* chap. VI.). We need only mention here lecithin and cholesterin. Lecithin by itself has a comparatively weak antigenic action. It has been shown, however, by Browning, Cruickshank and Mackenzie that the addition of cholesterin to lecithin causes an increase in the amount of complement absorbed in the presence of syphilitic serum, but not in the presence of normal serum under the same conditions. Thus lecithin and cholesterin constitute a very delicate and reliable test for the detection of syphilitic sera (the practical application of the lecithin-cholesterin method is described in chap. IV.).

In conclusion, *the biological syphilis-reaction depends on the fact that syphilitic serum in the presence of certain lipoid tissue-*

constituents leads to the absorption of an amount of complement in excess of the total of the amounts absorbed by the serum and the tissue-constituents by themselves. The reaction is apparently quite analogous to that which occurs when complement is added to the mixture of an antigen with its corresponding antibody ; but while it is convenient to speak of the organ-extract as "antigen," it is understood that *the syphilitic reaction is not due to an antibody to the specific infecting agent (spirochaete pallida), acting along with the receptors of the latter.* It is quite possible that, as Wassermann insists, when a watery extract of syphilitic tissue rich in spirochaetes is employed, there is a reaction between spirochaete receptors and specific anti-substances in the patient's serum ; but as Muir [6] points out the fact of greatest importance is that after infection with syphilis the serum acquires the power of reacting with certain bodies of known constitution in such a way as to bring about fixation of complement.

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CHAPTER II

METHOD OF CARRYING OUT THE SYPHILIS REACTION

THE ORIGINAL METHOD—MODIFIED METHOD CONSISTING IN THE ACCURATE ESTIMATION OF THE AMOUNT OF COMPLEMENT-DEVIATION — SUBSTANCES EMPLOYED IN THE REACTION : THE HAEMOLYTIC SYSTEM, BLOOD CORPUSCLES AND IMMUNE BODY : THE SERUM COMPLEMENT : THE FLUID TO BE TESTED, SERUM AND CEREBRO-SPINAL FLUID : THE TISSUE EXTRACT (" ANTIGEN ").

Original Method

THE original method of carrying out the syphilis reaction, which was described by Wassermann, Neisser and Bruck, is essentially as follows : five reagents are required—

(1) the patient's serum, recently drawn off and heated for half an hour at 55° C. just before the experiment ;

(2) watery extract of congenital syphilitic liver (*v.* p. 21) ;

(3) complement, freshly drawn guinea-pig's serum ;

(4) haemolytic immune body for sheep's blood obtained from the rabbit (minimum haemolytic dose 1 : 2000 –1 : 3000) ;

(5) 5 per cent. suspension of washed sheep's blood.

The haemolytic dose of immune body is on each occasion determined just before the experiment, with the same blood and complement in the same amounts as are to be employed in the test.

The following series of tubes are set up :

No.	Content			
1	0·2 c.c. patient's serum (55° C.)	+ organ-extract 0·1–0·2 c.c.	+ complement 0·05–0·1 c.c.	+ 1 c.c. 5 per cent. sheep's blood + 2–4 D. of IB.
2	0·2 c.c. patient's serum (the same amount as in No. 1)	—	+ complement 0·05–0·1 c.c.	+ „
3	0·4 c.c patient's serum (double the amount in No. 1)	—	+ complement 0·05–0·1 c.c.	+ „
4	—	organ-extract 0·1–0·2 c.c. (the same amount as in No. 1)	+ complement 0·05–0·1 c.c.	+ „
5	—	organ-extract 0·2–0·4 c.c. (double the amount in No. 1)	+ complement 0·05–0·1 c.c.	+ „
6	0·2 c.c. normal human serum (55° C.)	+ organ-extract 0·1–0·2 c.c.	+ complement 0·05–0·1 c.c.	+ „
7	—	—	+ complement 0·05–0·1 c.c.	+ „

The dotted lines each indicate incubation at 37° C. for 1 hour.

It is arranged that the total volume in each tube shall be the same (5 c.c.). Thus each reagent is, where necessary, diluted so that the required amount is contained in 1 c.c. and in the controls saline is added in place of the omitted components.

A positive result is obtained when there is no lysis of the test blood-corpuscles in tube No. 1; at the same time all the controls (tubes No. 2–7) must be completely lysed.

The blood-corpuscles are sensitised with immune body a quarter of an hour before they are to be added to the tubes.

The amount of organ-extract is arbitrarily chosen as the result of a preliminary examination of its behaviour in varying amounts along with 40 nonsyphilitic and 40 (secondary) syphilitic sera (Bruck [2]).

A modification which has found very general acceptance is the employment of an alcoholic instead of a watery organ-extract (*v.* p. 23).

Modified Method Consisting in the Accurate Estimation of the Amount of Complement-Deviation

It has been pointed out in the preceding chapter that the biological syphilis-reaction depends on the fact that haemolytic complement is rendered inactive by a mixture of syphilitic serum and an extract of certain tissue-constituents. The essential characteristic of the reaction is that more complement is absorbed by a mixture of syphilitic serum and tissue-extract than the sum of the amounts absorbed by these two substances alone. In view of the fact that different specimens of complement vary in their capacity for being fixed by the mixture of serum and organ-extract, and also that the controls as carried out by the above method do not really give precise information as to the behaviour of the various reagents, we have considered it advisable to employ a method whereby it is possible to estimate the actual amounts of complement absorbed, in the first place by the serum and tissue-extract alone, and again by these two substances combined; the complement absorbed should be measured in terms of haemolytic doses. As Muir[6] says, the method takes a little longer and requires more serum than the one tube method; but it has the great advantage of showing exactly the degree of deviation in each case, and thus gives greater confidence to the observer.

For this purpose three series of tubes * are employed: series A, containing in each tube 0·6 of a cubic centimetre of emulsion of organ-extract (*v.* p. 25); series B, containing 0·05 of a cubic centimetre of the serum to be tested (previously heated for half an hour at 55° C., *v.* p. 19) in 0·6 of a cubic centimetre of 0·85 per cent. NaCl solution; and series C, containing the mixture of the test-amount of emulsion of organ-extract (0·6 c.c.) and of serum (0·05 c.c.). The complement-containing serum is then added: to series A and B, 1, 2, 3, 4, etc., minimum haemolytic doses, and to series C, 5, 7, 10, 14, 20, 28, 40, etc., doses.† It is advisable to make a preliminary estimation of the

* Small test-tubes measuring 3″ by ½″ or 4″ by ½″ are most satisfactory.

† For measuring the sera, etc., the following pipettes are employed: (1) 0·1 c.c. pipettes of thermometer-tubing; external diameter $\frac{1}{16}$ in.; the length of the column of fluid corresponding to 0·1 c.c. is $6\frac{1}{8}$ in. The pipette tapers for the last $\frac{3}{4}$ inch and the external diameter at the orifice is $\frac{5}{64}$ in. They are graduated to 0·002 c.c. (2) 1 c.c. pipettes; external diameter $\frac{1}{4}$ in.; length of column of fluid corresponding to 1 c.c. = $8\frac{1}{2}$ in. External diameter at orifice $\frac{1}{16}$ in.; they

haemolytic dose of the complement (*v.* p. 19), and this is further controlled by incubating amounts of complement in 0·6 c.c. NaCl solution along with the above series. After one and a half hours at 37° C. the test-corpuscles, one cubic centimetre of a 5 per cent. suspension of washed ox-blood sensitised a quarter of an hour previously with at least five haemolytic doses of immune body from the rabbit, are added. The tubes are again placed in the incubator, and they are shaken every twenty minutes. The result is read at the end of one and a quarter hours, when the tubes are removed from the incubator. After standing overnight at room-temperature the reading is again taken; both results are almost identical in every case.* The accompanying example gives the details of an experiment performed according to this method, showing the comparative deviating properties of a syphilitic and a normal serum. In this experiment the amount of complement necessary to lyse one cubic centimetre of sensitised ox blood-corpuscles, i.e. the dose, was 0·01 cubic centimetre.

Series A shows that, whereas 0·01 cubic centimetre of complement is sufficient to lyse 1 cubic centimetre of sensitised ox's red blood-corpuscles, 0·02 cubic centimetre of complement (two doses) is necessary when 0·1 cubic centimetre of alcoholic extract of syphilitic liver is present; that is to say, 0·6 cubic centimetre of the emulsion of organ-extract is by itself able to inhibit † one dose of haemolytic complement.

Series B shows that as regards power of complement-absorption the heated syphilitic and the normal serum are alike, each by itself inhibiting † one dose of haemolytic complement.

Series C shows that the syphilitic serum in presence of the liver-extract has absorbed over thirty doses of complement,

are graduated to 0·01 c.c. Both kinds of pipettes are graduated to the orifice. For use these pipettes are attached by indiarubber tubing to a glass mouthpiece. The pipettes can be obtained from Messrs. Thomson, Skinner and Hamilton, 38, Sauchiehall Street, Glasgow.

* When a number of sera are being examined simultaneously, for purposes of economy half amounts of all the reagents may be employed without affecting the accuracy of the test.

† Where a substance has a certain degree of inhibitory action on complement, it is convenient to express this quantitatively by saying that the substance absorbed or inhibited a number of doses of complement which is one less than the number required to cause just complete lysis. Thus, if three doses of complement are required to produce just complete haemolysis in the presence of organ-extract, the organ-extract is said to inhibit or absorb two doses of complement.

whereas the normal serum with the same extract has not been able to absorb two and a half doses.

EXAMPLE

SHOWING THE COMPARATIVE DEVIATING PROPERTIES OF A SYPHILITIC AND A NORMAL SERUM ALONG WITH ALCOHOLIC EXTRACT OF SYPHILITIC LIVER.

Series A.	(1)	(2)	(3)	(4)		
Emulsion of extract of syphilitic liver	0·6	*0·6*	*0·6*	*0·6*		
Complement	0·01	*0·02*	*0·03*	*0·04*		
Sensitised ox red blood-corpuscles (added after 1½ hours)	1·0	*1·0*	*1·0*	*1·0*		
Series B.	(5)	(6)	(7)	(8)		
Syphilitic serum* (in 0·6 c.c. normal saline)	0·05	*0·05*	*0·05*	*0·05*		
Complement	0·01	*0·02*	*0·03*	*0·04*		
Sensitised ox red blood-corpuscles (added after 1½ hours)	1·0	*1·0*	*1·0*	*1·0*		
	(9)	(10)	(11)	(12)		
Normal serum* (in 0·6 c.c. normal saline)	0·05	*0·05*	*0·05*	*0·05*		
Complement	0·01	*0·02*	*0·03*	*0·04*		
Sensitised ox red blood-corpuscles (added after 1½ hours)	1·0	*1·0*	*1·0*	*1·0*		
Series C.	(13)	(14)	(15)	(16)	(17)	(18)
Liver extract emulsion	0·6	0·6	0·6	0·6	0·6	0·6
Syphilitic serum*	0·05	0·05	0·05	0·05	0·05	0·05
Complement	0·025	0·05	0·1	0·15	0·2	0·3
Sensitised ox red blood-corpuscles (added after 1½ hours)	1·0	1·0	1·0	1·0	1·0	1·0
	(19)	(20)	(21)	(22)	(23)	(24)
Liver extract emulsion	*0·6*	*0·6*	*0·6*	*0·6*	*0·6*	*0·6*
Normal serum*	*0·05*	*0·05*	*0·05*	*0·05*	*0·05*	*0·05*
Complement	*0·025*	*0·05*	*0·1*	*0·15*	*0·2*	*0·3*
Sensitised ox red blood-corpuscles (added after 1½ hours)	*1·0*	*1·0*	*1·0*	*1·0*	*1·0*	*1·0*

* The sera were heated for half an hour at 55° C.
Italics mean that lysis has occurred in this tube.
Dose of complement = 0·01 c.c.

As the result of the examination of a large number of cases, and the repeated examination of the same sera under different conditions, *the reaction has been arbitrarily defined as positive when lysis is incomplete with five haemolytic doses of complement in addition to the sum of the amounts inhibited by serum and by organ-extract alone.* This arbitrary definition of a positive result, as obtained by the foregoing method, is subject to the considerations dealt with in the next chapter, where it is

pointed out that variations in the individual properties of the reagents employed may give rise to anomalous results. Of course, in many cases the mixture of serum and organ-extract absorbs an amount of complement so greatly in excess of the sum of the amounts absorbed by each component separately that the positive result of the reaction is quite undoubted.

Substances Employed in the Reaction

In carrying out the test attention should be paid to the following practical points bearing on the various substances used in the reaction :

(1) The haemolytic system. (2) The serum-complement. (3) The serum or cerebro-spinal fluid to be tested. (4) The tissue extract or "antigen."

The Haemolytic System

A haemolytic system which has been employed with very satisfactory results is that in which *ox-corpuscles* are sensitised with the homologous immune serum from the rabbit, fresh guinea-pig's serum being used as complement. Five cubic centimetres of defibrinated ox blood * are mixed with two to three volumes of 0·85 per cent. NaCl solution and centrifugalised ; the supernatant fluid is then pipetted off and saline is again added and the suspension again centrifugalised : after the third washing, the sediment, which should measure about three cubic centimetres, is made up to 100 cubic centimetres with 0·85 per cent. NaCl solution. The blood-suspension is sensitised by adding five minimum haemolytic doses of immune body, which is the optimum sensibilisation when fresh guinea-pig's serum is used as complement. As the amount of sediment of red blood-corpuscles obtained from different specimens of blood varies, a convenient method is to make the suspension by adding 3 c.c. of sediment to 97 c.c. of saline.

Immune body is the serum of a rabbit which has received several intraperitoneal injections of washed ox-blood. A rabbit which has received injections of four, five, and six cubic centimetres of blood sediment at intervals of a week usually yields ten days after the last injection an antiserum of which

* The blood is received into a sterile bottle and defibrinated with a sterile wire switch ; it will remain fresh for almost a week if the bottle is kept on ice and the amount for an experiment is withdrawn and washed as required.

about 0·001 to 0·002 cubic centimetres is the minimum haemolytic dose, that is, this amount of the serum of the treated rabbit is sufficient to lyse one cubic centimetre of a 5 per cent. suspension of ox-blood corpuscles after one hour at 37° C. in the presence of an excess of complement. A small amount of blood for a preliminary test is withdrawn from the rabbit's ear and is switched and centrifugalised, or allowed to clot spontaneously. The serum is heated for an hour at 55°–57° C. before the immune body is titrated. If the serum is sufficiently active the animal is then bled from the carotid artery.

To estimate the minimum haemolytic dose of the immune serum the following procedure is adopted: three or four cubic centimetres of fresh complement-containing serum from a guinea-pig are, to begin with, treated for an hour at 0° C. with an equal volume of washed ox-corpuscles to remove any natural immune body which may be present, as it would prevent an accurate titration. This is effected by immersing the serum and the blood-sediment first of all in separate tubes in chopped ice; after five minutes, when both are cooled, they are mixed. Separation is brought about by rapid centrifugalising, and the treated complement is then pipetted off. To a series of tubes each containing one cubic centimetre of a 5 per cent. suspension of ox-corpuscles, 0·05 cubic centimetre of the treated guinea-pig's serum is added (that is, an excess of complement representing from 5 to 10 haemolytic doses for fully sensitised corpuscles). There are next added varying amounts of the immune serum to be tested, from 0·0001 c.c. to 0·0025 c.c. Accurate measurement is effected by diluting the immune serum a hundred times. The minimum haemolytic dose of the immune serum is the amount which is present in the tube where lysis is just complete after incubation for one hour at 37° C. It has been found that the minimum haemolytic dose of the same immune serum may vary considerably for different samples of ox-blood; the serum should be tested against ox-corpuscles from four or five different sources, and an average taken. As the haemolytic power of the immune serum may apparently fall somewhat within the first few days after it has been withdrawn, the determination of the average dose should be made a week after the rabbit has been bled. The average minimum haemolytic dose of the immune serum where the process of immunisation has been successful is about 0·0001 c.c. but it may be considerably less; if the dose is higher than 0·0025 c.c. the rabbit

should receive further injections of blood until the serum becomes sufficiently powerful. Some animals, however, are refractory, while others again yield a very active immune body after two injections of blood. The serum is conveniently stored in lengths of quill-tubing which are drawn out at the ends ; about one cubic centimetre is drawn up into each tube, and this is sealed and heated at 57° C. for an hour on three or four successive days, so as to sterilise it and to destroy the complement. The immune body then usually remains fairly constant for some months when kept in the dark at room-temperature or in the ice-chest.

Sheep's blood may be employed instead of ox blood. The procedure of obtaining the corresponding immune body, etc., is, of course, the same as that described. Gilmour (unpublished observations) has carried out a series of complement-deviation experiments with syphilitic sera, employing for the test sensitised sheep's blood ; at the same time, by way of control, parallel series were sent up in which the test was made with sensitised ox blood. The results were practically identical with the two haemolytic systems.

The Complement

The complement is contained in the fresh serum of a guinea-pig. The hair of the animal's neck is cut or shaved, and after cleansing with a pad of cotton wool moistened with salt solution, the blood is obtained by cutting the vessels transversely with a single sweep of a very sharp knife ; it is collected in a sterile glass cylinder, into the mouth of which the narrow end of a sterile glass funnel dips. The wide end of the funnel should be about 7 or 10 in. in diameter ; it serves to catch the blood flowing from the severed vessels. The clot must usually be separated from the sides of the glass, which should have been previously washed out with sterile normal saline solution. The serum is allowed to separate for 18 to 24 hours at room-temperature, and is then pipetted off ; if preserved for further use on succeeding days it should be frozen or kept on ice. The most reliable results are obtained with complement used on the first and second days after obtaining the blood ; this question will be dealt with more fully in a succeeding chapter (p. 28). The serum may also be separated from the blood by switching immediately after bleeding the animal, and then centrifugalising ; but, as has been indicated already,

such serum should be allowed to stand at room-temperature or in the ice-chest overnight before use.

Inasmuch as the method of performing the test is based on the principle of measuring the actual amount of the complement absorbed, it is advisable to make a preliminary estimation of the haemolytic dose of the complement-containing serum. This is done by taking a dilution of one part of serum with three parts of normal saline solution, and of this 0·01, 0·02, 0·03, 0·04, 0·05, 0·06, 0·08 c.c. are added to a series of tubes each containing one cubic centimetre of blood suspension to which 5 doses of immune body have been added at least a quarter of an hour previously (for the measurement of these amounts, the 0·1 c.c. capillary pipette graduated to 0·002 c.c. is employed). The tubes are then placed at 37° C., and after shaking every quarter of an hour, the amount of complement in the tube, which is just clear at the end of an hour, is taken as the dose for the subsequent estimation. It is to be noted that not infrequently the complement-content falls slightly in the control tubes, where the serum is incubated with salt solution prior to adding the test-corpuscles. Variations in the individual properties of the complement-containing serum, and the fallacies to which these variations may give rise, are treated in detail later (p. 31).

The Fluid to be tested, Serum or Cerebro-Spinal Fluid

The *blood* to be examined is most conveniently obtained by puncturing a vein with an ordinary 10 c.c. serum-syringe. The following procedure is recommended; a rubber tourniquet with a clip (or several turns of a cloth bandage) is fixed round the upper arm so as to obstruct the venous flow, but leaving the arterial pulse at the wrist; the flexure of the elbow is cleansed and sterilised, and the most superficial and prominent vein is chosen; it is advisable to enter the vein from the front so that the needle makes an angle of about 45 degrees with the line of the vein. The grooved side of the needle point is turned away from the skin, and the needle is of course inserted in the direction opposite to that of the blood stream in the vein. In the case of an adult the diameter of the lumen of the needle should be about $\frac{1}{32}$ in.; the point should be sharp, and about $\frac{1}{16}$ in. long. In the case of children, or where the veins are small, a proportionately smaller needle may be necessary. After drawing off the blood the tourniquet is removed and the arm elevated. As

a rule it is unnecessary to apply a bandage to the punctured wound, a piece of cotton wool and collodion being sufficient. We have taken blood in this way over a thousand times, and are convinced that it is not more difficult for the operator or inconvenient for the patient than puncture of the lobe of the ear for an ordinary haematological examination.

Although 10 c.c. of blood is more than is necessary for an ordinary examination, it is well to have at least 5 c.c., so that if necessary the test may be repeated without again resorting to puncture. The blood is immediately put into a sterile test tube, previously rinsed out with sterile salt solution, and allowed to coagulate; if in the process of drawing up the blood into the syringe there is an entrance of air with frothing, it is advisable not to empty the frothy material into the test-tube, as it renders separation of the clot more difficult. The clot should be separated from the side of the tube. After 12 to 24 hours the serum is pipetted off and centrifugalised, and then heated at 55°–57° C. for half an hour immediately before testing; this exposure to heat diminishes somewhat the deviating power of a syphilitic serum, but it is necessary in view of the fact that a considerable proportion of sera in other diseases react positively when unheated, though negatively when heated. Again, there are sera which, when preserved for some time, acquire the power of inhibiting complement of themselves; this power is destroyed on reheating at 55°–57° C. for half an hour. The fallacies arising from neglect to heat the sera are dealt with more fully in chapter VI.

The experience derived from a large series of cases has shown that 0·05 c.c. of serum is in general the optimum amount for eliciting the Wassermann reaction (*v.* p. 84).

In certain nervous diseases the *cerebro-spinal fluid* is examined for the syphilitic reaction. There are several points of practical importance which may be noted in performing lumbar puncture. Where the patient is very restless and excited it is advisable to give a small amount of chloroform, just sufficient to dull the senses without producing complete anaesthesia; this necessity does not often occur in cases where a syphilitic test is to be performed, but where necessary it may be resorted to with great advantage both to the patient and operator, as was found by Mackenzie and Martin in dealing with cases of cerebro-spinal meningitis. A local anaesthetic such as ethyl chloride is useless. A well-tempered sharp-pointed hollow steel needle is used. For an adult the needle

should be at least 3 in. in length, and the diameter of the lumen $\frac{1}{16}$ in.; for a child a proportionately shorter and narrower needle suffices. The patient is placed on his side, with the knees drawn up and the uppermost shoulder slightly inclined forwards so that the lumbar concavity is straightened out. The skin should be carefully sterilised, also the hands of the operator and the needle. To determine the site of puncture, a spinous process on a level with the iliac crest is chosen; the needle is inserted slightly below the process in the middle line and is directed slightly upwards with a firm thrust through the skin and superficial tissues, but afterwards more slowly in the direction of the canal. If too great force be used in the case of muscular patients who are struggling, the body of a vertebra may be punctured, giving rise to considerable pain and bleeding. If the needle becomes blocked it should be cleared by means of a stilette; if the fluid be not obtained even after clearing with the stilette, it is advisable not to probe further with the needle, but to withdraw it and puncture in a neighbouring interspace. Headache and slight sickness may follow the drawing off of fluid, especially when it has been under considerable pressure. With due precautions, however, the operation is simple and harmless. From advanced cases of general paralysis 50 to 60 c.c. of fluid may be withdrawn; from cases of severe epilepsy 30 to 40 c.c.; from cases of locomotor ataxy and chronic nervous disease 20 to 25 c.c.

A specimen of cerebro-spinal fluid which is to be examined for the syphilitic test should be free from blood, and if cellular elements are present, it should be centrifugalised. The cerebro-spinal fluid possesses as a rule weaker deviating powers than the corresponding blood-serum. It should not be heated before use, as it does not contain haemolytic complement, and as fresh cerebro-spinal fluids from cases other than syphilis do not react positively. It is further necessary to use a larger amount of the cerebro-spinal fluid—at least 0·4 c.c. instead of 0·05 c.c. as in the case of the blood-serum. Further details as to the procedure to be adopted in carrying out tests, with specimens of cerebro-spinal fluid, are given later (*v.* p. 134).

The Tissue-Extract ("Antigen")

Watery Extracts.

Syphilitic Organs.—The extract originally recommended

by Wassermann, Neisser, and Bruck,[1] was that made by macerating congenital syphilitic liver, finely minced or ground with sand, in 0·85 per cent. NaCl solution containing 0·5 per cent. of carbolic acid, in the proportion of four parts of fluid to one of tissue. Extraction is allowed to proceed for 24 hours at room-temperature in the shaking machine. The clear fluid is obtained, preferably by decanting, as Bruck[2] has found that centrifugalising tends to yield a less efficient extract. It is kept in the ice-chest and is not exposed to air or light. According to the majority of workers the disadvantages of watery extracts are, (1) a tendency to become highly anti-complementary by themselves on keeping, or (2) loss of power to react with syphilitic sera. These changes may occur comparatively suddenly and have led to the general discarding of watery extracts. Citron,[3] however, finds that the watery extract preserves its properties excellently, provided that it is kept continually in the ice-chest (not frozen), being removed only for the time necessary to enable the amount required for the day's tests to be pipetted off. The precipitate which forms must not be shaken up. Wassermann[4] still adheres to the use of the watery extract. Marie and Levaditi,[5] and also Bruck[2] find that the watery extract keeps well in the dry state. It is evaporated in vacuo over H_2SO_4 and kept in sealed tubes. Bruck dissolves 0·1 gram of the dried extract in 1 c.c. of distilled water before use. Watery extracts may have a haemolytic action by themselves on the test-corpuscles.

The amount of watery extract recommended for use is 0·1 to 0·2 c.c. along with 0·1 to 0·2 c.c. serum. If the mixture absorbs entirely the complement of 0·1 c.c. of guinea-pig's serum the reaction is positive (more recently 0·05 c.c. of complement-containing serum has been employed). In the controls, double the amount of serum and of antigen separately should fail to absorb the complement, so that complete lysis of the test corpuscles occurs (*v.* p. 12).

Normal Organs.—It has been found (Marie and Levaditi,[5] and Bruck[2]) that extracts from normal organs prepared as described above are inefficient as syphilitic "antigens." Prolonged extraction with salt solution (3 days) or the addition of KOH 1 : 5,000 to 1 : 10,000, and extraction for 24 hours have been found to yield active extracts from normal organs. In these ways Bruck obtained efficient extracts from normal monkey's spleen.

Alcoholic Extracts.

These are usually prepared by adding four parts of 96 per cent. alcohol to one part of the minced organ. Extraction is allowed to proceed for from 6 to 24 hours or longer, and the clear extract is then filtered through paper and kept in the dark in stoppered bottles ; the extraction may be facilitated by using a shaking apparatus. For the filtration, fat-free paper should be employed, or the paper should be washed first of all with ether and alcohol, so as to remove haemolytic substances which may be present.

With regard to the tissue which should be employed to obtain the most satisfactory "antigen," there is a variety of opinions. Apparently almost any animal-tissue will yield an alcoholic extract which is capable of causing deviation of complement in the presence of syphilitic serum. The question then arises as to what extract will enable the differentiation of syphilitic from non-syphilitic sera to be made in the largest number of cases. To begin with, congenital syphilitic liver was most employed and Citron [3] considers that the extract is enhanced in efficiency if autolysis has occurred beforehand. Guinea-pig's heart has been recommended by many workers as next to syphilitic liver in efficiency. A disadvantage of using tissue from a small animal is, of course, the limited amount which is available. We have regularly employed an extract made by digesting minced ox-liver, obtained within three or four hours after death, in the proportion of one part with four parts of 95 per cent. alcohol for three or four days at room-temperature, the mixture being stirred up at least once a day. After filtering there separates out from the solution in the course of several days a slight deposit which is allowed to remain undisturbed when the clear fluid is pipetted off for use. This extract keeps very well for many months and consequently may be made up in large amount. Thus the extract can be kept a constant over a long period. It is best kept at room-temperature in the dark.

The properties of an alcoholic organ-extract which have to be considered when it is used as syphilitic "antigen" are the following: (1) its haemolytic action by itself, (2) its anti-complement effect by itself, (3) its power of causing absorption of complement in the presence of syphilitic serum. These functions are to a great extent correlated and therefore are best considered together. Thus, in general, a liver-extract

which is strongly haemolytic by itself is also by itself markedly inhibitory to complement. In the presence of syphilitic serum such a haemolytic and anti-complementary extract causes a greater absorption of complement than a non-haemolytic extract. Probably the haemolytic and anti-complement functions depend to a great extent on autolysis of the tissue prior to extraction. Thus organs which have undergone aseptic autolysis yield extracts which are highly haemolytic (Korschun and Morgenroth,[7] Levaditi,[8] Browning, Cruickshank and Mackenzie). The same result follows if organs such as liver, kidney or heart, are heated at 57° C. for several days before extraction. The artificial extract of Sachs and Rondoni[9] seems to imitate to some extent the constitution of an extract of an autolysed organ, as it contains oleate of soda and oleic acid in addition to lecithin. We have found that in contrast to the other organs, the brain of the guinea-pig when autolysed or heated at 57° C. may yield an extract which has little haemolytic action and which, while only slightly anti-complementary in itself, produces very marked absorption of complement along with syphilitic serum. This result is no doubt to be attributed to the content in cholesterin, which we have found has a powerful action in increasing the deviation of complement which occurs with lecithin in the presence of syphilitic serum. At the same time, as is well known, cholesterin inhibits the haemolytic effect of bodies such as oleic acid and lecithin. Heated pig's brain, however, failed to give an efficient extract under the same conditions. In general it may be said that those extracts which by themselves exert a powerful haemolytic and anti-complement action should be avoided. It is true that they produce very marked complement-deviation with syphilitic sera; but their use is inadvisable because (1) it is impossible to estimate their effect by themselves on complement, and (2) they may give what is apparently a positive reaction with certain normal sera. These points are discussed in detail in Chapter III.

We have found that satisfactory extracts in the amount used, viz. 0·1 c.c. extract plus 0·5 c.c. salt solution, caused no lysis of the test-corpuscles after 2 to 3 hours at 37° C.; after standing for 18 hours longer at room-temperature lysis may be slight or more or less complete. M. Stern[10] has pointed out that the red blood-corpuscles of different individuals vary in their susceptibility to the haemolytic action of the extract. We can confirm this; the above result, however, indicates

our general experience. Along with the test amount of extract of fresh ox-liver by itself three or four doses of guinea-pig's complement are usually required to cause complete lysis of the test-corpuscles, that is to say, the extract by itself has always a certain inhibitory effect on the complement, which, however, may show wide variations. This is dealt with further in Chapter III.

Preparation of the Watery Emulsion of Alcoholic Extract.

One part of the alcoholic organ-extract is emulsified in five parts of NaCl solution. Sachs and Rondoni [11] have found that when the emulsion of alcoholic extract is prepared by adding the extract rapidly to the salt solution and mixing as quickly as possible, a fairly clear emulsion is produced, but that when the alcoholic extract is measured off and then the salt solution is added to it slowly, with continuous shaking, a dense milky fluid is produced. It was then ascertained that the turbid emulsion deviated more complement than the clear emulsion, both when tested alone and in the presence of syphilitic serum. These authors pointed out the great importance of such differences in technique in view of their effect on the results. We have fully corroborated these findings as to the differences in the turbidity of the emulsions depending on the mode of mixture and also as to the increased deviating power of the more turbid emulsion in the presence of syphilitic serum. Working with 5 c.c. of 0·85 per cent. NaCl solution and 1 c.c. of alcoholic extract, which is the usual proportion, we have found that when the alcoholic extract is added to the salt solution it makes practically no difference whether the whole amount of extract is rapidly added and the mixture quickly shaken, or whether the salt solution is placed in a wide beaker, which is continually shaken, while the alcoholic extract is added in single drops of 0·02 c.c., the whole procedure lasting some minutes. On the other hand, if the alcoholic solution is floated on to the salt solution in a test-tube of ½-inch diameter, and the mixture is gradually effected by rotation and shaking of the tube, which is held in a slightly sloping position, a much more turbid emulsion is produced. This procedure has yielded the same results with alcoholic extracts of ox's liver, human syphilitic liver, guinea-pig's liver, and guinea-pig's muscle.

The differences in the deviating power of emulsions depending on the turbidity are well shown by the following example :—

Emulsion of 1 c.c. alcoholic extract of syphilitic liver in 5 c.c. 0·85 per cent. NaCl solution.

(*a*) Rapidly mixed: a slightly opalescent fluid.

(*b*) Extract floated on to the salt solution and gradually mixed: an opaque milky fluid.

In each tube 0·6 c.c. emulsion + 0·05 c.c. syphilitic serum (½ hour 55° C.) + guinea-pig's complement, incubated 1½ hours at 37° C.; tested with 1 c.c. suspension of ox blood + five doses of immune body; complement (18 hours old) dose = 0·005 c.c.

Amount of Complement.	Syphilitic Serum (½ hour 55° C.); 0·05 c.c.	
	(*a*) Clear Emulsion, 0·6 c.c.	(*b*) Turbid Emulsion, 0·6 c.c.
0·05 c.c.	Lysis just complete	Faint trace of lysis
0·065 c.c.	Complete lysis	↓
0·08 c.c.	,, ,,	
0·1 c.c.	,, ,,	
0·125 c.c.	,, ,,	Almost complete lysis

Emulsion alone 0·6 c.c.

(*a*) 0·01 c.c. of complement = trace of lysis.
0·02 c.c. ,, = complete lysis.

(*b*) 0·01 c.c. ,, = trace of lysis.
0·02 c.c. ,, = complete lysis.

Serum alone 0·05 c.c. + 0·6 c.c. saline + 0·01 c.c. of complement = complete lysis.

Thus, along with syphilitic serum the turbid emulsion absorbed more than twice as much complement as the clear emulsion, but each emulsion by itself inhibited the same amount of complement, namely, two doses. Accordingly, in the presence of syphilitic serum the turbid emulsion caused a relatively greater deviation of complement.

Sachs and Rondoni found that the inhibitory power of the extract by itself was more marked with the turbid than with the clear emulsion. In our experience this is usually the case when the complement from freshly shed blood is employed. When the complement has been kept for 18 to 24 hours before use, however, the difference in inhibitory effect of clear and turbid emulsions by themselves differs usually by about a dose. In the presence of syphilitic serum the difference in the amount of complement deviated with the turbid as compared with the clear emulsion is very marked with complement which is 18 to 24 hours old, as the above example shows. This

question of the influence of the age of complement is referred to further in Chapter III.

Of course, the effect of turbidity holds of necessity only when comparing emulsions of the same extract. Accordingly, *for the detection of syphilitic sera the emulsion of organ-extract should be made so as to secure the maximum possible turbidity.* This is effected as described above. The emulsion should be prepared within at most a few hours of the time of use.

The Effect of the Alcohol present in the Extract.

The alcohol present in the extract-emulsion is not an indifferent factor. It is, of course, well known that the addition of sufficient alcohol to a mixture of salt solution and complement will destroy the latter. The presence in the extract-emulsion of alcohol in an amount short of that which is actively destructive by itself for the complement, plays a part in augmenting the amount of complement deviated in the presence of syphilitic serum (Sachs and Rondoni,[11] Browning and Mackenzie.[12]) The effect of varying the amount of alcohol, the other factors being constant, is shown in the table on p. 72.

Substitutes for the alcoholic organ-extract have been suggested in order to provide a more constant reagent for use in the test. The mixtures of Sachs and Rondoni (*v.* pp. 24, 74) have a marked haemolytic action. We have found that the mixture of ox liver lecithin and cholesterin (*v.* chap. IV.) is a very efficient substitute for the alcoholic organ-extract.

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CHAPTER III

FALLACIES DUE TO VARIATIONS IN THE PROPERTIES OF COMPLEMENT-CONTAINING SERUM AND ORGAN-EXTRACT

THE COMPLEMENT-CONTAINING SERUM: HAEMOLYTIC POWER: DEVIABILITY OF COMPLEMENT, THE EFFECT OF AGE OF COMPLEMENT-CONTAINING SERUM ON DEVIABILITY, THE EFFECT OF INDIVIDUAL PROPERTIES OF THE COMPLEMENT-CONTAINING SERUM ON DEVIABILITY—THE ORGAN-EXTRACT: TURBIDITY OF THE EMULSION: HAEMOLYTIC AND ANTI-COMPLEMENT ACTION.

The Complement containing Serum

IT is customary for those who follow the technique recommended by Wassermann and his collaborators to use an arbitrarily fixed amount of complement-containing serum in the test. This amount is as a rule 0·1 c.cm. of fresh guinea-pig's serum, when 1 c.cm. of a 5 per cent. suspension of sensitised red blood-corpuscles is added, after the patient's serum along with organ-extract and complement have been in contact for an hour or an hour and a half at 37° C. This procedure, consisting as it does in observing the effect of the reagents on a fixed amount of complement, would not be open to objection were it not that the complement itself and also the extract are variable factors in the reaction. If 0·1 c.cm. of fresh guinea-pig's serum represented a constant number of haemolytic doses for the test corpuscles to be used, and if equal quantities of different samples of complement behaved similarly in the presence of the same syphilitic serum and organ-extract, then the employment of a definite amount of complement in the experiment would be the most simple and satisfactory method of carrying out the test. There is ample

evidence, however, to show that considerable variations may manifest themselves in fresh guinea-pig's serum as regards (1) the amount of the minimum haemolytic dose, and (2) the deviability of the complement by organ-extract alone, and by serum plus organ-extract, as measured in haemolytic doses. For these reasons we emphasise the importance of performing the test by the method described on p. 13.

The Haemolytic Power of the Complement-containing Serum

According to this method, by which the complement absorbed is measured in terms of haemolytic doses, it is possible to detect abnormal variations in the constituent elements employed in the test, and therefore to avoid fallacious results. The procedure adopted in estimating the haemolytic dose of complement has already been described in detail (*v.* p. 19).

It has been found that if guinea-pig's serum is kept in an ice-chest the haemolytic dose of the complement may vary very little in three or even in four days; the dose of complement from different samples of serum, however, may exhibit considerable variations; sera of the same age have, with the test-corpuscles (1 c.c. suspension of ox's blood sensitised with five doses of immune body from the rabbit), varied in dosage from 0·0025 c.cm. to 0·02 c.cm. Thus, if the complement of 0·1 c.cm. of guinea-pig's serum must be absorbed before the Wassermann reaction is regarded as positive, this amount of serum may represent in one case forty doses of haemolytic complement and in another case only five.

Deviability of Complement

The complement in different specimens of guinea-pig's serum may show great variation in deviability, and the variation in this property of the complement manifests itself as regards the inhibiting influence both of organ-extract alone, and also of organ-extract along with syphilitic serum. In this connection two factors must be taken into consideration: (1) the age of the complement-containing serum; (2) individual properties of the complement-containing serum independent of its age.

The Effect of Age of the Complement-containing Serum on Deviability.—In general, the age of the complement-containing serum is a factor affecting the deviability of the complement. After standing for upwards of 24 hours in the ice-chest,

although there is little or no change in the amount of the haemolytic dose, the complement becomes less deviable, both by the emulsion of organ-extract alone and also by the emulsion along with syphilitic serum. The following table (I) gives an example of the difference between a fresh and an old complement in this respect.

TABLE I

	Complement I (four days old).		Complement II (fresh).	
Syphilitic serum (half hour at 55° C.) 0·05 c.cm. + turbid emulsion of organ extract 0·6 c.cm.	0·096 c.cm. (12 doses)	No lysis	0·09 c.cm. (11¼ doses)	No lysis
	0·144 c.cm. (18 doses)	Trace of lysis	0·15 c.cm (18¾ doses)	,,
	0·192 c.cm. (24 doses)	Marked lysis	0·2 c.cm. (25 doses)	,,
	0·24 c.cm. (30 doses)	Lysis just complete	0·25 c.cm. (31¼ doses)	Faint trace of lysis
	—	—	0·3 c.cm. (37½ doses)	Marked lysis
	—	—	0·35 c.cm. (43¾ doses)	Lysis just complete

Extract Emulsion 0·6 c.c. + Complement I 0·016 c.c. (2 doses) = almost complete lysis.

Extract Emulsion 0·6 c.c. + Complement II 0·035 c.c. (4½ doses) = trace of lysis.

Haemolytic dose of each complement = 0·008 c.c.

In this and the following tables, unless otherwise stated, the result indicates the amount of lysis of 1 c.c. ox blood suspension sensitised with five doses of immune body from the rabbit.

Here it is seen that, although the haemolytic dose is identical with the two complements (0·008 c.cm.), the fresh complement (II) is deviated to a greater extent, both by the extract alone and also by the extract along with the syphilitic serum. In combination with the syphilitic serum 0·192 c.cm. of complement I (24 doses) gives marked lysis, whereas with complement II 0·3 c.cm. (37½ doses) is required to cause the same amount of lysis, and the same ratio holds with respect to the amounts necessary to produce just complete lysis. Accordingly, the deviating power of the syphilitic serum in the presence of the same amount of emulsion of the same organ-extract is half as great again when measured with the fresh complement

as it is with the old complement, although the haemolytic power of both complements is identical.

It is difficult to find a satisfactory explanation for the effect of age on the deviability of complement. The possibility of conversion of complement into complementoid on standing naturally suggests itself, but the fact that there is not a corresponding fall in haemolytic value makes such an explanation improbable.

In view of the fact that the complement of freshly drawn blood tends to be hypersensitive to the anticomplement action of organ-extract and of mixtures of organ-extract with normal sera, it is best to keep the complement for 18 *to* 24 *hours before use, either on ice or at room-temperature.*

The Effect of Individual Properties of the Complement-containing Serum on Deviability.—It might be supposed that when a complement was only slightly absorbed by an organ-extract or by a mixture of organ-extract and syphilitic serum, this was due to the presence in the guinea-pig's serum of a natural immune body for ox corpuscles which was specially suited to act in conjunction with the guinea-pig's complement. We have, however, shown that this is not so.

The lytic power of three complement-containing sera was tested as follows :—

Complement I (3 days old), dose = 0·01 c.cm. With extract alone + 2 doses = lysis complete.

Complement II (24 hours old), dose = 0·0075 c.cm. With extract alone + 2 doses = lysis complete.

Complement III (fresh), dose = 0·0075 c.cm. With extract alone + 2 doses = lysis complete.

Along with 1 c.cm. of unsensitised suspension of washed ox corpuscles, 0·1 c.cm. of Complement I produced only a trace of lysis ; 0·1 c.cm. of Complement II produced no lysis ; 0·1 c.cm. of Complement III produced almost complete lysis (incubation for 1¼ hours at 37° C., reading taken next day).

It was thus found that Complement III, though possessing much more natural immune body than I or II, was inhibited to as great an extent as I or II by the emulsion of organ-extract alone. Further, the natural immune body was removed from Complement III by treatment with washed ox-blood at 0° C. (0·26 c.cm. of the treated serum caused no lysis of 1 c.cm. of ox-blood suspension), yet the complement of the treated serum was not inhibited to a greater extent than that of the untreated serum.

In the same way, experiments were carried out to test whether the amount of complement absorbed by organ-extract together with syphilitic serum was influenced by the amount of natural immune body for the test-corpuscles in the complement-containing serum. These same three sera were tested both in the untreated condition, and also after being in contact with washed ox-blood at 0° C. Table II gives the result of the experiments.

TABLE II

	No. of Doses of Comple-ment.	Complement I (three days old).		Complement II (twenty-four hours old).		Complement III (fresh).	
		Untreated.	Treated.	Untreated.	Treated.	Untreated.	Treated.
Syphilitic serum (½ hour at 55° C.) 0·05 c.cm. + turbid emulsion of organ-extract 0·6 c.cm.	7	No lysis	No lysis	No lysis	No lysis	No lysis	No lysis
	10	Faint trace of lysis	,,	Trace of lysis	Distinct trace of lysis	,,	,,
	15	Very marked lysis	Trace of lysis	Lysis just complete	Lysis just complete	Very faint trace of lysis	,,
	20	Complete lysis	Complete lysis	Complete lysis	Complete lysis	Trace of lysis	Trace of lysis
	30	,,	,,	,,	,,	Complete lysis	Complete lysis

The results show that in spite of the fact that Serum III contained a considerable amount of immune body for ox corpuscles, still, exactly the same number of doses of complement were absorbed from the treated as from the untreated serum. The estimation of the haemolytic value of the treated as compared with the untreated sera showed a slight falling off in the former, probably due to dilution. Accordingly, it is clear that the fresh complement (III) was deviated to a greater extent than that of the older sera, even although it contained the largest amount of natural immune body for the ox corpuscles. Again, the variation in deviability of different complements does not depend on the procedure by which the complement-containing serum is obtained. Certain sera were obtained by defibrinating the blood immediately and then centrifugali-

sing and pipetting off the serum, which was then allowed to stand for 18 to 24 hours; in other instances the blood was allowed to clot, and the serum allowed to separate at room-temperature for 18 to 24 hours; sera obtained in both ways were compared, but no definite and constant difference could be attributed to the variation in the procedure.

Specimens of complement-containing serum have occasionally been encountered which were so sensitive to the organ-extract emulsion as to render their use in the Wassermann test impossible. Thus, *if more than five doses of complement along with organ-extract emulsion or the patient's serum are required to cause complete lysis of the test-corpuscles, the complement is hypersensitive and the results should be discarded.*

In two such instances the extract alone absorbed the complement in 0·06 (8 doses) and 0·1 c.cm. (13½ doses) of the serum; both specimens were 18 hours old at the time of the test. All the patients' sera which were tested in these experiments including negative controls, gave positive results. In such cases the results must be discarded, and the experiments repeated with complement which is less sensitive. In one of these two specimens this peculiar sensitiveness was present when the serum was 18 hours old; but after a further interval of 24 hours the serum was found to be much less sensitive. Thus here also age appears to be a factor. Whether this extraordinary sensitiveness is in every case merely an extreme example of the greater deviability possessed by fresh complement has not been determined. In some instances the alcohol present in the organ-extract may be the agent which destroys the complement; but this is not generally the case under the conditions which we have observed. Thus we found that complements which were hypersensitive to crude organ-extract were very frequently not hypersensitive to an emulsion of lecithin containing practically the same amount of alcohol (*v.* pp. 41, 47). It may be, however, that the complement is, in certain cases, hypersusceptible to the action of certain components of the crude extract along with alcohol. This effect of alcohol would be similar to its action in increasing the haemolytic power of triolein (Arrhenius).

The Organ-Extract

Three properties of the organ-extract have to be considered: (1) the turbidity of the emulsion, also (2) its haemolytic and

(3) its anticomplement action. The latter two may best be taken together.

The Turbidity of the Emulsion depends on the manner in which extract and saline solution are mixed (*v.* p. 25). It has been seen that more complement is deviated by a turbid emulsion with syphilitic serum than by a clear emulsion. As normal sera do not, under the conditions already specified, produce deviation of a normal complement when a turbid emulsion is used, it is advisable in every instance to employ such a turbid emulsion. Syphilitic sera which react only weakly with a turbid emulsion do in some cases react negatively with a clear emulsion. The following table (III) is taken from an experiment in which the resulting complement deviation was markedly affected by the turbidity of the emulsion of organ-extract.

TABLE III

	Amount of Complement.	Result.
Turbid emulsion of organ-extract alone 0·6 c.cm.	0·025 c.cm. (5 doses)	No lysis.
	0·03 c.cm. (6 doses)	Trace of lysis.
Clear emulsion of organ-extract 0·6 c.cm.	0·01 c.cm. (2 doses)	Almost complete lysis.
	0·015 c.cm. (3 doses)	Complete lysis.
Turbid emulsion of organ-extract 0·6 c.cm. + 0·05 c.cm. syphilitic serum	0·15 c.cm. (30 doses)	Trace of lysis.
	0·2 c.cm. (40 doses)	Complete lysis.
Clear emulsion of organ-extract 0·6 c.cm. + 0·05 c.cm. syphilitic serum	0·1 c.cm. (20 doses)	Almost complete lysis.
	0·15 c.cm. (30 doses)	Complete lysis.

Here it is seen that the turbid emulsion alone absorbed more than twice as much complement as the clear emulsion; and in the presence of syphilitic serum almost twice as much complement was absorbed by the turbid emulsion as by the clear emulsion. It is also to be noted that if the result were read according to the single tube method a negative result would be recorded with the clear extract (almost complete lysis with 0·1 c.cm. complement), whereas with the turbid emulsion the result would be positive.

Haemolytic and Anticomplement Action.—The extract by itself should not have a marked lytic effect on the test-cor-

puscles, otherwise it is impossible to estimate the influence of the extract on the complement. In performing the test an estimation should be made of the amount of complement absorbed by the serum alone, and also by the extract alone. A true deviation of complement has resulted only where the amount absorbed by the serum along with the extract markedly exceeds the sum of the amounts absorbed by serum and extract alone. Now, if the extract has a lytic effect on the corpuscles, its effect on the complement cannot be estimated, and thus a control which is indispensable to a satisfactory test is left out.

Again, lytic extracts have practically always a marked destructive effect on complement, and under some circumstances, and especially when the single tube method is employed, a lytic extract may, with a normal serum, give what is apparently a positive reaction. The possible fallacy from employing a lytic extract should be emphasised in view of the facts that its use has recently been recommended, and that some authorities still persist in advocating the use of extract of syphilitic liver in the test. A syphilitic liver is, as a rule, either undergoing autolysis or in a state of fatty degeneration when obtained. Alcoholic extracts of such livers contain a larger amount of haemolytic substances than an extract of fresh normal liver does, so that the quantity of syphilitic liver extract employed in the Wassermann test is frequently more than sufficient to cause complete lysis of the test amount of ox corpuscles. The apparent advantage of using an extract of syphilitic liver is undoubtedly to some extent due to the abnormal character or amount of the lipoid substances which it contains. A syphilitic liver containing spirochaetes and without autolytic change or fatty degeneration was extracted with alcohol and the extract used in the Wassermann test. Titration against an alcoholic extract of fresh guinea-pig's liver showed that the extract of syphilitic liver did not, with syphilitic serum, deviate more complement than the guinea-pig's liver extract. It has been found that the lytic property of an alcoholic tissue extract can be inhibited by the addition of blood serum, and it has been suggested that when a lytic extract is employed in the Wassermann test its lytic effect is inhibited by the serum whose syphilitic property is being tested. This would, of course, depend on two things, first on the strength of the lytic effect of the extract, and secondly, on the inhibiting power of the human serum. Extracts are

known to vary considerably in their lytic effect on blood-corpuscles, and Cruickshank has found that sera vary in their inhibitory effect on the same extract. If the human serum employed does not completely inhibit the lytic property of the extract, then it is found that the complement of the guinea-pig's serum is destroyed to a greater or less extent. In this way, with an extract of a certain lytic strength, a negative serum may appear to give a positive Wassermann reaction. Our results in this connection are in agreement with Facchini.*

Conclusions

To avoid the fallacies which may arise from variations in the individual properties of the complement-containing serum and the organ-extract it is important that the following precautions be taken.

1. An estimation of the deviation of complement should be made in terms of the number of haemolytic doses absorbed by the serum along with the extract, and as controls the number of haemolytic doses of complement absorbed (1) by the serum alone and (2) by the extract alone, must always be accurately measured.

2. The complement-containing serum should be allowed to stand 18 to 24 hours before use, and should not be more than three days old.

3. The results obtained from the use of a complement-containing serum which is specially sensitive to the emulsion of organ-extract or the patient's serum should be discarded. A complement is hypersensitive when more than five doses are required to cause complete lysis of the test-corpuscles in the presence of organ-extract emulsion or patient's serum.

4. The emulsion of organ-extract should be as turbid as possible and the amount employed in the test should not cause more than a trace of lysis of the test-corpuscles in 2 to 3 hours at 37° C.

5. Known negative and positive sera should be used as controls in every experiment, in order to detect whether the deviability of the specimen of complement which is being employed is markedly abnormal.

6. A number of workers have recommended the use of a "pooled" complement from several guinea-pigs as likely to give a specimen of average deviability.

* Facchini, *Zeitschr. f. Immunitätsforsch.*, Bd. II., 1909, p. 257.

CHAPTER IV

LECITHIN AND CHOLESTERIN AS REAGENTS FOR THE DETECTION OF SYPHILITIC SERA (Method of Browning, Cruickshank and Mackenzie)

PREPARATION OF LECITHIN FROM OX'S LIVER—BIOCHEMICAL PROPERTIES OF LECITHIN—THE ACTION OF CHOLESTERIN ALONG WITH LECITHIN IN THE SYPHILIS REACTION—PRACTICAL APPLICATION OF THE LECITHIN-CHOLESTERIN METHOD FOR PURPOSES OF DIAGNOSIS.

IT has already been pointed out that an emulsion of cholesterin, together with lecithin, causes the absorption of more complement in the presence of heated syphilitic serum than is absorbed by the serum along with the same amount of lecithin in the absence of cholesterin. Under the same conditions the addition of cholesterin causes no increase in the amount of complement absorbed with heated non-syphilitic sera. In addition, the inhibitory effect of the lecithin on complement is not perceptibly increased by the addition of the cholesterin. Accordingly, Browning, Cruickshank and Mackenzie have suggested that lecithin and cholesterin should be employed as reagents for the detection of syphilitic sera. *If a serum absorbs more complement in the presence of lecithin plus cholesterin than it absorbs in the presence of lecithin alone, then this is evidence of its syphilitic nature.*

Lecithin by itself has only a moderate capacity for absorbing complement in the presence of syphilitic serum, as many workers have found. The term lecithin is applied in general to those alcohol-soluble lipoid substances which are precipitated by acetone from ethereal solution. It is practically certain that (1) lecithin prepared from any one source consists of a mixture of analogous bodies and (2) lecithins from different sources vary in their composition. The lecithin which we have employed is prepared from the crude alcoholic

extract of fresh ox-liver. As will be seen later (p. 68) lecithins from other sources may behave somewhat differently.

Preparation of Lecithin from Ox's Liver

The crude alcoholic liver-extract, prepared as already described (*v.* p. 23) is evaporated in an open flat porcelain dish on the water bath at 60°C. till, after 4 to 5 hours, a syrupy mass remains. This is rubbed up with quartz sand (which has previously been washed with water and dried) till a firm mass results. The mixture of dried extract and sand (about 50 grams of sand to the residue of 1,000 c.c. of extract) is placed in a spherical flask closed with a perforated rubber stopper through which runs a short piece of quill tubing drawn out to a capillary point at the end. This tube serves to prevent the vapour in the flask from forcing out the stopper. Ethyl-acetate is placed in the flask, which is then stoppered, and immersed up to the neck in water at 60° C. The flask is shaken repeatedly, and after 10 minutes the ethyl-acetate is poured off into a hot water filter funnel such as is used for filtering agar. The water in the funnel-jacket is kept at 60° C., and the solution is filtered through fat-free paper (*v.* p. 23). A further portion of ethyl-acetate is added to the sand, and the extraction repeated. After a third treatment practically all the soluble matter has been extracted. In all about 170 c.c. of ethyl-acetate should be used to extract the residue of 1,000 c.c. of crude extract. The portion which falls out when the ethyl-acetate solution is placed in the ice chest over night is again dissolved in ethyl-acetate at 60° C., and the solution put in the ice chest over night. Finally the portion insoluble in ethyl-acetate in the cold is dissolved in water-free ether (sp. gr. 0·717) at room-temperature. To the ethereal solution in a glass cylinder four volumes of acetone are added, which cause a precipitate to separate out. Precipitation is aided by shaking the mixture for several minutes. Separation is complete within 10 minutes. The separated fluid is poured off and the crude precipitate of lecithin is redissolved in ether and reprecipitated with acetone twice further. Finally the precipitate is rubbed up with sand and the soluble portion taken up by extraction with absolute ethyl-alcohol for 24 hours at room-temperature; the last traces of lecithin may be removed by extracting the residue with a further small quantity of alcohol.*

* The ordinary commercial "pure" reagents serve satisfactorily for the preparation of lecithin.

The crude lecithin of 1,000 c.c. extract should be extracted with about 100 c.c. of alcohol. The strength of the solution is estimated by evaporating a measured amount (5 to 10 c.c.) at 57° C. and weighing. The alcoholic lecithin solution is kept in a stoppered bottle at room-temperature in the dark.

Biochemical Properties of Lecithin

Lytic Properties.—The pure lecithin is only slightly lytic for ox's red blood-corpuscles; thus 2 c.c. of a 1 in 5 dilution of 1·7 per cent. alcoholic solution of lecithin caused not quite complete lysis of 1 c.c. of 5 per cent. suspension of washed ox blood after 3 hours at 37° C. (It is to be noted that some specimens of ox blood are more sensitive to the lytic action than others.)

Haemolytic Action along with Cobra-Venom.—0·0017 c.c. of a 1 per cent. emulsion of lecithin caused just complete lysis of 1 c.c. blood suspension with 0·1 c.c. of 1 : 1,000 cobra-venom after 3 hours at 37° C. (in the absence of lecithin, ox corpuscles, of course, undergo no lysis). The ratio of the lytic dose of lecithin in the presence of venom to its lytic dose without venom is thus 1 : 400. It is evident, therefore, that an active lecithin preparation has been obtained (Kyes and Sachs *).

Action on Complement.—After preliminary tests with different concentrations of lecithin a 0·75 per cent. solution in alcohol was adopted as satisfactory for purposes of diagnosis; 0·6 c.c. of the emulsion made by diluting 1 part of the alcoholic solution with 7 parts of saline so as to yield the maximum turbidity (*v.* p. 25) usually caused scarcely any lysis of 1 c.c. of the test-corpuscles in 24 hours. This quantity of emulsion has only a very slight inhibitory effect on complement by itself, lysis usually being complete with two to three doses. In general this amount of lecithin emulsion has less inhibitory action on complement than has the standard amount of crude extract emulsion. The inhibitory effect of the lecithin emulsion also is more uniform with different specimens of complement than that of the crude extract. This is well seen in Table II, Nos. 1, 6 and 9.

Wassermann Reaction.—Along with syphilitic serum the lecithin emulsion, in the amount mentioned above, caused increased absorption of complement; but this is much less

* In Ehrlich's *Studies on Immunity*, New York, 1909.

marked than in the case of a crude extract of fresh ox liver (vide Table I), even although the amount of lecithin in the latter represents less than that present in the standard amount of pure lecithin emulsion. With sera which yield only a

TABLE I

Syphilitic Serum (½ hour at 55° C.) 0·05 c.c. + Emulsion, 0·6 c.c.	Amounts of Guinea-pig's Complement.			
	0·075 c.c.	0·1 c.c.	0·15 c.c.	0·2 c.c.
Lecithin . . .	Just complete lysis	Complete lysis	Complete lysis	Complete lysis
Crude extract . .	0	Faint trace of lysis	Distinct lysis	Complete lysis

CONTROLS.

Lecithin emulsion, 0·6 c.c. + 0·01 c.c. complement = just complete lysis.
Crude extract emulsion, 0·6 c.c. + 0·01 c.c. complement = just complete lysis.
Syphilitic serum, 0·05 c.c. + 0·6 c.c. NaCl solution + 0·01 c.c. complement = complete lysis.
Dose of complement = 0·005 c.c.

slight positive reaction with a crude extract there may be practically no increase in complement-absorption with the pure lecithin (vide Table IV *A* ; also Table VIII). With regard to the action of different concentrations of lecithin *v.* p. 71.

THE ACTION OF CHOLESTERIN ALONG WITH LECITHIN IN THE SYPHILIS REACTION

To the 0·75 per cent. solution of lecithin in alcohol, cholesterin is added in excess, and the mixture is shaken repeatedly. As saturation occurs only slowly at room-temperature, the mixture should be allowed to stand for a week before the clear fluid is used. If it is desired to prepare the lecithin-cholesterin solution rapidly, 1 to 1·2 grams of cholesterin should be added to 100 c.c. of alcoholic lecithin and solution should be brought about by brief and gentle warming on a water bath.* The lecithin-cholesterin solution is preserved in the same way as the alcoholic lecithin. The emulsion, prepared so as to give the maximum turbidity, by floating one part of the saturated alcoholic solution on the surface of seven parts of 0·85 per cent. NaCl solution, and then mixing slowly, gives a uniform white emul-

* The cholesterin is obtainable from Kahlbaum or Poulenc Frères. The alcoholic solutions of lecithin and lecithin-cholesterin can be obtained from Messrs. Thomson, Skinner and Hamilton, 38, Sauchiehall Street, Glasgow.

sion, from which microscopic crystals of cholesterin separate out in some hours; shaking restores this suspension to its original state.

Lytic Action.—The haemolytic action of lecithin is diminished by the cholesterin, as Kyes and Sachs have pointed out.

Effect on Complement.—The lecithin-cholesterin emulsion by itself scarcely inhibits complement to any greater degree than does the lecithin alone; Table II shows the comparative effects of lecithin emulsion, lecithin-cholesterin emulsion, and crude extract emulsion in the usual amounts in a series of estimations with different specimens of complement. The increase of inhibitory effect on complement due to the cholesterin is in general almost negligible. In forty-two experiments the amount of complement inhibited by lecithin-cholesterin emulsion alone only twice amounted to as much as one dose more than the amount inhibited by lecithin emulsion; only rarely has a greater difference been met with.

TABLE II

No. of Experiment.	No. of Doses of Complement inhibited by Emulsion of—		
	Lecithin.	Lecithin-cholesterin.	Crude Extract (Ox Liver).
I	0·3	1	3·5+
II	1	1	2
III	3	3	5
IV	2	2	2
V	2	1	4
VI	1·5	2	6+
VII	1	1	1
VIII	2·5	2·5	5
IX	1	2	8
X	1	1	2·5

Wassermann Reaction.—The effect of the addition of cholesterin to the lecithin is most striking in its effect on the amount of complement absorbed in the presence of syphilitic serum. This has been tested with serum both from secondary and tertiary syphilitic and from parasyphilitic cases, and with sera which with a crude extract gave a weak positive reaction as well as with powerful sera. Table III gives the results of a comparison between the deviating power of (*a*) the pure lecithin emulsion; (*b*) the lecithin-cholesterin emulsion, and (*c*) an emulsion of a crude extract, in each case tested simultaneously and with the same complement.

TABLE III (*A*)

Syphilitic Serum, powerful, (½ hour at 55°C.), 0·05 c.c. +0·6 c.c. Emulsion.	Amounts of Guinea-pig's Complement.					
	0·075 c.c.	0·1 c.c.	0·135 c.c.	0·18 c.c.	0·22 c.c.	0·26 c.c.
(*a*) Lecithin	Trace of lysis	Complete lysis	Complete lysis	Complete lysis	Complete lysis	Complete lysis
(*b*) Lecithin-cholesterin	0	0	0	0	Faint trace of lysis	Distinct lysis
(*c*) Crude extract	0	Faint trace of lysis	Faint trace of lysis	Faint trace of lysis	Trace of lysis	—

CONTROLS.

Emulsions alone—(*a*) and (*b*)+0·015 c.c complement =complete lysis.
(*c*)+0·04 c.c. complement =just complete lysis.
Syphilitic serum, 0·05 c.c.+0·6 c.c. NaCl solution+0·01 c.c. complement =complete lysis.
Dose of complement =0·005 c.c.

TABLE III (*B*)

Syphilitic Serum, weak, (½ hour at 55° C.), 0·05 c.c. +Emulsion 0·6 c.c.	Amounts of Guinea-pig's Complement.				
	0·015 c.c.	0·025 c.c.	0·04 c.c.	0·065 c.c.	0·1 c.c.
(*a*) Lecithin . .	Very marked lysis	Complete lysis	Complete lysis	Complete lysis	Complete lysis
(*b*) Lecithin-cholesterin	0	0	Faint trace of lysis	Trace of lysis	Complete lysis
(*c*) Crude extract	0	0	Faint trace of lysis	Trace of lysis	—

CONTROLS.

0·6 c.c. emulsions (*a*) and (*b*)+0·01 c.c. complement =complete lysis.
0·6 c.c. emulsion (*c*)+0·02 c.c. complement =almost complete lysis.
Syphilitic serum, 0·05 c.c.+0·6 c.c. NaCl solution+0·01 c.c. complement =complete lysis.
Dose of complement =0·005 c.c.

It will be seen that the Wassermann effect with the lecithin-cholesterin mixture is here quite as marked as with the crude extract, and the former has this advantage, that by itself it usually inhibits complement to a less extent than does the crude extract; further, with different specimens of comple-

ment the lecithin-cholesterin mixture is more uniform in its inhibitory effect by itself.

TABLE IV

Syphilitic Serum (55° C. for ½ hour), 0·05 c.c.	Emulsion, 0·6 c.c.	Amounts of Guinea-pig's Complement.				
		0·02 c.c.	0·035 c.c.	0·05 c.c.	0·075 c.c.	0·1 c.c.
A (a weak serum)	(*a*) Lecithin saturated cholesterin (1·3 per cent.)	0	0	Trace of lysis	Very marked lysis	Complete lysis
	(*b*) Lecithin ⅔ saturated cholesterin	0	Faint trace of lysis	Very marked lysis	Complete lysis	Complete lysis
	(*c*) Lecithin ⅓ saturated cholesterin	0	Trace of lysis	Complete lysis	Complete lysis	Complete lysis
	(*d*) Lecithin alone	Marked lysis	Complete lysis	Complete lysis	Complete lysis	Complete lysis
		0·1 c.c.	0·14 c.c.	0·2 c.c.	0·275 c.c.	
B (a powerful serum)	(*a*) Lecithin saturated cholesterin (1·3 per cent.)	0	Trace of lysis	Distinct trace of lysis	Complete lysis	
	(*b*) Lecithin ⅔ saturated cholesterin	0	Trace of lysis	Distinct lysis	Complete lysis	
	(*c*) Lecithin ⅓ saturated cholesterin	Trace of lysis	Distinct lysis	Complete lysis	Complete lysis	
	(*d*) Lecithin alone	Very marked lysis	Complete lysis	Complete lysis	Complete lysis	

CONTROLS.

Emulsions alone, 0·6 c.c.—(*a*), (*b*), and (*c*) + 0·015 c.c. complement = complete lysis.
(*d*) + 0·01 c.c. complement = just complete lysis.

Syphilitic serum, 0·05 c.c. + 0·6 c.c. NaCl solution + 0·01 c.c. complement = complete lysis.

Dose of complement = 0·005 c.c.

Table IV shows the effect of varying the amount of choles-

TABLE V

Human Sera (55° C. for ½ hour), 0·05 c.c.	Emulsion, 0·6 c.c.	Amounts of Guinea-pig's Complement.					
		0·015 c.c.	0·04 c.c.	0·075 c.c.	0·1 c.c.	0·15 c.c.	0·2 c.c.
Normal No. I . . .	(*a*) Lecithin	Complete lysis	Complete lysis	Complete lysis	Complete lysis	Complete lysis	Complete lysis
„ No. II . . .	(*b*) Lecithin-cholesterin	Complete lysis	Complete lysis	Complete lysis	Complete lysis	Complete lysis	Complete lysis
„ No. III . . .							
„ No. IV . . .							
„ No. V . . .	(*c*) Crude extract of ox liver	Complete lysis	Complete lysis	Complete lysis	Complete lysis	Complete lysis	Complete lysis
Syphilitic	(*a*) Lecithin	0	Trace of lysis	Just complete lysis	Complete lysis	Complete lysis	Complete lysis
	(*b*) Lecithin-cholesterin	0	0	0	0	Faint trace of lysis	Just complete lysis
	(*c*) Crude extract of ox liver	0	0	0	Faint trace of lysis	Distinct lysis	Complete lysis

CONTROLS.

Sera alone, 0·05 c.c. + 0·6 c.c. NaCl solution + 0·01 c.c. complement = just complete lysis.

Emulsions alone, 0·6 c.c.

(*a*) Lecithin + 0·01 c.c. complement = almost complete lysis.

(*b*) Lecithin-cholesterin + 0·01 c.c. complement = trace of lysis.

„ „ + 0·015 c.c. complement = complete lysis.

(*c*) Crude extract + 0·01 c.c. complement = just complete lysis.

Dose of complement = 0·005 c.c.

terin in a lecithin emulsion in the case both of (*a*) a weakly reacting and (*b*) a powerful serum. The result is, that increase in the cholesterin content of such mixtures causes a fairly uniform increase in the amount of complement absorbed in the presence of syphilitic serum. Hence it is advantageous that a large amount of cholesterin should be present.

In Table V are given the results of a comparative test made with five normal sera and a parasyphilitic serum in the presence of emulsions of (*a*) pure lecithin, (*b*) lecithin-cholesterin, and (*c*) crude extract, along with a complement which was readily deviated. It is thus seen that, as contrasted with the parasyphilitic serum, the addition of normal serum causes no increase in the amount of complement absorbed. Further, lysis proceeded at the same rate in corresponding tubes in the series with lecithin alone and with cholesterin in addition. With seventy negative sera the lecithin-cholesterin emulsion only once absorbed as much as one dose more than the lecithin emulsion.

TABLE VI

Syphilitic Serum (55° C. for ½ hour), 0·05 c.c.	No. of Doses of Complement necessary to cause Just Complete Lysis.					
	In the Presence of Serum : + 0·6 c.c. Emulsion of			Without Serum : + 0·6 c.c. Emulsion of		
	Lecithin.	Lecithin + Cholesterin.	Crude Extract.	Lecithin.	Lecithin + Cholesterin.	Crude Extract.
M. (general paralytic) .	14	20+	20	4	3	4+
J. (general paralytic) .	12	30+	—	2	3	—
P. (general paralytic) .	8	15+	—	—	—	—
J. G. (general paralytic)	7—	15+	—	—	—	—
G. (general paralytic) .	6	15+	—	2	3	—
C. (general paralytic) .	25	40+	—	—	—	—
M.	15	28	—	3	4	—
M.	20—	52+	52	3	3	8
T. (general paralytic) .	5	15	17	2	2	5
G. (general paralytic) .	5	16	16	—	—	—
P. (general paralytic) .	5	16	18	—	—	—
R. (tertiary)	40	52+	—	—	—	—
D. (tertiary)	20	36	—	—	—	—
C.	15	40	40—	2	3	2

Where a number of sera are included in a bracket, this indicates that these sera were tested at the same time and with the same specimen of complement.

The general result may be expressed as follows: in the presence of syphilitic serum, ox liver lecithin causes the absorption of a certain amount of complement, which may be almost negligible with such sera as act weakly when tested with a crude extract; the amount of complement absorbed is greatly increased in the case of syphilitic sera by the addition of cholesterin; on the other hand, in the presence of normal serum, or where serum is absent altogether, the presence of cholesterin causes practically no increase in the amount of complement absorbed.

Table VI gives the results obtained with a series of syphilitic sera tested with the different emulsions on separate occasions. Certain sera have also been tested repeatedly, and it is strikingly shown, e.g. with the serum "M," that the amount of complement which is deviated on a particular occasion depends on individual properties of the complement-containing serum.

Practical Application of the Lecithin-Cholesterin Method for Purposes of Diagnosis

The practical value of lecithin and lecithin-cholesterin as reagents for the diagnosis of syphilitic sera has been established by Gilmour, who has examined in this way upwards of 150 sera, fully half of which were from syphilitic or parasyphilitic cases (of sixty-five cases of general paralysis 96 per cent. reacted positively); at the same time, by way of control, the behaviour of the sera with crude alcoholic extract of ox-liver was tested. Five or six sera were examined with the same specimen of complement at one time. The method adopted was to estimate accurately the amount of complement absorbed by the patient's serum along with emulsions of (*a*) lecithin and (*b*) lecithin-cholesterin respectively: the amounts employed were 0·05 c.c. of the patient's serum (previously heated for half an hour at 55° C.) and 0·6 c.c. of emulsion (1 part of alcoholic solution plus 7 parts of saline) in each case of maximum turbidity (*v.* pp. 39, 40). The usual controls, of course, were always made, viz. the estimation of the inhibitory effects of the emulsions and of the sera by themselves on complement. The results show that where the series containing serum and lecithin-cholesterin shows more complement absorbed than the series with serum and lecithin alone, then the reaction is positive. *Even a very small difference in the two series (two to three doses more of complement absorbed with lecithin-*

cholesterin than with lecithin) *is in favour of the serum being syphilitic, and a more marked difference* (*five doses or more*) *is practically conclusive under the conditions laid down.* As the result of Gilmour's observations we have adopted the lecithin-cholesterin method for the routine examination of all sera. Gilmour has found that sheep's blood with the corresponding immune body is quite as efficient as sensitised ox blood when carrying out the syphilis test by the lecithin-cholesterin method.

The absolute amount of complement fixed by lecithin-cholesterin emulsion along with syphilitic serum is in most cases somewhat inferior to that absorbed with crude ox-liver extract, but at the same time the crude extract by itself usually absorbs more complement than lecithin-cholesterin. We have found, however, that certain sera repeatedly absorb more complement with lecithin-cholesterin than with crude extract, so that the result is independent of the complement used. This fact serves to emphasize how complicated the nature of the processes involved in the Wassermann reaction must be.

In the ordinary method of carrying out the test with an organ-extract, the criterion of a positive reaction is, of course, the absolute amount of complement absorbed (measured in haemolytic doses), as compared with the amounts absorbed in the controls. Now it has been found that frequently the complement is abnormally susceptible to the inhibitory action of the extract or the heated serum, separately or together. Along with such deviable complements a normal serum may cause the absorption of much more complement than the sum of the amounts absorbed by serum and emulsion separately, so that the reaction is apparently positive (*v.* chap. III). For this reason we have suggested that where with the extract or serum alone more than five doses of complement are required to cause complete lysis the results should be rejected. In a series of forty estimations made by Browning and Gilmour the results were on ten occasions vitiated by the use of complements which were abnormally deviable so far as the crude extract was concerned. On the other hand, with the lecithin-cholesterin method, where the difference of the amounts of complement absorbed in the two series is taken into account, we have found that with highly deviable complements two sera absorbed the same amount of complement in the presence of lecithin ; but, on the addition of cholesterin, the one ab-

sorbed much more complement, thus showing its syphilitic character, whereas the other, from a non-syphilitic subject, deviated exactly the same amount as without cholesterin. The advantage of the use of lecithin and lecithin-cholesterin is thus apparent. The following is an illustrative experiment (Table VII).

TABLE VII

Serum.	No. of Doses of Complement necessary to produce just complete lysis with—		
	Lecithin.	Lecithin-cholesterin.	Crude Extract.
Syphilitic	9	25	25
Normal	10½	10½	10½

CONTROLS.

Amount of complement which produced just complete lysis with—lecithin alone, 4 doses; lecithin-cholesterin alone, 3 doses; crude extract alone, 3 doses; syphilitic serum alone, 3 doses; normal serum alone, 4½ doses.

It is to be noted that the proportional increase in the amount of complement absorbed under the influence of cholesterin, is often especially marked in the case of weakly positive sera. Thus, in the following experiment, we see the results when a strong and a weak positive serum were tested at the same time (Table VIII).

TABLE VIII

Serum.	No. of Doses of Complement necessary to produce just complete lysis with—	
	Lecithin.	Lecithin-cholesterin.
Weak syphilitic	4	16
Powerful syphilitic . . .	21	37

CONTROLS.

Amount of complement necessary to produce just complete lysis with—lecithin alone, 2 doses; lecithin-cholesterin alone, 2 doses; sera alone, 2 doses.

In every instance the inhibitory power of the sera and of the emulsions by themselves must be carefully measured. It is also of great importance by way of control always to test a

positive serum which has been examined previously and a known negative serum simultaneously with the patient's serum. The result of the examination of the patient's serum is to be accepted only when the series with the negative serum behaves in the way above described. This applies especially to the interpretation of weak positive reactions.

The advantages of the lecithin-cholesterin method are (1) its reliability, as the use of the two series minimises the disturbing effect of variations in deviability of the complement, and (2) its delicacy in the case of weakly reacting sera.

The application of this method in the examination of cerebrospinal fluids is described later (*v.* p. 134).

CHAPTER V

COMPLEMENT DEVIATION IN EXPERIMENTAL TRYPANOSOMIASIS

THE WASSERMANN REACTION WITH THE SERUM OF RABBITS INFECTED WITH THE TRYPANOSOMES OF NAGANA: THE EFFECT OF TREATMENT WITH ARSENOPHENYLGLYCIN (EHRLICH): THE ACTION OF EXTRACTS FROM VARIOUS SOURCES ALONG WITH THE SERA OF INFECTED RABBITS: THE ACTION OF UNHEATED RABBIT'S SERUM.

AS will be seen later, the relation between infectivity, or the presence of the living syphilitic virus, and the presence of those substances in the serum, which give rise to a positive Wassermann reaction, has not been definitely determined. This applies especially to the so-called parasyphilitic diseases. Animal experiment might be expected to clear up such points. In general, however, animals' sera tend to behave differently from human sera in regard to complement-deviation along with lipoid bodies. Thus, heated normal sera of *lower* apes (Bruck and Stern [3,13], and Blumenthal [14]), dogs (Rossi [12]) and rabbits may, in some instances, give a positive reaction. In the case of monkeys infected with syphilis Bruck and Stern have found that the reaction develops or increases with the appearance of the primary lesion, but this may occur before the development of the sore. Subsequently the reacting power of the serum fluctuates; but it tends to be marked one to two years after infection.

A number of observers (Landsteiner, Müller, and Pötzl,[6] etc.) have found that the serum of rabbits experimentally infected with trypanosomes might develop the property of reacting positively in the Wassermann test.* At the same

* McIntosh (*Zeitschr. f. Immunitätsforsch.*, Bd. VIII., 1910, p. 183) has found that in the serum of rabbits infected with nagana try-

time, in such infections the presence of the pathogenic agent can usually be readily determined by the biological method, and the parasites can be killed in the host's body by suitable drugs. Thus the trypanosomes of nagana are markedly pathogenic for rabbits. The presence of parasites in the rabbit's blood can usually be shown by injecting 1 to 2 c.c. subcutaneously into a mouse. The infected rabbit can be cured with practical certainty by the injection of a sufficient dose of Ehrlich's arsenophenylglycin.

It was our intention to examine the serum of infected rabbits from time to time, and to prolong the course of the disease by the injection of minute doses of arsenophenylglycin, so as to produce a state of affairs comparable with syphilitic and trypanosome infections in the human subject. The attainment of such a result is obviously attended with considerable difficulty. The investigation illustrates also the precautions which must be observed in drawing conclusions from series of observations, either in clinical or experimental work, on such a property of the serum as the power of giving a positive Wassermann reaction (*v.* p. 54).

The Infection

The strain of nagana trypanosomes was carried through normal rabbits. A mouse was inoculated with blood from an infected rabbit and when abundant parasites were present, its blood was used to inoculate rabbits intravenously. The resulting infection was fairly virulent, since marked symptoms were usually present within a fortnight, and death resulted in two to five weeks after inoculation. No spontaneous recovery was observed.

The Wassermann Reaction in Infected Rabbits

It has been shown by numerous workers that the blood serum of normal rabbits may give a positive Wassermann reaction (*vide* Dohi [11], also Schilling and Hoesslin [10], who give full references to earlier literature), and some have considered this due to the presence of coccidiosis. Manteufel [8], however, denies this, and several of our animals which reacted positively after infection with trypanosomes showed no evidence of coccidiosis post-mortem. The question as to whether

panosomes specific antibodies also develop, which absorb complement in the presence of suspensions of trypanosomes. These specific immune bodies, however, do not concern us here.

infection with a non-pathogenic protozoon, e.g. the trypanosome described by Ashworth and MacGowan,[7] may not in some instances cause the alterations in the serum, merits consideration. We employed only such rabbits as reacted negatively to begin with.

Out of ten animals which were inoculated with nagana trypanosomes, one (No. IX) gave a marked positive reaction nineteen days after inoculation.

It received an injection of 0·1 grm. arsenophenylglycin per kilo, and died thirteen days later—cause not ascertained. The serum taken post-mortem reacted strongly positive.

Another rabbit (No. V) reacted negatively after thirteen days, but gave a marked positive reaction after thirty-nine days. It was evidently unusually resistant to the disease. (For the further fate of this animal, *v.* p. 55.) Three other rabbits gave only a slight positive reaction two to three weeks after inoculation.

Two of these (Nos. VIII and X) were treated (*v.* pp. 54, 55); the third was not further observed.

The rest remained negative at this period.

Of the latter, three (Nos. III, XII, XIV) were treated (*v.* pp. 54, 55); the remainder were not observed further.

The method of carrying out the Wassermann reaction is that already described in Chapter II, an emulsion of alcoholic extract of fresh ox-liver being employed.

Table I shows the results of the tests of the serum of the two animals which developed a marked positive reaction after infection, along with the tests of control animals.

TABLE I

(*A*)

Rabbit.	Doses of Complement.	
	Three.	Seven.
No. IX.—Before inoculation.	Almost complete lysis	Complete lysis
Control: No. XII.—Before inoculation.	0	Complete lysis

Extract emulsion alone + 3 doses of complement = complete lysis.
Sera alone + 2 „ „ = „ „

Dose of complement = 0·0075 c.c.

Rabbit.	Doses of Complement.			
	Ten.	Fifteen.	Twenty.	Thirty.
No. IX.—Nineteen days after inoculation.	0	0	0	Faint trace of lysis
Control : No. XII.—Nineteen days after inoculation.	Complete lysis	Complete lysis	Complete lysis	Complete lysis

Extract emulsion alone +3 doses of complement = complete lysis.
Sera alone +2 ,, ,, = ,, ,,
Dose of complement = 0·005 c.c.

(*B*)

Rabbit.	Doses of Complement.		
	Four.	Seven.	Ten.
No. V.—Thirteen days after inoculation.	Very marked lysis	Almost complete lysis	Complete lysis
Control	Complete lysis	Complete lysis	Complete lysis

Extract emulsion alone +4 doses of complement = just complete lysis.
Sera alone +2 ,, ,, = complete lysis.
Dose of complement = 0·006 c.c.

Rabbit.	Doses of Complement.			
	Seven.	Ten.	Fifteen.	Twenty.
No. V.—Thirty-nine days after inoculation.	0	0	0	0
Control	Complete lysis	Complete lysis	Complete lysis	Complete lysis

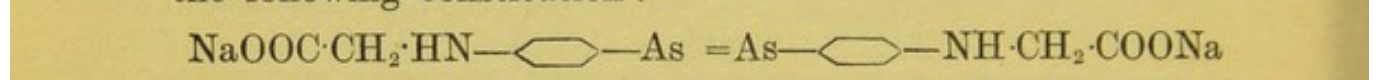
Extract emulsion alone +4 doses of complement = complete lysis.
Serum No. V alone +4 ,, ,, = ,, ,,
Control alone +2 ,, ,, = ,, ,,
Dose of complement = 0·005 c.c.

With regard to the conclusions which may be drawn from a series of consecutive tests, we have already pointed out that individual properties of the complement-containing serum influence, to a marked degree, the amount of complement

absorbed by a given serum in the Wassermann reaction (*v.* p. 31). Accordingly, accurate information can be got only by testing a number of sera simultaneously on the different occasions. Where all the sera show a similar variation in the same direction in one test as compared with another, the results must be looked on with suspicion, as possibly due merely to individual differences in the deviability of the complements.

Treatment with Arsenophenylglycin

Arsenophenylglycin (Ehrlich-Bertheim, *vide* Ehrlich [4]) has the following constitution :—



This drug, for the use of which we are indebted to Professor Ehrlich, is very soluble in water. In air it oxidises to poisonous compounds ; accordingly it is kept in sealed tubes *in vacuo* and the solution is prepared immediately before use. It is interesting that the toxicity of this substance for mice is practically constant (Röhl [9]), whereas, to para-amido-phenyl-arsenic acid (atoxyl) great variations in susceptibility occur (Ehrlich and Browning, *vide* Browning [2]). Arsenophenyl-glycin, which was introduced as a chemotherapeutic agent in trypanosome infections by Ehrlich, is the best, both for prophylaxis and in curative effect, so far discovered. Thus Röhl has found that in rabbits a single intravenous injection of 0·08 to 0·06 gram per kilo. of body weight cures with certainty an infection with nagana trypanosomes. These quantities represent only a third to a fourth of the minimum lethal dose.

Although we did not intend to test the curative effect of arsenophenylglycin, the results which we have obtained with comparatively minute doses strikingly confirm the originally published accounts of its action. Thus a single intravenous injection of 0·035 to 0·04 grm. per kilo. of body weight, two to three weeks after inoculation, cured two out of five animals (Nos. X and XIV). This is a somewhat smaller proportion of cures than Röhl [9] obtained with similar doses (about 65 per cent.) ; but it is to be noted that in every instance we delayed treatment until the animal was very ill, and in several series some of the rabbits had already died of the infection before the rest were treated. Recurrences were treated in three instances by an injection of 0·08 to 0·11 grm. per kilo. One of these animals (No. III) was cured. It was originally

treated with 0·04 grm. per kilo. on the twelfth day after inoculation. The recurrence was cured by an injection of 0·08 grm. per kilo. forty-three days later.

The criterion of cure in these cases is that the animals have remained alive, and have shown no symptoms of nagana infection twelve (No. XIV), fourteen (No. X) and fifteen (No. III) months after cessation of treatment; and further, that repeated inoculations of 1 to 2 c.c. of blood into mice have not caused an infection of the latter.

It is interesting to note that certain animals are exceptionally resistant to treatment.

Thus, rabbit No. V, which had a particularly chronic form of infection, was treated on the thirty-ninth day with 0·09 grm. of arsenophenylglycin per kilo. (its weight at this time being 1,247 grms. as compared with 1,843 grms. when inoculated). At the time of treatment there were the usual symptoms of advanced nagana infection. These disappeared within a week. Four weeks after the injection symptoms recurred, and a mouse injected with 1 c.c. of blood rapidly showed parasites. The weight was now 1,153 grms.; 0·11 grm. per kilo. of arsenophenylglycin was injected. Twenty-four days later a mouse received 1 c.c. of the blood, and remained free of parasites during the three weeks that it was kept under observation. Forty-five days after the last injection the rabbit appeared quite well in the morning; in the afternoon it was found dying. There was slight lachrymation from the right eye, but none of the usual signs of nagana infection. Trypanosomes were swarming in the blood. The spleen was not enlarged.

The Effect of Treatment on the Wassermann Reaction

Non-sterilising Doses.—Three animals (Nos. III, VIII, and XII) were treated with 0·04 grm., 0·04 grm., and 0·036 grm. of arsenophenylglycin per kilo., the first twelve and the others twenty-one days respectively after inoculation, when Nos. III and XII reacted negatively to the Wassermann test, and No. VIII gave a slight positive reaction. The first had a recurrence thirty-three days later, but continued to react negatively, although not treated until ten days later. As has been seen, this animal was cured by the second injection (p. 54). Rabbit No. XII gave a marked positive reaction twenty-four days after the injection; accordingly, 0·08 grm. of arsenophenylglycin per kilo. was injected; three weeks later the Wassermann reaction was still positive. The animal was free from signs of disease fourteen days after the first injection, and had remained so. During four months following the last injection it continued to react positively on frequently repeated examinations, and mice were frequently inoculated with 1 to 2 c.c. of blood, but did not become infected. Then the animal again showed signs of nagana illness; but a mouse injected with 1 c.c. of blood showed no trypanosomes during the eight days that it was kept under observation. That the illness was really a

recurrence appears practically proved by the fact that a further injection of arsenophenylglycin (0·1 grm. per kilo.) caused disappearance of the signs. The Wassermann reaction remained positive, and the animal died, most probably from the trypanosome infection, two months after the last injection, having lived seven months after inoculation. Rabbit No. VIII had a relapse twenty-four days after the injection. Its serum continued to give only a slight positive Wassermann reaction. It was not further treated and died six weeks after inoculation.

Thus it appears that the administration of a non-sterilising dose of arsenophenylglycin, which caused a protraction of the infection, was followed by the development of a marked positive Wassermann reaction in one out of three cases. In the positively reacting animal the degree of the reaction was quite independent of its clinical condition.

Curative Doses.—Of the three animals which were cured, two reacted negatively at the time of treatment, namely, Nos. XIV and III, the latter having continued to give a negative reaction during the relapse. Thus in the latter instance the reaction failed to develop in spite of an infection which lasted for eight weeks. The third (No. X) gave only a very weak positive reaction. After the cessation of treatment the reaction with the sera of these animals continued unchanged for some months, and then intermittently positive reactions began to be obtained. The results on the last occasion on which the sera were tested are given in Table II. At the same time, the negative result obtained with a non-syphilitic human serum is given by way of control.

TABLE II

Rabbit.	Doses of Complement.			
	Five.	Eight.	Twelve.	Sixteen.
No. V.—Fourteen months after treatment.	0	Very marked lysis	Complete lysis	Complete lysis
No. X.—Thirteen months after treatment.	0	0	0	Very marked lysis
No. XIV.—Eleven months after treatment.	Distinct lysis	Complete lysis	Complete lysis	Complete lysis
Control: Non-syphilitic human serum.	Complete lysis	Complete lysis	Complete lysis	Complete lysis

Extract emulsion alone + 3 doses of complement = just complete lysis.
Sera alone + 2½ ,, ,, = complete lysis.
Dose of complement = 0·0085 c.c.

Alt [1] has found that in general paralytics arsenophenylglycin caused the positive Wassermann reaction to diminish or disappear in 44 per cent. of cases.

The Action of Extracts from Various Sources along with the Sera of Infected Rabbits

It has frequently been suggested that unsatisfactory results in carrying out the Wassermann test have been due to the use of an unsuitable organ-extract. Thus the extract of liver from a case of congenital syphilis has usually been considered as the most efficient for obtaining a reaction with syphilitic sera. We have found (*v.* p. 34) that, in general, organ-extracts which are actively haemolytic as the result of autolytic or similar processes, produce very marked deviation of complement in the presence of syphilitic sera ; but such extracts have, by themselves, a very active inhibitory effect on complement. In order to test whether any specific factor was concerned in the organ-extract when employed along with positively reacting sera from rabbits affected with trypanosomiasis, we compared the following extracts, using the same rabbit's serum and the same complement throughout :— alcoholic extracts of (1) congenital syphilitic liver, (2) liver of rabbit dead of infection with nagana trypanosomes, (3) liver of mouse dead of nagana trypanosome septicaemia, and (4) a watery extract of the same liver as No 2 (extraction was allowed to proceed for 24 hours ; the centrifugalised extract was then used). All the alcoholic extracts were of practically equal efficiency. The watery extract of the rabbit's liver was markedly inferior to the others.

The Action of Unheated Rabbit's Serum

It has been found by various observers (Sachs and Altmann [15]) that syphilitic sera when unheated give a more marked Wassermann reaction than after heating. Sera from a variety of other conditions, however, may also react positively in the fresh state (*v.* p. 80). With rabbits' sera the opposite is the case. Thus Table III shows the result of testing the same serum from a rabbit infected with nagana trypanosomes (1) after heating for half an hour at 57° C., and (2) unheated. The result, after standing over night, is that the heated serum has caused marked absorption of the complement, whereas

the unheated serum reacts negatively. This has also been observed by Morgenroth and Halberstaedter (vide Hessberg [5]). It is to be noted that in the presence of the unheated serum the lysis of the test-corpuscles often occurs only very slowly, so that if a reading is taken when the test corpuscles have been incubated for 1 hour at 37° C., the result is practically equal in a series containing fresh rabbit's serum and in one containing heated serum. By next morning diffusion of the haemoglobin from the sedimented blood-corpuscles is seen to have occurred in the series containing the unheated rabbit's serum.

TABLE III

Rabbit's Serum.	Doses of Complement.				
	Four.	Eight.	Fifteen.	Twenty.	Thirty.
Heated for half an hour at 57° C., one hour and twenty-four hour readings.	0	0	Trace of lysis	Very marked lysis	Complete lysis
Unheated, one-hour reading.	0	0	Marked lysis	Just complete lysis	Complete lysis
Unheated, twenty-four hours readings.	Almost complete lysis	Complete lysis	Complete lysis	Complete lysis	Complete lysis

Extract emulsion alone +5 doses of complement = complete lysis.
Heated serum alone +2 „ „ = „ „
Unheated serum alone = almost complete lysis.
Dose of complement = 0·005 c.c.

Hessberg [5] has found in certain cases that the unheated as well as the heated serum reacted positively; but among five animals, several of which were repeatedly tested, we have not met with a single instance in which the fresh serum gave a positive reaction.

If the negative reaction were due to a non-deviable complement present in rabbit's serum, then it appeared probable that fresh normal rabbit's serum added to guinea-pig's complement in the Wassermann test with syphilitic serum might prevent the occurrence of a positive reaction.

Accordingly, we added in one series 0·1 c.c. of fresh normal rabbit's serum (which contained about one dose of complement for the test corpuscles) to the usual mixture of emulsion of organ-extract and

syphilitic serum, and in a second series 0·1 c.c. of the same rabbit's serum previously heated for half an hour at 57° C. was added. Guinea-pig's complement was then added, and the test carried out as usual. The result was that a marked absorption of complement occurred, which was practically equal in both series.

Thus it would seem that the negative reaction with unheated rabbit's serum is not due to the presence of a small amount of normal rabbit's complement.

Summary and Conclusions

(1) Little is known as to the conditions which cause rabbits' sera to react positively in the Wassermann test. Apparently normal rabbits may react positively. On the other hand, trypanosome infection in rabbits does not determine a positive reaction with the same certainty that syphilitic infection in the human subject does. Even where a non-sterilising dose of arsenophenylglycin was administered, so that the infection had a protracted course, the sera did not invariably come to react positively. When the serum of an infected animal did give a positive Wassermann reaction the administration of non-sterilising doses of arsenophenylglycin did not lead to disappearance, or even to a definite diminution, of the Wassermann reaction, although the tests were carried out at a time when the signs of disease had receded and the infection was obviously latent. These facts, together with the spontaneous variations in the behaviour of the sera of animals which have practically certainly been cured, indicate that no safe conclusions as to the action of pathogenic protozoa, or the effect of treatment on infections with these, can be based on the Wassermann reaction in the case of rabbits, a conclusion which agrees with that of Schilling and Hoesslin [10] and Manteufel [8]. This irregularity of action is apparently fairly common among lower animals.

(2) Positively reacting sera from rabbits infected with nagana trypanosomes deviated complement equally well with a variety of alcoholic extracts. A watery extract of the liver of a rabbit dead from the trypanosome infection was markedly inferior to the alcoholic extracts in this respect.

(3) The sera of trypanosome-infected rabbits which after heating give a marked positive Wassermann reaction, in the fresh state may merely delay the progress of lysis of the test-corpuscles, the reaction ultimately becoming negative. Fresh

normal rabbit's serum did not prevent the occurrence of the Wassermann phenomenon with a syphilitic serum.

(4) The high efficiency of arsenophenylglycin as a therapeutic agent in trypanosome infections was shown by the fact that two out of five rabbits infected with nagana trypanosomes were cured at a very advanced stage of the disease by a single intravenous injection of 0·035 to 0·04 grm. per kilo. of body weight, i.e. about a sixth of the minimum lethal dose.

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CHAPTER VI

THE THEORY OF THE SYPHILIS REACTION

COMPONENTS CONCERNED IN THE SYPHILIS REACTION: THE INFLUENCE OF PHYSICO-CHEMICAL FACTORS—THE "ANTIGEN"; ANALYSIS OF ALCOHOLIC EXTRACTS; THE ANTIGENIC ACTION OF LIPOIDS AND OF LIPOID MIXTURES, LECITHIN, CHOLESTERIN, ETC.—THE SERUM; OTHER PROPERTIES OF SYPHILITIC SERA, LECITHIN-PRECIPITATION, THE KLAUSNER PHENOMENON, ANTICOMPLEMENT ACTION, REACTION, ALTERATIONS IN PROTEIN-CONSTITUENTS; CONSTITUENTS OF THE SERUM WITH WHICH THE SYPHILITIC REACTING BODIES ARE ASSOCIATED—THEORIES TO ACCOUNT FOR THE SYPHILIS REACTION AS A BIOLOGICAL PHENOMENON AND TO CORRELATE IT WITH PATHOLOGICAL CHANGES.

MUCH work has been done in connection with the nature of the components taking part in the syphilis reaction, and on the influence of physico-chemical factors. Certain of the facts elicited have already been discussed in detail, since they have an important bearing on the practical performance of the test; others which may throw light on the mechanism of the reaction will be more fully gone into here. The principal theories which have been devised to account for the reaction and to correlate it with pathological changes following syphilitic infection will also be considered.

Components concerned in the Syphilis Reaction: The Influence of Physico-chemical Factors

The "Antigen"

In the case of *watery tissue extracts* it is obvious that no satisfactory analysis can be made. The watery extract of syphilitic liver has, however, been found to differ in no material respect from a similar extract prepared from the liver of a case of acute yellow atrophy due to streptococcal sepsis in which syphilis

could be excluded (Ehrmann and Stern,[1] Seligmann and Pinkus [2]). Thus both were inactivated by heating, etc. Accordingly, the action of a specific factor even in the case of watery extracts of syphilitic organs must be regarded as not yet demonstrated with certainty.

Alcoholic Extracts are also highly complicated mixtures, and the fact that the extract of a syphilitic liver is usually more "efficient," that is, leads to the deviation of more complement along with syphilitic serum than that from a normal organ, has already been considered (*v.* pp. 23, 35). The properties of the syphilitic extract are most probably to be explained by chemical alterations which are reflected to some extent in the histological condition of the tissue, namely, fatty change, autolysis, presence of soaps (Beneke [6]), and of excess of cholesterin (Pighini [16 (1)]), and which are not specifically syphilitic in their nature, although often produced in a striking degree as the result of congenital syphilis. The alcoholic extracts also are apparently not true antigens in the biological sense, since when injected into animals, they do not give rise to antibodies (Schatiloff and Isabolinsky,[4] Seligmann and Pinkus [2]). An important fact in connection with alcoholic extracts is that the alcohol which is present intensifies the reaction (*v.* p. 72). Blanck and Friedemann [5] have found that some alcoholic extracts when kept in the ice-chest lose their efficiency, the loss being independent of any precipitation; exposure at 37° C. may restore the activity again. On the other hand, we have found that on boiling alcoholic extracts of normal ox liver with a reflux condenser for several hours their "antigenic" action is increased rather than diminished, and this agrees with the generally admitted fact that alcoholic extracts are coctostable. The effect of the turbidity of the watery emulsion of alcoholic extract has already been considered (p. 25, *v.* also Gatz and Inaba [49]).

Analysis of Alcoholic Extracts

Noguchi [7] has analysed alcoholic extracts of normal and syphilitic liver and blood. The extracts were dried, then treated with ether, and the ether-soluble substances were dried and finally extracted with acetone. Both the acetone-soluble and insoluble fractions were found to act as syphilitic "antigen."

We have analysed alcoholic extracts of fresh ox-liver on the lines already described (*v.* p. 38), and we would emphasise

the fact that the bodies contained in such extracts mutually influence the solubility of one another, so that great care must be exercised in concluding that solubility in any reagent excludes the presence of bodies which in a purer state are known to be insoluble. Further, precipitating reagents frequently act incompletely; thus on the addition of acetone to an ethereal solution of lecithin (from ox-liver), the latter is not completely precipitated even when the mixture has stood for several days in the ice-chest. If the mixture is evaporated, then taken up in ether and acetone again added, a further precipitate of lecithin forms. In addition to the *lecithin component* of ox-liver extract whose properties have already been described (*v.* p. 39), we have also examined the properties of that portion of the alcoholic extract which is soluble in cold ethyl-acetate. In the course of the analysis a considerable number of other constituents were separated, which have not been investigated further. The results of Noguchi and Bronfenbrenner [12] are in general agreement with our observations.

The portion soluble in ethyl-acetate in the cold was freed from the solvent by distillation under diminished pressure at 50° C. It dissolved readily in absolute alcohol, a 2 per cent. solution being prepared which had a deep yellow tint. With alcoholic $CdCl_2$ solution no precipitate occurs. One part of the 2 per cent. alcoholic solution floated on to 7 parts of 0·85 per cent. NaCl solution, and slowly mixed, produces a very turbid emulsion from which flocculi rapidly separate out; the reaction is slightly acid to litmus paper. (To avoid destructive effects from repeated treatment with reagents at higher temperatures, the portion intended for biological examination was prepared as described.) Further experiments showed that the portion soluble in ethyl-acetate in the cold was almost entirely soluble in water-free ether, and that the addition of acetone caused practically no precipitate from the ethereal solution even after several days in the ice-chest. This product is therefore apparently free from lecithin, but is probably a complex mixture, since cholesterin and other lipoids, such as oleic and linoleic acids, are soluble in ethyl-acetate. The importance of investigating this product lay in the fact that the lecithin derived from the crude extract was so much weaker than the latter in its deviating power along with syphilitic serum.

(*a*) *Lytic Properties.*—This component is distinctly more

lytic for ox-corpuscles than is the pure lecithin, 0·025 c.c. of a 1 per cent. solution causing just complete lysis of 1 c.c. of ox-blood suspension ; but the lytic action is weak as compared with oleic or linoleic acid (*vide* Table I *B*).

TABLE I (*A*)

Emulsions made by slow admixture (alcoholic solution 1 part + salt solution 3 parts).

Substance.	Concentration in Solution in 25 per cent. Alcohol.	Lytic dose along with 0·1 c.c. of 0·1 per cent. Cobra-Venom Solution +1 c.c. of 5 per cent. Ox Blood.	Dose lytic by itself for 1 c.c. of 5 per cent. Ox Blood.
Lecithin (ox liver) .	0·085 per cent.	0·017 c.c.	(0·34 per cent. solution in 20 per cent. alcohol) 2 c.c.
Ethyl-acetate soluble component . .	0·1 ,,	0·04 c.c.	0·25 c.c.
Oleic acid . . .	0·05 ,,	—	0·07 c.c.
Linoleic acid . .	0·05 ,,	—	0·07 c.c.

(*b*) *Lytic Effect with Cobra-Venom.*—The emulsion of this component is rendered more lytic by cobra-venom ; but only to a slight degree, as the ratio of the lytic dose with venom to the lytic dose by itself is only 1 : 6. Table I (*A*) shows the result of estimations of the lytic dose with cobra-venom and the lytic dose by itself of the lecithin * and of the ethyl-acetate soluble component, as well as the lytic doses of oleic acid (I. of Kahlbaum) and linoleic acid (Kahlbaum) The tests were all made at the same time and with the same specimen of ox-blood, and serve as a representative example of repeated observations. To ensure uniformity, the amounts of alcohol in the different series were kept, as far as possible, equal ; thus with the exception of the estimation of the lytic power of lecithin, stock dilutions containing 25 per cent. of alcohol were made up just before use. The amount of alcohol present in the doses employed was therefore by itself practically negligible. For the estimation of the lytic dose of lecithin by itself a solution containing 20 per cent. alcohol was employed.

* A clear emulsion of lecithin produces haemolysis more rapidly in the presence of cobra-venom than a turbid emulsion of the same concentration ; but after 24 to 36 hours, the lytic dose is practically the same with both emulsions.

The amounts of the various solutions were added to 1 c.c. of 5 per cent. washed ox-blood suspension, and after incubation at 37°C. for 2½ hours, with repeated shaking, the results were read. Table I (*B*) shows the respective doses in grammes of the various substances. Smaller amounts of the ethyl-acetate soluble component distinctly inhibit lecithin activation (cf. Kyes and Sachs,[66] in the case of alcoholic extracts of rabbit serum). The effect is practically the same whether the alcoholic solutions of lecithin and of the component soluble in ethyl-acetate are mixed and then emulsionised or are emulsionised separately and the emulsions then mixed.

TABLE I (*B*)

Substance.	Absolute Amounts.		Ratio.
	Lytic dose with 0·1 c.c. of 0·1 per cent. Cobra-Venom +1 c.c of 5 per cent. Ox Blood.	Lytic Dose for 1 c.c. of 5 per cent. Ox Blood.	
Lecithin (ox liver) .	0·000017 grms.	0·0068 grms.	1 : 400
Ethyl-acetate soluble component . .	0·00004 „	0·00025 „	1 : 6
Oleic acid . . .	—	0·000035 „	—
Linoleic acid . .	—	0·000035 „	—

(*c*) *Action on Complement.*—A slowly prepared emulsion of the ethyl-acetate soluble component has a very powerful anti-complement effect. This can be neutralised to a great extent by lecithin. The neutralising effect depends, however, on the manner in which the lecithin and the ethyl-acetate soluble product are brought together. When the alcoholic solutions are first mixed and the emulsion is then made slowly, the anti-complement effect is found to be neutralised ; on the other hand, when the emulsions are made separately in the same fashion and are then mixed, the anti-complement effect remains practically unaltered. These results are shown in Table II.

In series (*a*), 0·9 c.c. of 1·7 per cent. alcoholic solution of ox-liver lecithin was mixed with 0·1 c.c. of 2 per cent. alcoholic solution of the ethyl-acetate soluble portion and 0·1 c.c. of absolute alcohol, and the mixture was slowly emulsionised

in the usual way with 7 c.c. of NaCl solution. In series (*b*), the same quantity of alcoholic lecithin solution was emulsified in 3·5 c.c. salt solution, and the ethyl-acetate soluble component mixed with the absolute alcohol was emulsified in another 3·5 c.c. of salt solution; the two emulsions were then mixed. In both cases the mixtures were neutral to litmus paper and were of approximately equal turbidity. To 0·6 c.c. of the emulsions increasing amounts of guinea-pig's complement were added, and after 1½ hours' incubation at 37° C. 1 c.c. of the test suspension of sensitised ox-corpuscles was added to each tube and the mixtures were again incubated as usual. The result was that in series (*a*) 0·025 c.c. of complement caused complete lysis, whereas in series (*b*) more than 0·1 c.c. of complement required to be added before complete lysis occurred.

TABLE II

Lecithin + Ethyl-Acetate Soluble Component.	Amounts of Guinea-pig's Complement.						
	0·02 c.c.	0·025 c.c.	0·035 c.c.	0·05 c.c.	0·075 c.c.	0·1 c.c.	0·15 c.c.
(*a*) Alcoholic mixture emulsionised	Very marked lysis	Complete lysis	Complete lysis	Complete lysis	Complete lysis	Complete lysis	Complete lysis
(*b*) Emulsions made separately, then mixed	0	0	0	Faint trace of lysis	Distinct lysis	Marked lysis	Complete lysis

Dose of complement = 0·0075 c.c.

Similar phenomena in other colloid reactions would suggest as an explanation, that where the alcoholic solutions are mixed before the emulsion is made, lecithin comes into more intimate contact with the other components, and thus protects the complement from the latter, whereas, when the substances are mixed in the form of emulsions, they persist for a considerable period in the form of isolated globules, so that absorption processes must be delayed.

(*d*) *Wassermann Effect.*—The presence of syphilitic or normal serum may sometimes, inhibit slightly the anti-complement effect in the amounts employed, but no Wassermann reaction takes place. Table III shows that the test amounts of a syphilitic serum and a normal serum had practically no influence on the complement inhibition due to this component.

TABLE III

2 per cent. Alcoholic Solution of the Ethyl-Acetate Soluble Component 0·05 c.c.+Absolute Alcohol 0·45 c.c. slowly emulsionised in 7 c.c. Salt Solution : 0·6 c.c. of the Emulsion.	Amounts of Guinea-pig's Complement.			
	0·08 c.c.	0·12 c.c.	0·17 c.c.	0·25 c.c.
+ 0	Trace of lysis	Marked lysis	Very marked lysis	Just complete lysis
+normal serum (55° C. for ½ hour) 0·05 c.c.	Faint trace of lysis	Trace of lysis	Marked lysis	Very marked lysis
+syphilitic serum (55° C. for ½ hour) 0·05 c.c.	Trace of lysis	Marked lysis	Very marked lysis	Just complete lysis

Dose of complement = 0·005 c.c.

The Effect of a Mixture of Lecithin with the Component Soluble in Cold Ethyl-acetate.—When the two components are mixed in alcoholic solution and then slowly emulsified, it is found that the Wassermann reaction is increased as compared with that given with lecithin alone in the presence of syphilitic serum (*vide* Table IV). An effect equal to that produced by the crude extract has not been obtained, however.

TABLE IV

Syphilitic Serum (55° C. for 30 minutes), 0·05 c.c.+0·6 c.c. Emulsion.	Amounts of Guinea-pig's Complement.			
	0·05 c.c.	0·075 c.c.	0·1 c.c.	0·15 c.c.
Lecithin (1·7 per cent.) 0·4 c.c. +absolute alcohol 0·6 c.c. in 7 c.c. salt solution	Faint trace of lysis	Marked lysis	Just complete lysis	Complete lysis
Lecithin (1·7 per cent.) 0·4 c.c. +ethyl-acetate soluble component (2 per cent.), 0·165 c.c. + absolute alcohol, 0·435 c.c. in 7 c.c. salt solution	0	0	Trace of lysis	Almost complete lysis

CONTROLS.

Emulsions alone, 0·6 c.c. +0·02 c.c. complement = complete lysis.
Serum, 0·05 c.c. +NaCl solution, 0·6 c.c. +0·01 c.c. complement = complete lysis.

Dose of complement = 0·0065 c.c.

The Antigenic Action of Lipoids * and of Lipoid Mixtures

The property of causing deviation of complement in the presence of syphilitic serum has been claimed for a large number of substances more or less pure chemically, and the results of different workers have been contradictory in numerous instances. The discordant results may be due, Sachs and Rondoni [8] point out, to the fact that the mode of preparation of the lipoid emulsion has a marked effect on its "antigenic" value. This important point has been referred to in detail, *v.* p. 25. In general, none of these substances brings out so markedly the difference between syphilitic and non-syphilitic sera as does a crude alcoholic extract, and none of them has been found to serve as an efficient substitute for such an extract. Much more satisfactory results have been obtained by the use of mixtures.

Lecithin.

Porges and Meier [62], and Landsteiner, Müller and Pötzl,[63] found that lecithin could to a certain extent replace crude alcoholic organ-extract. This has been confirmed by many others. We have tested *the behaviour of lecithins uniformly prepared from various sources* by a modification of the method already described (*v.* p. 38). The minced fresh organs of healthy adult animals were employed. The alcoholic extracts were prepared and evaporated in the usual way. The treatment with ethyl-acetate was repeated three times, however, so as to remove as far as possible all traces of substances soluble in cold ethyl-acetate. The further treatment was carried out as usual. Lecithin from ox's liver, heart and kidney, sheep's liver, pig's heart, liver and brain, and also egg-yolk were tested. As a standard for comparison, a stock specimen of lecithin from ox's liver was always used simultaneously. The alcoholic solutions containing 0·75 per cent. of lecithin were emulsified as usual so as to give the maximum turbidity. The general result was, that the heart lecithins gave the most marked deviation of complement in the presence of syphilitic serum, the liver lecithins being slightly inferior. Brain and egg-yolk lecithin caused least deviation.

* The term *lipoid* ("fat-like") is applied to substances of very different chemical natures. Bang (*Chemie und Biochemie der Lipoide*, Wiesbaden, 1911) defines lipoids as compounds which are soluble in organic solvents, such as ether, alcohol, chloroform and benzol. But every lipoid is not soluble in all of these reagents.

Several specimens of commercial ovolecithin (Kahlbaum, Merck, Poulenc Frères, Riedel I and II) were also found to be comparatively inactive. By themselves all the emulsions had a practically equal and almost negligible inhibitory effect on complement. The iodin-values of the various lecithins and also their haemolytic action along with cobra-venom on ox blood-corpuscles did not show variations corresponding with those in the syphilis reaction. The lecithin emulsions by themselves were only slightly haemolytic. The results of comparative tests with ox-liver lecithin, ox-heart lecithin and egg-yolk lecithin are given in Table V.

TABLE V

0·75 per cent. Alcoholic Solution of Lecithin 1 part+7 parts saline : Emulsion of maximum turbidity, 0·6 c.c.+Syphilitic Serum (½ hour at 55° C.), 0·05 c.c.	Amounts of Guinea-pig's Complement.			
	0·07 c.c.	0·1 c.c.	0·14 c.c.	0·2 c.c.
Ox-liver lecithin	No lysis	Faint trace of lysis	Trace of lysis	Almost complete lysis
Ox-heart lecithin. . . .	No lysis	No lysis	No lysis	Faint trace of lysis
Egg-yolk lecithin. . . .	Faint trace of lysis	Very marked lysis	Complete lysis	Complete lysis
Alcoholic solutions of lecithin as above, but containing in addition 1 per cent. of cholesterin ; emulsions as above	0·12 c.c.	0·17 c.c.	0·24 c.c.	0·32 c.c.
Ox-liver lecithin + cholesterin	No lysis	No lysis	Trace of lysis	Trace of lysis
Ox-heart lecithin + cholesterin	No lysis	No lysis	No lysis	No lysis
Egg-yolk lecithin + cholesterin	Faint trace of lysis	Complete lysis	Complete lysis	Complete lysis

CONTROLS.

All lecithin emulsions, 0·6 c.c. + complement 0·03 c.c. = just complete lysis.

0·6 c.c. emulsion of ox-liver lecithin + cholesterin + complement 0·03 c.c. = just complete lysis.

0·6 c.c. emulsion of ox-heart lecithin + cholesterin + complement 0·05 c.c. = just complete lysis.

0·6 c.c. emulsion of egg-yolk lecithin + cholesterin + complement 0·03 c.c. = just complete lysis.

Syphilitic serum 0·05 c.c. + saline 0·6 c.c. + complement 0·015 c.c. = just complete lysis.

Dose of complement = 0·006 c.c.

Thus it appears that the differences are due to variations in the chemical constitution of lecithin from different sources. Further purification of the ox-liver lecithin was attempted by prolonged exposure of the ethereal solution to the temperature of a mixture of ice and salt in the following way; the solution of lecithin in ether was kept in the freezing mixture for 24 hours, the precipitate which had formed was then separated and the clear solution was precipitated with

TABLE VI (*A*)

Alcoholic Solution of Ox-liver Lecithin 1 part + Saline 7 parts: Emulsion of maximum turbidity 0·6 c.c. + Syphilitic Serum (55° C., ½ hour) 0·05 c.c.	Amounts of Guinea-pig's Complement.				
	0·09 c.c.	0·12 c.c.	0·15 c.c.	0·18 c.c.	0·24 c.c.
Weak positive serum + emulsion of lecithin 0·6 per cent.	Very faint trace of lysis	Marked lysis	Very marked lysis	Complete lysis	Complete lysis
Weak positive serum + emulsion of lecithin 0·2 per cent.	Trace of lysis	Very marked lysis	Complete lysis	Complete lysis	Complete lysis
Weak positive serum + emulsion of lecithin 0·06 per cent.	Distinct lysis	Almost complete lysis	Complete lysis	Complete lysis	Complete lysis
Strong positive serum + emulsion of lecithin 0·6 per cent.	No lysis	No lysis	No lysis	Faint trace of lysis	Almost complete lysis
Strong positive serum + emulsion of lecithin 0·2 per cent.	No lysis	Very faint trace of lysis	Distinct lysis	Marked lysis	Complete lysis
Strong positive serum + emulsion of lecithin 0·06 per cent.	Trace of lysis	Very marked lysis	Complete lysis	Complete lysis	Complete lysis

CONTROLS.

All emulsions, 0·6 c.c. + complement 0·04 c.c. = just complete lysis.
Syphilitic sera, 0·05 c.c. + saline 0·6 c.c. + complement 0·035 c.c. = complete lysis.
Dose of complement = 0·01 c.c.

acetone. The acetone precipitate was again dissolved in ether and the solution was cooled for a further period of 24 hours by the freezing mixture. After repeating this procedure five or six times no further precipitate formed on cooling. The last acetone precipitate was then taken up in alcohol as usual. The lecithin purified in this way showed practically no difference from a specimen prepared from the same crude extract by the ordinary method, either as regards its action as syphilitic "antigen" or its haemolytic action with cobra-venom.

Experiments have been carried out with a view to determine *the effect of varying the amount of lecithin* (Browning Cruickshank and Gilmour—unpublished observations). The procedure was as follows: the alcoholic ox-liver lecithin solution was diluted with alcohol in varying proportions, and then emulsions as turbid as possible were prepared in the usual way, thus the amount of alcohol was constant throughout. The "antigenic" effect of the different dilutions was then tested in the ordinary fashion. Illustrative experiments are given in Tables VI and X. The results show that by increasing

TABLE VI (*B*).

Alcoholic Solution of Ox-liver Lecithin 1 part + Saline 7 parts: Emulsion of maximum turbidity 0·6 c.c. + Syphilitic Serum (½ hour, 55° C.) 0·05 c.c.	Amounts of Guinea-pig's Complement.				
	0·08 c.c.	0·12 c.c.	0·17 c.c.	0·24 c.c.	0·36 c.c.
Emulsion of 0·75 per cent. alcoholic lecithin	No lysis	No lysis	Very faint trace of lysis	Almost complete lysis	Complete lysis
Emulsion of 0·25 per cent. alcoholic lecithin	No lysis	No lysis	No lysis	Marked lysis	Almost complete lysis
Emulsion of 0·083 per cent. alcoholic lecithin	No lysis	No lysis	No lysis	Very marked lysis	Complete lysis
Emulsion of 0·027 per cent. alcoholic lecithin	Faint trace of lysis	Trace of lysis	Almost complete lysis	Complete lysis	Complete lysis

CONTROLS.

Emulsions alone, 0·6 c.c. + complement 0·03 c.c. = complete lysis.
Syphilitic serum, 0·05 c.c. + saline 0·6 c.c. + complement 0·04 c.c. = distinct lysis.
Dose of complement = 0·015 c.c.

the amount of lecithin up to a certain point, the amount of complement absorbed in the presence of syphilitic serum is also increased. Emulsions containing more than $\frac{1}{8}$ per cent. lecithin (1 per cent. alcoholic lecithin 1 part + 7 parts saline) do not lead to further complement-deviation. Occasionally, further amounts of lecithin may cause some diminution in the complement-absorption, that is, a slight zone-phenomenon may occur (*v.* Tables VI *B* and X.) But with lecithin of the strength employed in practice, there is no marked diminution in the degree of complement-absorption, as will be seen in the experiment where a weak and a strong positive serum were tested simultaneously. The inhibitory effects of the various strengths of lecithin on the complement were practically equal. As in the case of the crude extract, so also with lecithin, the alcohol present intensifies the Wassermann effect. This is well seen in Table VII. It is to be noted that the addition of alcohol did not increase the inhibitory action of the emulsion by itself on complement.

TABLE VII

2·37 per cent. solution of ox heart lecithin in alcohol, 1 part emulsionised with 7 parts of saline, so as to produce the maximum turbidity = stock emulsion.

A. 2·5 c.c. of stock emulsion + 5·5 c.c. saline.
B. 2·5 c.c. „ „ + 5·2625 c.c. saline + 0·2375 c.c. alcohol.
C. 2·5 c.c. „ „ + 4·8125 c.c. „ + 0·6875 c.c. „

Syphilitic Serum (55° C., ½ hour) 0·05 c.c. + Lecithin Emulsion 0·6 c.c.	Amounts of Guinea-pig's Complement.					
	0·06 c.c.	0·09 c.c.	0·13 c.c.	0·17 c.c.	0·22 c.c.	0·3 c.c.
Emulsion A . .	Faint trace of lysis	Trace of lysis	Distinct lysis	Very marked lysis	Complete lysis	Complete lysis
Emulsion B . .	No lysis	Very faint trace of lysis	Faint trace of lysis	Trace of lysis	Very marked lysis	Complete lysis
Emulsion C . .	No lysis	No lysis	Very faint trace of lysis	Faint trace of lysis	Trace of lysis	Very marked lysis

CONTROLS.

Emulsions, 0·6 c.c. + complement 0·02 c.c. = complete lysis.
Serum, 0·05 c.c. + saline 0·6 c.c. + complement 0·02 c.c. = complete lysis.
Dose of complement = 0·01 c.c.

Cholesterin

According to Fleischmann[13] and Walbum,[14] cholesterin can act as "antigen"; but this is denied by Levaditi and Yamanouchi,[11]

and also by Noguchi [7] who tested with negative result preparations of cholesterin from sheep's brain, gall-stones and a sample prepared by Merck.

The action of colloidal cholesterin has been tested by Browning and Cruickshank (unpublished observations). The colloidal solution was prepared by the method of Porges and Neubauer.[15] A concentrated solution of cholesterin (Kahlbaum) in acetone, is poured drop by drop into a large volume of distilled water. Finally the solution is filtered through paper and the acetone evaporated by gentle warming on the water-bath ; the solution is again filtered. The amount of cholesterin present is estimated by evaporating a measured quantity of the solution and weighing. To prevent precipitation of the cholesterin the following procedure was adopted. The colloidal solution was measured off and mixed in bulk with the correct amount of normal or syphilitic serum (previously heated for half an hour at 55° C. as usual). To each test tube of the series concentrated NaCl solution was added, so as to make the final concentration of salt 0·85 per cent., then the mixture of cholesterin and serum was added, and finally the complement. In the control series without serum, the complement was added to the concentrated salt solution, and then the cholesterin. The test was made in the usual way. The results showed that the cholesterin by itself had a powerful anti-complement action (cf. Pighini [16] and Iscovesco [17]). The effect of adding serum varied in different experiments. In some instances normal serum diminished the anti-complement effect of the cholesterin, while a powerful syphilitic serum increased the inhibitory action, and a moderately strong syphilitic serum rather diminished the inhibition (*v.* Table VIII) ; on other occasions normal sera and syphilitic sera, both powerful and weak, had practically no effect. In other experiments the colloidal condition of the cholesterin was preserved by mixing an alcoholic solution of cholesterin quickly with serum in salt solution. Here also the results were unsatisfactory. Thus cholesterin by itself is not a suitable reagent to elicit the Wassermann effect. The action of colloidal cholesterin, along with lecithin, is referred to below.

Other Lipoids

Soaps.—Sodium oleate has been employed by Sachs and Altmann.[9] Hessberg [10] found, in addition, that *potassium stearate* and *sodium palmitate* react with syphilitic serum.

Salts of the Bile-acids.—*Sodium glycocholate* and *taurocholate* can act as syphilitic antigens (Levaditi and Yamanouchi [11]). Noguchi [7] confirms this, and adds *sodium cholate*; but he considers these substances less active than other lipoidal bodies.

Protagon and Cholin are stated to give the Wassermann reaction with syphilitic serum (Levaditi and Yamanouchi [11]). Noguchi [7] has been unable to confirm this, and has also found *neurin* inactive.

TABLE VIII

In each tube 2·55 per cent. NaCl solution 0·2 c.c. +complement +a mixture of serum (55° C. ½ hour) 0·05 c.c. with 0·4 c.c. distilled water, containing 0·05 per cent. colloidal cholesterin.

Serum.	Amounts of Guinea-pig's Complement.				
	0·08 c.c.	0·13 c.c.	0·2 c.c.	0·29 c.c.	0·4 c.c.
Normal . . .	Distinct lysis	Complete lysis	Complete lysis	Complete lysis	Complete lysis
Syphilitic, moderately strong	Trace of lysis	Marked lysis	Complete lysis	Complete lysis	Complete lysis
Syphilitic, powerful	No lysis	No lysis	Very faint trace of lysis	Distinct lysis	Complete lysis
Control, no serum	Very faint trace of lysis	Faint trace of lysis	Distinct lysis	Complete lysis	Complete lysis

CONTROLS.

Serum alone, 0·05 c.c. +0·6 c.c. saline +0·04 c.c. complement=complete lysis.

Dose of complement=0·008 c.c.

MIXTURES OF LIPOIDS

Lecithin and Sodium Oleate, etc.

Sachs and Rondoni [8] have shown that the "antigenic" property of lecithin together with oleate of sodium is much greater than that of either of these substances by itself; while, in addition, the lecithin neutralises to a great extent the haemolytic and anti-complement effects of the soap. These workers have accordingly suggested an alcoholic solution of lecithin, oleate of soda, and oleic acid as a substitute for the crude organ-extract. Comparatively small amounts of this mixture cause haemolysis of the test corpuscles; it thus resembles extracts made from organs which have autolysed or which have been heated at 57° C. rather than extracts

of fresh organs (*v.* pp. 24, 35). Eisenberg and Nitsch [18] confirm Sachs and Rondoni's results, but consider that the oleic acid may be omitted. Schürmann [19] recommends a mixture of lecithin, sodium glycerophosphate, lactic acid and ammonium vanadinate, the latter component being essential.

Lecithin and Cholesterin

The action of cholesterin dissolved in alcoholic lecithin has already been described (*v.* p. 40). It has also been found that colloidal cholesterin behaves similarly when the alcoholic lecithin is floated on to the surface of the watery colloidal cholesterin solution, and then an emulsion prepared in the usual fashion. The emulsion prepared in this way, however, tends to have a more marked inhibitory effect on complement than that prepared with the alcoholic lecithin-cholesterin solution.

Cholesterin acts with lecithin from various sources, but the greatest increase due to cholesterin in the amount of complement absorbed in the presence of lecithin and syphilitic serum,

TABLE IX

Emulsions in every case of maximum turbidity.

Syphilitic Serum (55° C., ½ hour) 0·05 c.c. + Emulsion 0·6 c.c.	Amounts of Guinea-pig's Complement.			
	0·1 c.c.	0·14 c.c.	0·2 c.c.	0·32 c.c.
Ox-heart lecithin	No lysis	No lysis	Faint trace of lysis	—
Ox-heart lecithin + cholesterin 1 per cent.	No lysis	No lysis	No lysis	No lysis
Ox-liver lecithin	Faint trace of lysis	Trace of lysis	Almost complete lysis	Complete lysis
Ox-liver lecithin + cholesterin 1 per cent.	No lysis	No lysis	Faint trace of lysis	Trace of lysis
Egg-yolk lecithin. . . .	Very marked lysis	Complete lysis	Complete lysis	Complete lysis
Egg-yolk lecithin + cholesterin 1 per cent.	No lysis	Distinct lysis	Complete lysis	Complete lysis

CONTROLS.

Emulsions alone, 0·6 c.c. + 0·03 c.c. complement = complete lysis.

Serum alone, 0·05 c.c. + saline 0·6 c.c. + 0·015 c.c. complement = complete lysis.

Dose of complement = 0·005 c.c.

was found in the case of heart lecithin, liver lecithin being slightly inferior, and the least increase was with egg-yolk and brain lecithin (*v.* Tables V and IX). It was found, however, that with certain specimens of ox-heart lecithin, cholesterin caused an increase in the amount of complement absorbed with some normal sera (heated for half an hour at 55° C.) so that for practical purposes the heart lecithin may be unsuitable.

The effect of cholesterin in a fixed amount (1 *per cent.*) *along with varying strengths of ox-liver lecithin* has been tested. The amounts of complement absorbed by the syphilitic serum along with similar strengths of lecithin were ascertained at the same time (*v.* Table X). The result showed that with

TABLE X

Emulsions in every case of maximum turbidity.

Syphilitic Serum (55° C., ½ hour) 0·05 c.c. + 0·6 c.c. Emulsion of—	Amounts of Guinea-pig's Complement.					
	0·08 c.c.	0·12 c.c.	0·18 c.c.	0·24 c.c.	0·32 c.c.	0·4 c.c.
0·075 per cent. lecithin	Trace of lysis	Very marked lysis	Complete lysis	Complete lysis	Complete lysis	Complete lysis
Ditto + 1·3 per cent. cholesterin	No lysis	No lysis	No lysis	Very faint trace of lysis	Very marked lysis	Complete lysis
0·2 per cent. lecithin	Trace of lysis	Almost complete lysis	Complete lysis	Complete lysis	Complete lysis	Complete lysis
Ditto + 1·3 per cent. cholesterin	No lysis	No lysis	No lysis	Very faint trace of lysis	Very marked lysis	Complete lysis
0·75 per cent. lecithin	Distinct lysis	Just complete lysis	Complete lysis	Complete lysis	Complete lysis	Complete lysis
Ditto + 1·3 per cent. cholesterin	No lysis	No lysis	Very faint trace of lysis	Trace of lysis	Very marked lysis	Complete lysis

CONTROLS.

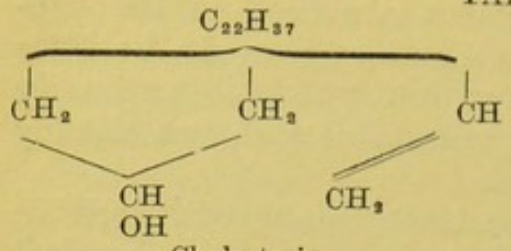
Lecithin emulsions alone, 0·6 c.c. + 0·03 c.c. complement = complete lysis.
Lecithin-cholesterin emulsions alone, 0·6 c.c. + 0·04 c.c. complement = complete lysis.
Serum, 0·05 c.c. + saline 0·6 c.c. + 0·02 c.c. complement = complete lysis.
Dose of complement = 0·015 c.c.

each concentration of lecithin the cholesterin caused an increase in the amount of complement absorbed in the presence of syphilitic serum, so that there appears to be little danger of the concentration of lecithin which is ordinarily employed (0·75 per cent.) proving unsuitable and thus causing failure to detect a syphilitic serum.

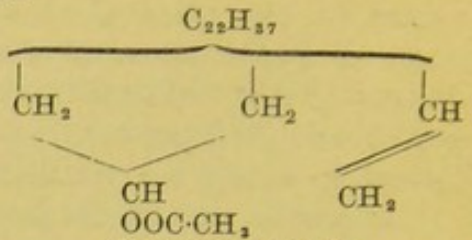
Cholesterin derivatives have been tested along with lecithin with a view to determining the relationship between the chemical constitution of the compounds and their action along with lecithin and syphilitic serum (observations of Browning and Cruickshank). The compounds were dissolved in 0·75 per cent. ox-liver lecithin in ethyl alcohol in amounts equimolecular with 1 per cent. cholesterin. Emulsions were made of the maximum turbidity and the tests carried out in the usual fashion. The action of lecithin and of lecithin-cholesterin were always tested simultaneously by way of control. The constitutional formulae of the substances tested, so far as they have been determined, are given in Table XIA (for the chemistry of these bodies *v.* Windaus [67]).

The results show that practically any alteration in the cholesterin-molecule diminishes to some extent the effect in the syphilis reaction. At the same time, none of the three known characteristics of the cholesterin-molecule appears to be absolutely essential: (1) *the alcoholic hydroxyl*; (*a*) *cholesteryl esters*, in which the secondary alcoholic hydroxyl-group has been replaced by an acid radical (*acetate, chloride, benzoate, oleate*); (*b*) *cholestenone*, the corresponding ketone ($\lessdot^{H}_{OH}$ replaced by $=O$) all give the reaction in some degree: (2) *the unsaturated carbon double-bond; cholesterin dibromide*, in which the carbon double-bond has been saturated by a molecule of bromine, gives the reaction, so also does *β-cholestanol* (*dihydrocholesterin*) the normal reduction product of cholesterin; *dibromcholesterylacetate*, from which both the unsaturated bond and the alcoholic hydroxyl are absent, gives the reaction slightly: (3) *the open chain* ($-CH{:}CH_2$—*terminal vinyl-group*); the conversion into a closed ring may greatly reduce the action, thus *a-cholestanol*, which according to Windaus may be *cyclocholesterin*, has a very weak effect, and *dehydrocholestanonol* is the only one of all the substances examined which has practically no action. On the other hand *oxycholestendiol*(*dehydrocholestandionol*) and *dehydrocholestandion* have a moderate action, while *oxycholestenone* (*dehydrocholestendion*) and its ethyl(enol)-

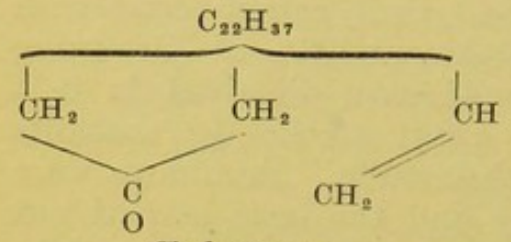
TABLE XIA



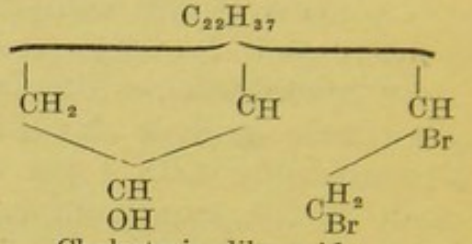
Cholesterin.



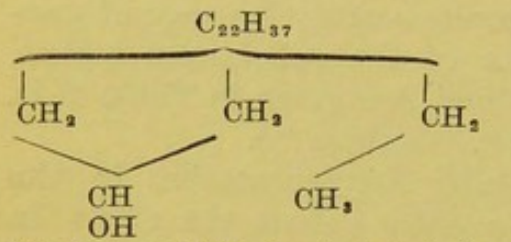
Cholesteryl acetate.



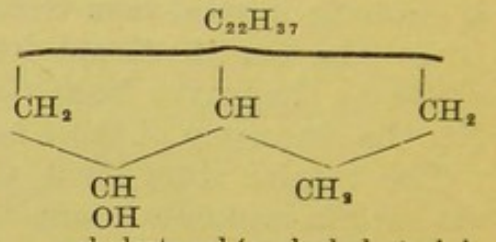
Cholestenone.



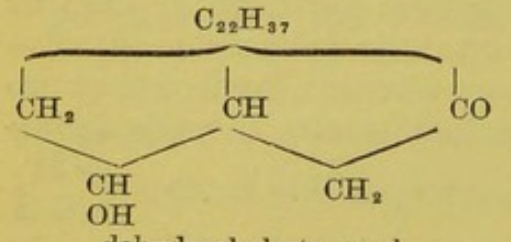
Cholesterin dibromide.



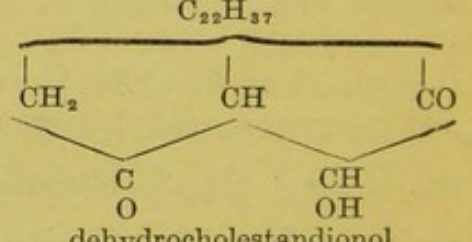
β-cholestanol (dihydrocholesterin).



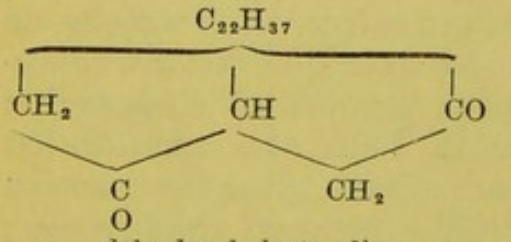
α-cholestanol (cyclocholesterin).



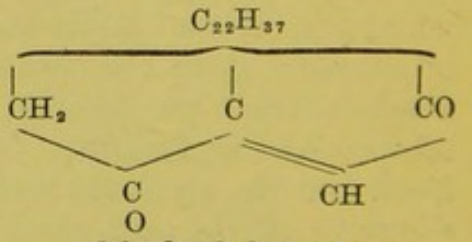
dehydrocholestanonol.



dehydrocholestandionol
(oxycholestendiol).



dehydrocholestandion.



dehydrocholestendion
(oxycholestenone).

ether are the most efficient of all the cholesterin derivatives. It appears, therefore, that in the case of cholesterin and its derivatives the relationship between constitution and biochemical action is exceedingly complicated, and it is not

possible to formulate any general law. The action of these bodies in inhibiting the haemolytic effect of lecithin (from ox's-liver) along with cobra-venom has also been investigated, and it has been found that no constant parallel exists between the antihaemolytic effect and the behaviour in the syphilis reaction (*v.* Table XIB). Thus, cholesterin is efficient in both reactions; but dehydrocholestendion and cholesteryl-esters, which are very efficient in the syphilis reaction, inhibit the haemolytic action only to a slight extent. Dehydrocho-lestanonol, on the other hand, which has no effect in the

TABLE XIB

Substance.	Effect in Syphilis Reaction.	Inhibition of Lecithin Venom Hæmolysis.
Cholesterin	Very marked	Very marked
Cholesteryl esters	Marked	Practically none
Cholesterin dibromide	Marked	Marked
Dehydrocholestendion	Marked	Practically none
Dehydrocholestanonol	Practically none	Marked

syphilis reaction, is markedly antilytic. In general the physical condition which gives the optimum effect differs in the two reactions, thus the turbid emulsion is most efficient in elicit-ing the syphilis reaction, whereas the colloidal solution made by rapidly mixing the alcoholic solution of lecithin *plus* cholesterin or its derivative with NaCl solution is least lytic with cobra-venom, as Walbum [14] also found. Dehydrochole-stanonol, however, forms the exception so far as antilytic effect of emulsions is concerned, since the slowly made turbid lecithin-dehydrocholestanonol emulsion is less lytic with cobra-venom than the colloidal solution.

The Serum

THE EFFECT OF PHYSICO-CHEMICAL FACTORS ON THE "SYPHILITIC ANTIBODY"

Temperatures under 0° C. do not appear to have any harmful effect on the reacting power of syphilitic serum (Seligmann and Pinkus [2]). Syphilitic serum which has been kept frozen or in the ice-chest for long periods may still give a positive reaction.

Heat has a marked effect on the reacting power of the serum: 30 minutes at 55° to 57° C. diminishes the reaction, and pro-longed exposure at these temperatures can destroy the reacting

power altogether. Sachs [20] has shown that exposure to 62° C. for 30 minutes abolishes the reaction. Bruck [21] found that exposure to a temperature of 60° C. greatly increases the inhibitory action of sera by themselves on complement. Again, Seligmann and Pinkus have shown that human sera in the fresh state may have an inhibitory effect on guinea-pig's complement, which is abolished by heating for 30 minutes at 57° C. Certain non-syphilitic sera may react positively in the fresh state, but cease to react after heating. Thus we obtained the following results with an alcoholic extract of congenital syphilitic liver—

Cases.		Number which gave a positive Wassermann reaction with Unheated serum.	Heated serum (30 minutes at 55°C.)
Scarlet fever	28	8	0
Enteric „	8	4	0
Typhus „	6	2	0
Eclampsia	4	2	0
Dementia (early)	1	1	0

The fresh sera by themselves had no inhibitory effect on complement.

Accordingly, *it is essential that sera which are to be tested for the syphilis reaction should be heated beforehand for* 30 *minutes at* 55°–57° *C.* (*v.* also Thomsen and Boas [34]).* Heating at 55° C. increases the globulin content of serum (Moll [22]), it also increases the alkalinity (Liebermann [78]), and this latter effect explains, in part at least, the diminution in reacting power which occurs when syphilitic sera are heated. Thus Sachs and Altmann [23] have shown that the addition of alkali ($\frac{1}{800}$ to $\frac{1}{3200}$ normal NaOH) decreases the amount of complement absorbed by syphilitic serum along with organ-extract, while conversely, acid ($\frac{1}{1000}$ to $\frac{1}{2000}$ normal HCl) increases the absorption (the acid and alkali being without any other demonstrable effect in the concentrations employed). The action of acid is said to favour the formation of lecithalbumin (Mayer and Terroine [24]). In the case of a true antigen-antibody reaction, namely globin from guinea-pig's haemoglobin acting along with the homologous antibody from the rabbit, Browning and Wilson [25] have found the same effects with alkali and acid. It is to be noted that Sachs and

* In the case of rabbits' sera a different effect as regards the Wassermann reaction has been found to result on heating. Thus, rabbits' sera which after heating at 55° C. gave a marked Wassermann reaction, when tested in the fresh state caused merely a temporary inhibition of haemolysis (*v.* p. 57).

Altmann did not succeed in obtaining a positive reaction by adding acid to normal sera. Changes in the reacting power of syphilitic serum subsequent to heating may occur. Such an experiment is shown in Table XII. A specimen of serum was divided into two portions, each of which was heated once for 30 minutes at 55° C. The one was heated 48 hours before the test, the other immediately before. They were then tested simultaneously with the same complement and organ-extract emulsion. The result shows that the portion of serum which had been heated first absorbed the greater amount of complement.

TABLE XI

Organ-Extract Emulsion 0·6 c.c.+0·05 c.c. Syphilitic Serum (55° C., ½ hour).	Amounts of Guinea-pig's Complement.			
	0·03 c.c.	0·045 c.c.	0·06 c.c.	0·0825 c.c.
(*a*) Heated on the day of the test	Marked lysis	Complete lysis	Complete lysis	Complete lysis
(*b*) Heated 48 hours before the test	Trace of lysis	Distinct trace of lysis	Marked lysis	Complete lysis

CONTROLS.

Organ extract emulsion 0·6 c.c. +complement 0·0075 c.c. =complete lysis.
Serum (*a*) and (*b*) 0·05 c.c. +saline 0·6 c.c. +complement 0·0075 c.c. =complete lysis.
Dose of complement =0·0075 c.c.

We have found that sera which have been kept at room-temperature or in the ice-chest in a sterile condition, unheated or after heating for 30 minutes at 55° to 57° C., may acquire a very powerful anti-complement action; thus 0·05 c.c. of unheated serum (3 months old) in 0·5 c.c. of saline inhibited entirely the action of ten to twenty doses of complement. Further exposure for ½–1 hour at 55° to 57° C. destroys this property; but in the case of a syphilitic serum the power of reacting with organ-extract is usually retained. Sera which have been exposed for 1 hour and upwards at 55° to 57° C. to begin with, do not acquire this anti-complement action. These facts in regard to the development of thermolabile anti-complement have been confirmed by Zinsser and Johnson.[26] Filtration *

* In regard to such experiments on the effects of filtration, we would draw attention to the fact that it is important to examine the properties of the filtrate at different stages of the process. Thus Muir and Browning (v. Muir's *Studies on Immunity*, Oxford, 1909) have shown that guinea-pig's complement is retained by a fresh Berkefeld

TABLE XIII

Serum (55° C., ½ hour).	Emulsion 0·6 c.c.	Amounts of Guinea-pig's Complement.						
		0·02 c.c.	0·04 c.c.	0·07 c.c.	0·1 c.c.			
Normal 0·01 c.c.	Lecithin	Just complete	Complete	Complete	Complete			
„ 0·01 c.c.	Lecithin-cholesterin	Complete	Complete	Complete	Complete			
„ 0·05 c.c.	Lecithin	Very marked	Just complete	Complete	Complete			
„ 0·05 c.c.	Lecithin-cholesterin	Almost complete	Complete	Complete	Complete			
„ 0·5 c.c.	Lecithin	0	Trace	Marked	Complete			
„ 0·5 c.c.	Lecithin-cholesterin	0	Trace	Marked	Complete			
		0·03 c.c.	0·05 c.c.	0·08 c.c.	0·12 c.c.	0·17 c.c.	0·23 c.c.	0·31 c.c.
Syphilitic "S" 0·01 c.c.	Lecithin	Just complete	Complete	Complete	Complete	Complete	Complete	Complete
„ 0·01 c.c.	Lecithin-cholesterin	—	Complete	Complete	Complete	Complete	Complete	Complete
„ 0·05 c.c.	Lecithin	Marked	Very marked	Complete	Complete	Complete	Complete	Complete
„ 0·05 c.c.	Lecithin-cholesterin	—	0	Faint trace	Distinct	Marked	Almost complete	Complete

„ 0·5 c.c.	Lecithin	—	Faint trace	Distinct	Very marked	Complete	Complete	Complete
„ 0·5 c.c.	Lecithin-cholesterin	—	—	0	Faint trace	Trace	Distinct	Marked
Syphilitic "L" 0·01 c.c.	Lecithin	—	—	Complete	Complete	Complete	Complete	Complete
„ 0·01 c.c.	Lecithin-cholesterin	—	—	Very marked	Complete	Complete	Complete	Complete
„ 0·05 c.c.	Lecithin	—	—	Complete	Complete	Complete	Complete	Complete
„ 0·05 c.c.	Lecithin-cholesterin	—	—	0	0	Trace	Marked	Almost complete
„ 0·5 c.c.	Lecithin	—	—	Almost complete	Complete	Complete	Complete	Complete
„ 0·5 .c.	Lecithin-cholesterin	—	—	—	—	Marked	Almost complete	Complete

CONTROLS.

Serum, normal, 0·01 c.c. + NaCl solution 0·6 c.c. + complement 0·03 c.c. = complete.
„ „ 0·05 c.c. + „ „ 0·6 c.c. + „ 0·03 c.c. = „
„ „ 0·5 c.c. + „ „ 0·6 c.c. + „ 0·08 c.c. = very marked.
„ syphilitic "S" 0·01 c.c. + „ „ 0·6 c.c. + „ 0·03 c.c. = complete.
„ „ 0·05 c.c. + „ „ 0·6 c.c. + „ 0·03 c.c. = „
„ „ 0·5 c.c. + „ „ 0·6 c.c. + „ 0·06 c.c. = very marked.
„ syphilitic "L" 0·01 c.c. + „ „ 0·6 c.c. + „ 0·03 c.c. = complete.
„ „ 0·05 c.c. + „ „ 0·6 c.c. + „ 0·03 c.c. = „
„ „ 0·5 c.c. + „ „ 0·6 c.c. + „ 0·06 c.c. = almost complete.

Emulsions, 0·6 c.c. + complement 0·02 c.c. = complete.

Dose of complement = 0·01 c.c.

through a Berkefeld candle has little effect on the serum (Seligmann and Pinkus [2]); but filtration through collodion sacs removes its reacting power (Mutermilch [54], McIntosh [55]), as also does filtration through porcelain and Doulton filters (McIntosh).

The effect of varying the amount of serum in the syphilis test has been studied by Gilmour (unpublished observations). The experiment shown in Table XIII illustrates well the general results. Three series were set up, each containing the standard amount of turbid emulsion of ox-liver lecithin (0·6 c.c. of a 1 + 7 dilution) and lecithin *plus* cholesterin 1 per cent., but with varying amounts of heated human serum, both normal and syphilitic, viz. 0·01, 0·05 and 0·5 c.c. respectively. The results show that—

1. Increasing the amount of serum by itself causes an increase in the amount of complement necessary to produce lysis of the test-corpuscles. The anti-complement effect of the human serum is not more marked with syphilitic than with normal sera;

2. Even large amounts of normal serum (0·5 c.c.) do not give a positive reaction, that is to say, the same amount of complement is absorbed by the serum along with lecithin and with lecithin-cholesterin;

3. In the case of syphilitic sera there is an optimum amount; thus with 0·05 c.c. of serum not merely is the amount of complement deviated along with the emulsions greater than when 0·01 c.c. of serum is used, but also the increase in complement-absorption due to the addition of the cholesterin is much more marked. On the other hand, when the amount of serum is increased to 0·5 c.c., a further increase in the difference between the lecithin and the lecithin-cholesterin series may fail to occur. Thus with 0·5 c.c. of serum "L" more complement is taken up in the lecithin series and less in the lecithin-cholesterin series than is taken up in the corresponding series with 0·05 c.c. of serum. A diminution in the complement absorbed by organ-extract along with large amounts of syphilitic serum as compared with that absorbed with smaller amounts, has been observed by various workers, and has been

filter to begin with, so that the first few cubic centimetres of filtrate are practically devoid of complement; but the complement passes through after further quantities have been filtered, whereas haemolytic immune body passes through quantitatively from the commencement of filtration, as also does immune opsonin (Browning [68]).

TABLE XIV

Serum (½ hour, 55° C.).	Emulsion 0·6 c.c.	Amounts of Guinea-pig's Complement.					
		0·02 c.c.	0·04 c.c.	0·07 c.c.	0·1 c.c.	0·14 c.c.	0·2 c.c.
0·05 c.c. . . .	Lecithin	Very marked	Complete	Complete	Complete	Complete	Complete
0·05 c.c. . . .	Lecithin-cholesterin	Marked	Very marked	Almost complete	Just complete	Complete	Complete
0·2 c.c.	Lecithin	Marked	Almost complete	Complete	Complete	Complete	Complete
0·2 c.c.	Lecithin-cholesterin	Distinct	Marked	Very marked	Almost complete	Complete	Complete
0·5 c.c.	Lecithin	—	Very marked	Complete	Complete	Complete	Complete
0·5 c.c.	Lecithin-cholesterin	—	Marked	Almost complete	Complete	Complete	Complete
C.S. Fluid 0·1 c.c. .	Lecithin	Faint trace	Marked	Almost complete	Complete	Complete	Complete
,, 0·1 c.c. .	Lecithin-cholesterin	0	Very faint trace	Trace	Marked	Almost complete	Complete

CONTROLS.

Serum, 0·05 c.c. + NaCl solution 0·6 c.c. + complement 0·03 c.c. = just complete.
,, 0·2 c.c. + ,, ,, 0·6 c.c. + ,, 0·03 c.c. = very marked.
,, 0·5 c.c. + ,, ,, 0·6 c.c. + ,, 0·04 c.c. = ,, ,,
C.S. Fluid, 0·1 c.c. + ,, ,, 0·6 c.c. + ,, 0·02 c.c. = complete.
Emulsions, 0·6 c.c. + complement 0·06 c.c. = complete.

Dose of complement = 0·015 c.c.

attributed to the increased amount of natural immune body for the test-corpuscles introduced with the larger amount of human serum. This explanation obviously cannot hold in the present instance, where increasing the amount of human serum has opposite effects in the lecithin and the lecithin-cholesterin series.

The question then arises as to whether a serum which in the usual amount gives only a very weak positive reaction may not show its syphilitic character when tested in larger amounts. The results of such an experiment are given in Table XIV. The case was one of general paralysis in which the serum was found on repeated examinations by the ordinary method to give at most a doubtful or a very weak positive reaction over an interval of several months.* The fresh cerebro-spinal fluid from the same case when tested in an amount of 0·1 c.c. along with 0·6 c.c. of the emulsions gave always a distinct positive reaction; accordingly, by way of control, the cerebro-spinal fluid was tested at the same time as the serum. From the results it appears that in this case with 0·2 c.c. of serum a slightly more definite positive reaction is obtained than with 0·05 c.c. With 0·5 c.c. of serum, however, the reaction is again less marked.

The practical conclusion to be drawn from these experiments is, that if repeated examinations of a serum in the usual amount fail to give a definite positive result, different amounts may be tested simultaneously in view of the possibility of finding an optimum; but the procedure is not likely to yield much more reliable information.

Other Properties of Syphilitic Sera

Lecithin-precipitation

Porges and Meier [27], Elias, Neubauer, Porges and Salomon [28] etc., have found that syphilitic sera caused precipitation of lecithin from a watery suspension, as well as of other hydrophilic colloids,† e.g. glycocholate of soda. Normal sera have the same action, and the latter authors came to the conclusion that the phenomenon was of identical nature with both syphilitic and non-syphilitic sera, since with

* The diagnosis of general paralysis was confirmed by post-mortem examination.

† Hydrophilic colloids are those which in solution in water cause an increase in the viscosity.

both, acid aided, and alkali and heat inhibited the phenomenon, and since both showed optima in their reaction-curves where all sera might be regarded as positive. The differences were held to be merely quantitative; thus with a fixed amount of lecithin a smaller quantity of syphilitic than of normal serum caused the appearance of precipitate, and a larger amount of syphilitic than of normal serum required to be added before the precipitate ceased to appear. The greater breadth of reaction-zone was attributed to the greater instability of the reacting substances in the syphilitic serum. In carrying out the syphilis-reaction in the ordinary way with serum no obvious precipitation occurs; but Bruck [29] has observed the formation of precipitates when syphilitic serum was mixed with alcoholic extract of syphilitic liver and kept in the ice-chest over night (Bruck and Hidaka [30]); Mackenzie and Morton have also observed the formation of precipitate on mixing positively reacting cerebro-spinal fluid with alcoholic organ extract (*v.* p. 138). Extraction with ether deprives syphilitic serum of its power of precipitating lecithin (Pick and Pribram [36]).

The Klausner [31] phenomenon

This consists in the appearance of a precipitate when a mixture of fresh syphilitic serum (0·2 to 0·3 c.c.) and distilled water (0·6 to 0·7 c.c.) is incubated for some hours. The reaction lacks specificity, thus Hayn (*v.* Bruck [21]) found that of 83 non-syphilitic sera, none of which reacted positively in the Wassermann syphilis-test, 19 gave a positive Klausner reaction. Sera from cases with fever give the Klausner-test. Heating at 55° C. or keeping the serum abolishes the effect (Sachs and Altmann,[32] Citron [33]), and acid restores it (Sachs [20]). Sachs and Altmann [32] have described precipitum formation when dilute alcohol and other reagents are added to syphilitic sera.

The phenomena of precipitum formation already described have been held to account for the absorption of complement in the syphilis reaction (Liefmann [46]); but the two processes do not run parallel. The reacting substances are colloids, and in their interactions they behave as colloids do, but it is obvious that this leaves the specificity of the complement deviation reaction quite unexplained. It has not been determined whether the specific alteration in the serum resulting

from syphilis is qualitative or merely quantitative. In the case of a positively reacting cerebro-spinal fluid the change appears to be definitely qualitative (*v.* p. 136).

Anticomplement Action

Ehrmann and Stern [1] state that syphilitic sera by themselves in large amounts (1 c.c.) have an anticomplement action, whereas normal sera have not. As has been already shown, we always estimate quantitatively the anticomplement effect of sera which are being tested for the syphilis reaction, and we have not found any constant difference in this respect between negative and positive sera (see also Hecht [3] and the examples in Table XIII). Pick and Pribram [36] found that syphilitic sera after treatment by ether—the ether being removed subsequently or allowed to evaporate—became inhibitory to complement. Satta and Donati [37] confirm the result; but find that this effect of ether is not specific for syphilis.

Reaction

Syphilitic sera possess a normal degree of alkalinity (Elias, Neubauer, Porges, and Salomon [28]).

Alterations in the Protein Constituents

The alterations in the protein constituents of serum following syphilitic infection are considered below.

Constituents of the Serum with which the Syphilitic Reacting Bodies are associated

Numerous workers have agreed that the reacting power of a syphilitic serum is resident in the globulin fraction (Landsteiner and Müller,[35] Gross and Volk,[38] Noguchi,[7] Bauer and Hirsch [39]); this is also true of cerebro-spinal fluids (*v.* p. 136).

The various workers, however, show considerable diversity in the details of their results. Thus Gross and Volk found that the euglobulin of human serum, both normal and syphilitic, had by itself in the heated as well as the unheated state, an inhibitory effect on complement; Noguchi, on the other hand, found that the euglobulin of syphilitic, but not of normal serum reacted positively. Landsteiner and Müller [35] found that globulin precipitated by CO_2 from syphilitic serum gave the Wassermann reaction after heating at 56° C., but normal globulin reacted only weakly after heating. Spiegler,[40] and Elias, Neubauer, Porges and Salomon [28] found no constant altera-

tion in the proportion of albumin to globulin in syphilitic sera, although in Spiegler's series a marked relative diminution of globulin was frequent, but had no definite relationship to treatment or any clinical factor. Noguchi, however, states that in untreated or slightly treated cases of primary and secondary syphilis the globulin content of the serum was increased; but there was no parallelism between the amount of globulin and the degree of the Wassermann reaction (for the controls necessary before such conclusions can be justifiably drawn *v.* p. 54). In the case of cerebro-spinal fluid from cases of general paralysis the correspondence between globulin-content and Wassermann reaction was even less marked (Noguchi, *v.* also p. 143). The reacting power of the globulin fraction of syphilitic serum is destroyed by tryptic and peptic digestion and cannot be extracted with alcohol (Noguchi). After precipitin-reaction takes place, i.e. after the addition of antihuman serum to syphilitic serum, the complement binding property in the presence of "syphilitic antigen" disappears (Noguchi [75]). Sachs [20] concludes from the experiments on the effects of heat and of acid and alkali that the active component in syphilitic serum may be a denaturalised protein of the nature of acid-albumin. The association of the syphilitic reacting body with the globulins finds an analogy in the fact that antitoxins (diphtheria, tetanus) are precipitated along with the serum globulins (*v.* Pribram [70], Ledingham [71]).

Of course, these analogies do not permit of any further identification of the syphilitic reacting body with immune antisubstances. This is obvious when the chemical character of the globulins is considered (*v.* Mann [72]); they are protein bodies of acid character which are more unstable and more readily denaturalised than the albumins. Their instability would suggest that they may be very actively concerned in biochemical processes, hence a qualitative and quantitative alteration in these bodies under a wide variety of abnormal conditions (infections, intoxications, etc.) is not surprising. The alteration peculiar to syphilitic globulin is, however, shown by the biological test more definitely than by ordinary chemical methods, a fact which corresponds with the delicacy of biological reactions in general. Whether an abnormal lipoid element is also concerned in the reacting substances of the serum is not definitely established (*v.* p. 93).

Friedemann [73] has recently carried out extensive experiments

on the constituents of the serum concerned in the Wassermann reaction and has come to the following conclusions.

(1) The globulin fraction of many unheated normal sera (obtained by half saturation with $(NH_4)_2SO_4$) deviates complement along with alcoholic organ-extract, but by itself does not inhibit complement, i.e. it gives a positive Wassermann reaction. The globulin of some normal sera, however, has by itself an anticomplement action.* The globulin of syphilitic sera reacts positively; it has no anticomplement effect by itself.

(2) The euglobulin of all sera, both normal and syphilitic (obtained by one-third saturation of unheated serum with $(NH_4)_2SO_4$), has by itself an anticomplement action.

(3) The globulin precipitated from normal serum which has previously been heated for half an hour at 56° C., does not give a positive Wassermann reaction and does not by itself inhibit complement. But a positively reacting globulin after precipitation from unheated normal serum continues to give a positive Wassermann reaction after heating at 56° C., although the complement deviation is more marked with the unheated globulin. On the other hand, the globulin precipitated from heated syphilitic serum gives the Wassermann reaction.

(4) The pseudo-albumin of both normal and syphilitic serum (i.e. the constituents of serum which remain in solution on one-third saturating with $(NH_4)_2SO_4$) when mixed with normal globulin—both being in concentrated solution—deprives the latter of its power to give a positive Wassermann reaction. *Syphilitic globulin, however, is not affected by the addition of pseudo-albumin.* Heating at 56° C. deprives the pseudo-albumin of its "antiglobulin" action.

(5) The antiglobulin action of albumin and the anticomplement action of globulin are not dependent on complement components—end-piece or middle-piece respectively (*v.* p. 92).

Friedemann considers that the anticomplement effect of globulin is due to globulin-soap compounds, and that the extract also acts in virtue of containing soaps. The Wassermann reaction would thus be due to the extract intensifying the anticomplement effect of the globulin. It is to be regretted that this explanation of the action of the "antigen" does not include a consideration of the facts available as to the

* The globulin of the same individual's serum may at one time have an inhibitory action by itself on complement and on another occasion be free from anticomplement effect.

effects of cholesterin along with lecithin in the syphilis reaction.

Mackenzie and Morton in their investigations on cerebro-spinal fluid have obtained results (unpublished) which differ from those of Friedemann in the examination of blood serum. Thus, Mackenzie and Morton found that the globulin fraction, precipitated by half saturation with $(NH_4)_2SO_4$, from unheated cerebro-spinal fluid in cases of acute meningitis and of epilepsy (non-syphilitic conditions with high proteins content of the cerebro-spinal fluid) did not in any instance either give a positive Wassermann reaction or inhibit complement by itself, whereas the globulin from positively reacting cerebro-spinal fluids invariably gave a positive syphilis reaction (*v.* p. 136).

Constituents of the serum which interfere with the syphilis reaction are present after heating at 55° C., according to Noguchi and Bronfenbrenner.[79] Wechselmann's[80] method of converting negatively reacting sera from secondary syphilitics into a positive state (0·9 c.c. heated serum + 3 c.c. saline +0·5 c.c. 7 per cent. suspension of freshly precipitated $BaSO_4$ shaken up and kept 1 hour at 37° C.; centrifugalise and pipette off the serum which is ready to be tested) depends on the removal of the inhibitory substances by the $BaSO_4$. Noguchi and Bronfenbrenner find that larger amounts of $BaSO_4$ may remove the syphilitic "antibody" from the serum also.

The property of absorbing complement in the presence of lipoids has also been found associated with milk (Gross and Volk,[38] Thomsen[65]) pleural and peritoneal exudates and albuminous urine (Bauer and Hirsch[39]) from syphilitic cases.

Effect of Temperature on the Complement-Absorbing Power of the Mixture of Syphilitic Serum and Antigen: Fate of the Complement

The complement is absorbed at 0° C., but perhaps more slowly than at 37° C. (Satta and Donati[61]). Guggenheimer[82] finds that with some sera more complement is absorbed at 0° than at 37° C. The amount of complement absorbed is the same when the "antigen" and serum are freshly mixed and complement added or when the mixture is allowed to stand for some time before the addition of the complement (*v.* Seligmann and Pinkus[2]).

Fate of the Complement.—Michaelis and Skwirsky[41] (confirmed by Henderson Smith[44] and Amako[45]) have shown

that in the syphilis-reaction only one constituent of the complement is fixed, namely the *middle-piece.*

Complement can be more or less successfully broken up by a variety of procedures, dialysis, treatment with dilute acid or acid salts, treatment with red corpuscles in the presence of excess of immune body at 0° C. Thus Michaelis and Skwirsky [43] have shown that if 0·5 c.c. blood suspension sensitised with a large amount of immune body (40 to 50 doses) is mixed with 1 c.c. of a solution containing 16 parts of $\frac{1}{7}$ normal NaH_2PO_4 and 1 part of $\frac{1}{7}$ normal Na_2HPO_4, and complement (0·05 c.c. guinea-pig's serum) is added, no lysis occurs at 37° C. If the mixture after incubation is neutralised by adding a similar amount of a mixture of the two phosphates in converse proportions (i.e. 1 c.c. of a mixture of $\frac{n}{7}$ NaH_2PO_4 1 part + $\frac{n}{7}$ Na_2HPO_4 16 parts) lysis occurs. If, however, the unlysed corpuscles are separated from the acid mixture by centrifugalising and are suspended in saline, they do not undergo lysis, and the separated fluid when neutralised does not lyse sensitised corpuscles. Accordingly, in the acid medium one portion of the complement, the *middle-piece,* has become attached to the RCS + IB, the corpuscles being said to be *persensitised,* while the other portion of the complement, the *end-piece,* remains in the fluid. Thus, if on introducing sensitised corpuscles into a fluid no lysis occurs, but on introducing persensitised corpuscles lysis occurs, then the fluid contained only the complement *end-piece.* This is the case in the mixture of syphilitic serum and antigen to which complement has been added in such amount that it has been "absorbed." This action on complement corresponds with what occurs in antibody reactions; thus mixtures of serum + antiserum, tuberculin + antituberculin, absorb only the *middle-piece.* On the other hand, where haemolysis occurs through the action of complement and immune body on red corpuscles, the whole complement is bound, and the same is the case with the non-specific absorption of complement by kaolin (Skwirsky [42]). It does not appear to have been determined whether the complement end-piece can be recovered quantitatively from the mixture of syphilitic serum and "antigen."

Theories to account for the Syphilis Reaction as a Biological Phenomenon, and to Correlate it with Pathological Changes

A number of hypotheses have been advanced to account

for the syphilis reaction. These will be only briefly mentioned as they do not rest on any very conclusive experimental evidence.

I. Is there a Specific Antisubstance to the Spirochaete Pallida in Syphilitic Serum?

The existence of such a specific anti-substance has been maintained by Wassermann, Neisser and Bruck, and others. In support of this view are the experimental results of Bruck [21], who found that the serum of monkeys which had been inoculated with watery extracts of syphilitic organs containing dead spirochaetes came to react positively. But such sera (Bruck and Stern), and also positively reacting human cerebrospinal fluid, have no protective effect when a mixture with living spirochaetes is injected into animals (Marie and Levaditi [69]). In the absence of pure cultures of the spirochaete pallida it is, of course, impossible to settle the question. The important fact, however, is that in syphilis the serum acquires the power of reacting with substances which do not contain any specific constituents of the spirochaete pallida, and that this "non-specific" reaction is the more prominent characteristic, although Wassermann [48] maintains that "whoever works with an extract derived from syphilitic organs determines those substances in the blood serum which have been developed against the cause of the disease," and cites the results of Dean, who found that the sera of congenital idiots practically never gave the reaction with the alcoholic extracts of normal organs, while they reacted typically with the aqueous extract of syphilitic organs. We have, however, obtained positive reactions with the sera of congenital idiots, using alcoholic extract of normal ox-liver (*v.* p. 125).

II. Theories advanced to explain the Reaction of Syphilitic Serum with Lipoids

(*a*) *Autoantibody Formation.*—Weil and Braun [50] consider that in the course of the disease tissue products (albumin-lipoid complexes) are absorbed and give rise to antibodies. The antibody thus formed is supposed to have the power of reacting *in vitro* with the lipoid component of the albumin-lipoid complex, the lipoid portion, of course, being alone present in alcoholic tissue extracts. Toyosumi's [77] observation that organ-cells, but not bacterial emulsions, absorb the reacting

bodies from the serum, has been cited in support of the auto-antibody theory (*v.* also Guth [81]).

(*b*) *Toxolipoid-Antibody.*—According to Citron's [51] view there is a syphilitic toxin which has an affinity for lipoids: the toxolipoid compound then acts as antigen and leads to the production of an antibody in the serum, which has the power of reacting in *vitro* with the lipoid by itself. The lipoid would thus stand in the same relationship to toxolipoid as diphtheria toxoid does to diphtheria toxin.

Lipoids such as lecithin do not act as antigens (*v.* Bruck [21]); accordingly Weil and Braun's and Citron's theories rest on a similar basis, namely, the assumption that some body belonging to the class of antigens (tissue protein, syphilitic toxin) combined with lipoids gives rise to an antibody.

These theories were strongly supported by the analogy of cobra-lecithid, but this body has now been shown by Manwaring [53] to be merely a modified lecithin containing no component of cobra-venom and devoid of the power of giving rise to an antibody. Further evidence against the view of antibodies being concerned is that filtration of syphilitic serum through a collodion sac or a porcelain filter deprives it of its reacting power (*v.* p. 81), whereas true antibodies pass through collodion (Frouin [64]). Accordingly, there is little definite evidence in favour of those views. As Sachs and Altmann [52] point out, an equally tenable theory would be that the tissues undergo such an alteration in syphilis that they can form antibodies to lipoid substances.

(*c*) Peritz [56] considers that (1) in syphilis the serum contains more lecithin than normal, and the sera of old syphilitics and tabetics who react negatively contain a great excess of lecithin, and (2) lecithin injections can transform a positively reacting serum into a negative one. Accordingly there is in syphilis a body (syphilis toxin) with an affinity for lecithin. If this affinity is unsatisfied then the serum reacts positively *in vitro*, and vice versa. Other observers have been unable to confirm this result of lecithin injections on the syphilis reaction (Bruck and Stern [57]).

(*d*) Manwaring [58] holds that there is a proteolytic ferment present in guinea-pig's serum which tends to destroy the complement; substances present in the syphilitic serum and organ-extract intensify and hasten this effect.

(*e*) According to Kiss [59] organ-extract tends to poison the complement, and syphilitic serum increases this action.

(*f*) As a result of the observations that sera may react positively after narcosis, Bruck [57] has given up the view that autoantibodies are concerned in the reaction. Bruck and Stern [57] hold that the complement fixation is due to the interaction of the extract with protein-lipoid bodies present in the serum, which are of allied character or identical with the substances in the organ-extract. They base this conclusion on the following experiments. Normal serum previously heated for 30 minutes at 55° C. was treated with syphilitic liver and normal guinea-pig's tissues (liver, heart, brain) for three days at 37° C., choloroform being added as antiseptic. They then found that the treated serum in some instances caused the fixation of complement along with alcoholic extract of syphilitic liver. It is to be noted that frequently the treated sera had by themselves either a powerful anticomplement effect or else were actively haemolytic. Hecht [60] considers that the phenomenon of Bruck and Stern is merely a summation effect. The theory of Bruck and Stern corresponds closely with the explanation of the syphilis reaction originally advanced by Levaditi and Yamanouchi,[11] who held that the active substances in the serum or cerebro-spinal fluid were non-protein colloidal bodies. A similar view is taken by Mott [74] in the case of parasyphilitic diseases : he holds that the nerve elements being perpetual and having acquired a habit of increased metabolic activity, will continue it during life and will contribute to the excess of lipoids in the blood, and he advances the theory that it is in virtue of the entrance of the products of the degeneration of nervous tissue into the blood and cerebro-spinal fluid that they yield the reaction. According to Peritz,[56] the syphilitic toxin deprives the nervous tissue of its lecithin, which is then found in excess in the serum. Noguchi, however, found no quantitative difference in the amount of alcohol soluble matter from normal and syphilitic serum and blood coagulum ; and Morton has observed that the cerebro-spinal fluid from cases of general paralysis does not contain a larger amount of lecithin than fluids with a corresponding protein-content from non-syphilitic cases. Friedemann's view has already been referred to (*v.* p. 90).

Summary of the Principal Facts

1. As the result of syphilitic infection the serum (and under certain conditions the cerebro-spinal fluid) acquires in a greatly enhanced degree the property of reacting with certain lipoid

substances in such a way as to lead to the absorption of a constituent of haemolytic complement (the middle-piece).

2. The principal lipoid substances with which syphilitic serum reacts are soaps, glycocholates, and especially "lecithin," heart and liver lecithin being most efficient. The reaction is more marked with mixtures of lipoids, especially lecithin and cholesterin. The degree of the reaction depends to a great extent on the physical state of the lipoids, other things being equal.

3. Cholesterin has the property of increasing the amount of complement which is absorbed by a mixture of syphilitic serum (or cerebro-spinal fluid) and lecithin. This action is specific.

4. The reacting power of sera is diminished by heating at 55° to 57° C. for 30 minutes and this treatment deprives all but syphilitic sera of their reacting power as tested under the conditions laid down.

5. The reacting power of the serum and cerebro-spinal fluid is resident in the globulin-fraction.

6. No other property of syphilitic serum or cerebro-spinal fluid, so far as is at present known, runs parallel with the power of deviating complement along with suitable lipoid mixtures. This property is the most delicate and constant indication of syphilitic infection.

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CHAPTER VII

THE CLINICAL APPLICATION OF THE SYPHILIS REACTION

SPECIFICITY OF THE REACTION—OCCURRENCE OF THE WASSERMANN REACTION IN THE VARIOUS STAGES OF ACQUIRED SYPHILIS AND ITS DURATION—CLINICAL SIGNIFICANCE OF A POSITIVE REACTION—DIAGNOSTIC VALUE OF THE REACTION—THE EFFECT OF MERCURIAL TREATMENT ON THE WASSERMANN REACTION—THERAPEUTIC BEARING—PARASYPHILITIC DISEASES—CONGENITAL SYPHILIS—SYPHILIS AND CONGENITAL MENTAL DEFICIENCY

Specificity of the Reaction

THE diagnostic value of the biological reaction rests, of course, on the fact that the abnormal property of the serum on which it depends is peculiar to syphilis. This has been found to be the case, with only rare exceptions. In the first place, it might be expected that a similar change should occur in diseases due to protozoal organisms closely allied to the spirochaete pallida. Thus, positive reactions have been obtained in framboesia (Bruck,[1] Hofmann and Blumenthal,[2] in which the causal organism, spirochaete pertenuis, is morphologically almost indistinguishable from the syphilitic spirochaete, in relapsing fever (Korschun and Leibfreid [13]), also in trypanosome infections ; but it has not been clearly determined whether, in the human subject, infection with trypanosoma gambiense (the cause of sleeping sickness) gives rise as a rule to a positive Wassermann reaction. According to some observers, a positive reaction may be obtained with the sera of malarial patients (Boehm [4]) but the reaction is said to disappear when the parasites disappear from the blood and symptoms cease (Meier and Bonfiglio [6]). Nanu-Muscel and Vasiliu,[5] however, examined twelve cases of malaria (four

chronic and eight acute) and obtained a negative result in all.

Another disease in which positive reactions are frequently obtained is leprosy of the tuberous type, but cases of anaesthetic leprosy (in which the nerves are the seat of infection) usually react negatively (Eitner,[7] Bruck,[1]). A means of differentiating between leprous and syphilitic sera is afforded by the fact that the former also react with tuberculin, whereas syphilitic sera do not. (Meier[8,9], Bruck,[1] Babes[10]). In addition, sera obtained after veronal-morphine-scopolamin-ether narcosis (Wolfsohn,[11] *v.* also Reicher[12]), and in many cases sera obtained just before death or post-mortem (Bruck[1]) may react positively without syphilitic infection existing. Apart from these conditions, it is only with the greatest rarity that positive reactions are obtained with non-syphilitic sera. It is obviously not a sufficient control to compare syphilitic sera with those, say, from cases of non-syphilitic skin affections, as was done by many observers when testing the reliability of the Wassermann reaction. Accordingly, several years ago, we examined a wide variety of acute and chronic diseases in a series of cases in which syphilis could in all likelihood be excluded, as shown in the following table:

Acute tuberculosis	6 cases.
Chronic tuberculosis	5 ,,
Malignant disease	5 ,,
Lobar pneumonia	4 ,,
Bronchopneumonia	3 ,,
Enteric fever	14 ,,
Typhus fever	14 ,,
Scarlet fever (at various stages)	37 ,,
Cerebro-spinal meningitis	3 ,,
Eclampsia	4 ,,
Surgical cases	6 ,,

Employing the sera after they had been heated for half an hour at 55°–57° C., according to the usual procedure, we found that of the 101 cases only one gave a positive reaction, viz., a case clinically diagnosed as chronic phthisis, but in which no tubercle bacilli were found in the sputum. It is to be noted that 37 cases of scarlet fever at various stages were examined, and in no instance was a positive result obtained. This is important in view of the fact that, according to some investigators, the serum from scarlet fever cases occasionally reacts positively. The results of the examination of many thousand

sera by workers in all parts of the world have now established the specificity and clinical value of the reaction. The frequency of a positive reaction with non-syphilitic sera is estimated by different observers at between one per hundred and one per thousand. The former figure probably places the percentage of error too high. It appears, therefore, that *so far as diseases common to this country are concerned, the alteration in the serum which gives rise to a positive Wassermann reaction is peculiar to syphilis.*

Occurrence of the Wassermann Reaction in the Various Stages of Acquired Syphilis and its Duration

Primary Stage.—Only exceptionally has a positive reaction been observed before the appearance of the chancre. Thus, in one instance, Lesser (cited by Bruck [1]) found a positive reaction in a young man eight days after inoculation ; and the sore did not appear until a fortnight later. In general, the property of giving rise to a positive reaction is acquired by the serum in the late period of the primary stage. Levaditi, Laroche and Yamanouchi [14] found that the reaction became apparent from the tenth to the thirtieth day after the appearance of the sore, that is to say, about five to eight weeks after inoculation. It is a fact of considerable importance both for diagnosis and treatment that an undoubted positive reaction is frequently obtained before the appearance of secondary symptoms. With increasing age of the infection the proportion of positive reactions increases until, in the *secondary stage*, 95 per cent. of cases are positive. Over 75 per cent. of *tertiary* cases react positively.

Latent Periods.—It is characteristic of syphilis, in common with other protozoal diseases, that the symptoms tend to disappear spontaneously at certain periods ; a state of equilibrium between host and parasite is set up, or, as Ehrlich terms it, a non-sterilising immunity. The continued existence of the living virus, however, renders the patient liable to recrudescence of the disease, and this is the rule in syphilis—in fact, it is doubtful if a spontaneous cure, in the sense of complete destruction of the parasites, ever occurs. During the latent periods it is found that an increased number of cases fail to react positively ; still, a positive reaction is obtained in 50 per cent., which is a very considerable proportion when it is remembered that such latent cases may be apparently in perfect health, and in the absence of a history, present no

clinical basis for diagnosis. In some cases during the latent period a negative reaction may be transformed into a positive one by the administration of a specific spirillicidal agent, e.g. mercury or salvarsan. This is no doubt a part of the phenomenon described under the name of the *Jarisch-Herxheimer reaction*. It has been attributed either to the effect of endotoxines liberated from spirochaetes destroyed as the result of the drug, or to a stimulation of the spirochaetes by a dose of the drug which did not suffice to cause their death (*v.* p. 174).

Valuable information as to the significance of the *law of Colles* has been obtained by the examination of the sera in such cases (Knöpfelmacher and Lehndorff [15], also *v.* Bruck [1]). It had long been known that the mothers of manifestly syphilitic children might themselves show no signs of infection, and also that they could suckle their infected children without developing a sore; such women have also proved resistant to inoculation with syphilitic material (*v.* Neisser [3]). Knöpfelmacher and Lehndorff found that the sera of 90 per cent. of the mothers when tested within a few months after the birth of the syphilitic child, reacted positively, and the mother may react positively at a time prior to the appearance of a positive reaction in the child. A positive reaction has further been obtained with the sera of fully 50 per cent. of such women at an interval of years after the birth of the last syphilitic child, that is to say, in this respect they belong to the same category as latent syphilitics. It has been held that the foetus became infected directly from the paternal side, and that then immune substances passed through the placenta into the maternal circulation. The immunity of the mother would thus be of a passive nature. There is little evidence in support of such a view. Thus, the positive reaction of the mother has been found in half of the cases to persist for a long time after the birth of the syphilitic child, a state of affairs which would be most unlikely were the reacting substances transferred merely passively from the foetal to the maternal tissues, since it is the rule that passive immunity is of short duration. Further, more or less characteristic tertiary lesions have been observed in some cases (McDonagh,[17] *v.* also Hutchinson [16]), and, as will be shown later, tertiary phenomena are the direct result of living spirochaetes, and not, as was at one time supposed, mere sequels. Hochsinger [30], however, maintains the existence of a purely paternal syphilis in at least some cases, but bases

his opinion on clinical observations; this view would also appear to be supported by the occurrence of certain exceptions to the law of Colles, i.e. women who after the birth of syphilitic children proved to be susceptible to syphilitic infection (*v.* Neisser [3]).

In general, it may be said that 85 *per cent. of cases, in which there are active syphilitic lesions, give a positive Wassermann reaction.* It is apparent from what has been said that a certain small proportion of cases may fail to give a positive reaction, in spite of the existence of an undoubted syphilitic lesion. In some instances, e.g. recurrences after treatment (*v.* p. 253), a probable explanation is, that the parasites which have persisted and have then caused the lesion, are too restricted in their distribution to give rise to that degree of metabolic alteration which is necessary to produce a positively reacting serum. No satisfactory explanation of other cases has been offered, beyond the general one that almost no biological phenomenon is found to be of universal occurrence.

With regard to the date after infection at which a positive reaction may still be obtained no limit can be set, as cases have been found to react positively forty to fifty years after the original inoculation (Wassermann [18]).

Clinical Significance of a Positive Reaction

As has been shown, a positive reaction affords conclusive evidence that infection with syphilis has occurred. Two other important points as to the significance of the reaction require to be considered: firstly, what relation does it bear to the dissemination of the virus through the tissues of the host, and, secondly, does a positive reaction indicate that the pathogenic agent is still alive and infective, or may the reaction persist for a long time after the spirochaetes have been killed off? It is conceivable also that a positive reaction once established as the result of syphilitic infection, might disappear after the death of the spirochaetes, and then reappear subsequently as the result of some other condition.

With regard to the first question, the positive reaction appears after dissemination of the spirochaetes has occurred, and is to be regarded as the expression of a chemical alteration in certain constituents of the serum resulting from the action of the spirochaetes on the tissues. The conclusion follows from clinical as well as experimental observations. Thus, a

positive reaction is not obtained usually until the primary sore has appeared, and it is well established that by the time there is a manifest chancre it is impossible to prevent the signs of generalisation of the virus (secondary stage) by amputation of the sore. In monkeys also, the serum acquires the power of reacting positively at the same time that the internal organs become infective (Bruck [1]). The appearance of the syphilis reaction in the human subject corresponds with the time of onset of immunity of the skin to fresh inoculations (Levaditi, Laroche and Yamanouchi [14]).

The question as to whether a positive reaction invariably denotes the presence of living spirochaetes is of great importance from the point of view of both diagnosis and treatment. No one has doubted the syphilitic nature of the secondary manifestations, since patients at this stage were seen to be actively infective. Similarly, the existence of the disease in a latent form had to be admitted, as infection might be transmitted by individuals presenting no signs of disease at the time. On the other hand, patients with massive tertiary lesions, as a rule, are non-infective, and so also are those with parasyphilitic affections. This led to the view that the latter conditions were more or less of the nature of sequels, to which the subject of a foregoing syphilitic infection became predisposed, just as scarlet fever, for example, predisposes to otorrhoea. The true syphilitic nature of tertiary lesions has been definitely proved, however, since the presence of spirochaetes has been demonstrated (e.g. in aortitis by Reuter [20]), and also since frequent positive results have followed the inoculation of material from closed gummata into monkeys (Neisser [21]); in one positive case of Hoffmann's the gumma used for inoculation was taken from a patient who had been infected twenty-four years before. The number of spirochaetes in tertiary lesions is very small, and this, together with the site and localised character of the lesions, probably explains why patients at this stage are practically non-infective. Thus, even when the gummatous material is utilised directly for experimental inoculation, the disease fails to develop in a considerable number of the animals employed. It may be asked, then, why the lesions in the tertiary stage should attain such large proportions as compared with those occurring at earlier periods. In explanation of this, the theory has been advanced that the tissues of the host become allergic in the tertiary stage, so that they react

excessively to the products of scanty parasites when compared with the slight reaction to abundant parasites in the secondary stage (Finger and Landsteiner, *v.* Levaditi [22]). This theory is based on the analogy of the allergic or anaphylactic phenomenon; this is well seen in the guinea-pig, for which a single dose of serum, e.g. of the horse, is quite innocuous, but for which a second dose under suitable conditions is highly toxic, and may be fatal; the tuberculin reaction is of similar nature. The question of the etiology of parasyphilitic affections is considered in detail later (*v.* p. 112), but it may be noted here that there is considerable evidence in favour of these conditions also being due to living spirochaetes, as parasyphilitics have been found relatively immune to reinoculation with syphilis (Krafft-Ebing,[43] Mott [23]) and Neisser[3], [21, 24] has shown experimentally that such immunity means that living parasites are still present.

Diagnostic Value of the Reaction

From what has been already said as to the constant occurrence of a *positive reaction* in the presence of active syphilitic manifestations, it follows that a positive result is of great value in determining the syphilitic character of extra-genital sores and of atypical lesions of all stages. It is scarcely necessary to go into this question further, since an almost endless variety of indefinite but refractory symptoms have been proved to be syphilitic by the Wassermann test, and the diagnosis has been confirmed by cure consequent on antisyphilitic treatment, e.g. chronic sore throat and nasal catarrh, neurasthenic conditions, etc. The Wassermann test enables the nature of the condition to be settled directly and not merely by inference from the effect of treatment or from transmission of a typical infection to others. It has been proved that primary syphilitic manifestations may show all gradations from the typical indurated chancre to a mere abrasion. More or less complete suppression of either primary or secondary appearances, or of both, is probably less uncommon than was at one time supposed; and as in other infectious diseases, the clinically unrecognisable cases constitute a considerable proportion, so that many patients may be sincere in denying knowledge of early symptoms. A remarkable instance of modification of the disease is afforded by the women who are examples of the "law of Colles." On the other hand, a *negative reaction*, even provided there is no fault in the method of the

test, does not definitely exclude syphilis, but it is most unlikely that a negative reaction will be obtained repeatedly if an active syphilitic lesion is present. Where some form of treatment has been adopted, e.g. as a prophylactic measure, the chance of atypical manifestations is, of course, increased; e.g. the case observed by Emery (*v.* Neisser [21]), where treatment of the glans penis with powdered corrosive sublimate immediately after infection apparently prevented the appearance of a chancre, but secondary lesions occurred after a latent period of three months.

Bassett-Smith [25] has proved by the Wassermann test that in many instances "*soft sores*" are in reality syphilitic.

In the *primary stage*, the serum reaction is supplementary to the demonstration of spirochaetes as a method of diagnosis. Spirochaetes are, of course, usually to be found in the secretion of the sore for a considerable time before the serum gives a positive reaction; but although they may be numerous and easily found, it is a matter of general experience that spirochaetes are frequently difficult to demonstrate. Again, if a case is seen when the primary lesion has healed, but before the onset of the secondary stage, one has to rely on the information supplied by the serum reaction. The following cases illustrate the application of the two methods of diagnosis in the primary stage.

Case I.—A man with an uncharacteristic sore on the prepuce of two weeks' duration, but without induration: Wassermann reaction negative. The dates of possible infection were six weeks and two weeks before the appearance of the sore. Amputation was performed, and spirochaetes were demonstrated in the tissue by the silver method.

Case II.—A woman, aged 50, had a septic ulcer of about the size of a shilling on the right side of the lower lip; there was no induration. It had been first noticed four weeks previously, when it was of the size of a pin's head. Two weeks after its appearance the submaxillary glands on the right side began to enlarge, and at the time of the examination they were of the size of a hen's egg. A positive Wassermann reaction was obtained with the blood serum, and numerous spirochaetes were also found in the secretion of the ulcer. Six days later a typical secondary rash appeared.

Case III.—A married woman, suckling her child, had an ulcer at the side of one nipple of six weeks' duration. The axillary glands on the same side were palpable. There had

been no rash. The Wassermann reaction was positive, and the ulcer healed after the administration of mercury.

Case I illustrates the value of the examination for spirochaetes in early cases before the serum reaction is apparent. In cases II and III the question of tumour had to be considered, and the results of the serum reaction confirmed the diagnosis of syphilis.

In cases where the diagnosis lies between a lesion of the *secondary stage* and a non-syphilitic condition, the Wassermann test is obviously of the utmost value, since a positive reaction is given almost without exception at this period. Accordingly a negative reaction will enable syphilis to be excluded with almost absolute certainty.

Where spirochaetes are extremely scanty, as in *tertiary lesions*, the Wassermann reaction is alone available. But it must be borne in mind that a positive reaction merely proves that the individual is syphilitic, and does not indicate that the lesion in question is necessarily due to the action of the parasites. An obvious application of this caution is to be observed in lesions of the tongue, for it is known that epithelioma frequently supervenes on syphilitic leukoplakia. Out of 12 cases of leukoplakia examined by Bruck,[1] 10 gave a positive reaction. Again, where there are signs of a focal cerebral lesion, the possible co-existence of syphilitic infection and tumour is to be emphasised. Accordingly, the necessity for histological examination should never be lost sight of. *Where the lesion is of the nature of a granuloma, with indeterminate histological characters, and the serum gives a positive reaction, then there is a very strong presumption that one is dealing with a syphilitic manifestation.* This is strikingly illustrated by the following case, for the clinical details of which we are indebted to Dr. Archibald Young.

The patient, a man aged twenty-three, was seen in June, 1910, with enlarged glands on the right side of the neck, the enlargement having been present intermittently since childhood. One of the glands was removed; the histological appearances excluded any malignant tumour, but were not characteristic of tuberculosis. By October, 1910, the glandular enlargement had increased greatly and extended down to the clavicle. There was no ulceration of the mass, although the overlying skin was reddened: the patient had an elevated temperature (102°–103° F.) and suffered from dyspnoea. In November, tracheotomy had to be performed to relieve the dsypnoea; tissue was at the same time removed from the neck; it was mainly caseous, with a small area of granulation tissue; the appearances did not suggest

tuberculosis, and no tubercle bacilli were found. The patient's condition became worse, especially the dyspnoea; a fluctuant swelling had by this time developed in the region of the left sternoclavicular joint. The prognosis of an early fatal issue was made. On December 10, a positive Wassermann reaction was obtained with the patient's serum. Potassium iodide was then prescribed, at first 2 grams and later 4 grams daily. Very rapid improvement in the general condition, dyspnoea, etc., occurred; by January 14, 1911, the glandular enlargement and the sternoclavicular swelling had almost disappeared.

The Wassermann test has shown the frequent association of syphilis with primary aortic valvular disease, as well as with aneurysm. Thus Bruckner and Galasesco [32] obtained a positive reaction in 17 out of 22 cases of aortic regurgitation, and 18 out of 22 cases were found to react positively by Longcope [33] (who gives full references to the literature). Three cases of paroxysmal haemoglobinuria which we examined recently, all had positively reacting sera. Two were children; one had a family history of syphilis; in the other two cases nothing suggestive of syphilis could be made out; but the mother of the second child had a positively reacting serum.

The fact that 50 per cent. of *latent syphilitics* react positively is of very considerable importance. Thus in the early latent period, that is, usually within the first two to three years after infection, absence of clinical signs does not exclude infectivity; a positive reaction, therefore, by leading to the detection of such cases, gives an indication for prophylactic measures. That infectivity may exist over a very long latent period is shown by a case of Hochsinger [30], in which the first and second pregnancies, separated by an interval of eighteen years, both resulted in syphilitic children. The parents denied syphilis and were not treated in the interval; neither showed any evidence of the disease. In addition, a positive reaction at any period most probably shows that the patient still harbours living spirochaetes and is therefore liable to a relapse or to later lesions; hence the necessity for further treatment. In connection with life insurance, too, the importance of detecting syphilitic infection can scarcely be overrated, since it has been estimated that a tenth to a third of those who have been infected with syphilis die from the results of the disease. It is a significant fact that in the experience of the American insurance companies the death rate among more than 5,000 syphilitics who were accepted for insurance, was a third over the expectation (*v.* Brockbank [31]).

The Effect of Mercurial Treatment on the Wassermann Reaction

The general result with regard to the effect of treatment is, that energetic mercurialisation early begun and long continued restores the serum to its normal condition (*v.* Citron [28]).* It is to be noted that both the date of commencement of treatment and the duration of it are important factors. Thus it has been found that a positive reaction is much more readily transformed into a negative one when treatment is begun immediately on the appearance of the primary sore, as compared with cases in which the commencement of treatment is delayed until six weeks after the chancre has appeared (Merz, *v.* Bruck [1]). The longer the treatment is continued the greater the likelihood of restoring the serum to normal. Thus, taking cases in which treatment had ceased several months before, and which were free of obvious lesions at the time of examination, it was found that the number of negatively reacting cases was proportional to the number of courses of treatment, up to six or eight (Pürckhauer,[26] Neisser's clinique). By a course of treatment was understood the injection of 10 c.c. (10 per cent.) calomel suspension, 2 c.c. (40 per cent.) grey oil, 30 to 40 c.c. (3 per cent.) sublimate solution, or 30 to 40 daily inunctions of 1 to $1\frac{1}{4}$ drachms of grey ointment. With lapse of time after inoculation it becomes increasingly difficult to restore the serum to normal. Thus, in tertiary cases it is only with great difficulty that a positive reaction can be transformed into a negative one, even by repeated courses of treatment. (Pürckhauer,[26] also *v.* Bruck [1]). Accordingly, it is clear that treatment which is directed merely to removal of the external manifestations is quite inefficient. The most favourable cases are those in which treatment is commenced before the serum has begun to react positively, and in which the reaction remains negative throughout. It has been found not infrequently that although the reaction was negative to begin with, it became positive in spite of treatment. Such a result shows that the treatment was inefficient. In this connection it is to be borne in mind that the Wassermann reaction indicates an alteration of the serum which is the most constant

* The view that the negative reaction after treatment was not due to the restoration of the patient's serum to a normal state, but was to be accounted for by the presence of mercurial compounds in the serum and could be reproduced *in vitro* by adding $HgCl_2$ to a positive serum, is quite erroneous (*v.* Ritz [34]).

symptom of syphilitic infection, and that the effect of treatment is greatly influenced by the individuality of the host, certain individuals possessing tissues which enable the therapeutic agent to act with much greater efficiency than others. It is interesting to note, however, that in some instances treatment apparently may have the effect of preventing the appearance of the positive Wassermann reaction without preventing the occurrence of localised syphilitic lesions. Thus in a case which we observed along with Dr. Haswell Wilson, mercurial treatment (1 grain of calomel by the mouth, thrice daily) was begun four days after the appearance of the chancre (diagnosis by demonstration of spirochaetes). There never was any secondary rash, but three and a half months later superficial ulcers of the throat, such as are typical of the secondary stage, developed. At this time the serum reacted negatively. The throat condition persisted for upwards of a fortnight, in spite of the continued mercurial treatment, but it healed within 48 hours as the result of one subcutaneous injection of Ehrlich's salvarsan, 0·6 gram as neutral emulsion.

The effect of treatment with salvarsan on the Wassermann reaction is discussed on pp. 192, 223.

Therapeutic Bearing

It is a matter of general experience that the primary and secondary stages of syphilis are comparatively amenable to treatment. But in the case of the late manifestations a variety of opinion exists, and some actually hold that tertiary and parasyphilitic manifestations are quite beyond the control of early specific treatment. This view is irrational as regards the tertiary lesions, which, as has been seen, are definitely proved to be due to the activity of the syphilitic spirochaete. In the case of parasyphilitic affections, the evidence is less conclusive but the analogy with sleeping sickness and the beneficial effect of treatment with an antiparasitic drug, such as Ehrlich-Hata's salvarsan, make the presumption very strong that here also one is dealing with pathogenic manifestations of the spirochaete pallida (*v.* p. 112).

There is good authority for the view that energetic treatment early begun is of value in preventing late manifestations, and we know from experimental work on the therapy of infections with trypanosomes, which most closely resemble spirochaetes in their pathogenic action, that the difficulty of sterilising the tissues increases with the length of time which

elapses before treatment is begun (Ehrlich-Browning [27]). Accordingly, the value of a method of early diagnosis, such as is afforded by the Wassermann test, is evident. The old procedure of waiting till the secondary eruption appears cannot be defended on scientific grounds. The discovery of a positive reaction in latent cases should be taken as an indication for further treatment. Since there is a good basis for the belief that a positive reaction indicates the presence of living spirochaetes, such positive latent cases are in danger of relapse, and this has been confirmed clinically. Further, such individuals may be infective, and in the case of apparently healthy women who repeatedly abort, the discovery of a positive reaction is an indication for treatment, and mercury has been found effective in some instances in protecting the foetus. According to Hochsinger,[30] treatment restricted to the father has been found to prevent the production of manifestly syphilitic foetuses by apparently healthy women. The susceptibility of the foetus to syphilitic infection obviously may be affected by the state of the father at the time of fertilisation; hence this observation does not exclude the presence of a latent syphilitic infection in the mother. A positive reaction in either parent of a seemingly healthy infant is an indication for treatment of the child also, and this is especially the case if the mother reacts positively.

The question then remains as to *the value of the Wassermann test in determining the duration of mercurial treatment.* It has been seen that the reaction may be positive at a time when there are no obvious manifestations. This might be due to lesions situated in the internal organs; but such is probably the case in only a proportion of instances, as the virus is known to remain alive in monkeys for long periods without producing obvious changes in any tissues (Neisser [3, 21]). At the same time it is clear that the spirochaetes may well exert their pathogenic effects by perverting the tissue-metabolism in such a way as, later on, to lead to premature senescence, without any localised histological lesion being observable at the time of examination. It is reasonable to regard a positively reacting serum as the most delicate sign of generalised pathogenic action on the part of the spirochaetes, and its presence as an indication that serious effects and gross local lesions are liable to occur at any time. Accordingly, a positive reaction would indicate the continuance of treatment. Conversely, a negative reaction, which means the restoration of the serum to its

normal state, signifies generally, if not complete destruction of the parasites, at least the establishment of a condition of equilibrium between the host and the spirochaetes, so that the latter assume the character of harmless saprophytes. If any treatment short of complete sterilisation is to be of value, it must maintain this relationship; hence the Wassermann reaction should be used to control the treatment, as it is usually the most subtle indicator of a disturbance of equilibrium with a tendency to the assumption of pathogenic activity on the part of the spirochaetes. The rational procedure would be to begin mercurial treatment as early as possible and to carry it on as energetically as possible, compatible with the maintenance of good general health, for several years—two at least—after the disappearance of all symptoms, including the positive Wassermann reaction. Thus the serum should be tested at intervals of three to four months during treatment, and at periods of three to six months and a year after treatment has ceased. The appearance of a positive reaction after cessation of treatment is to be taken as an indication for its immediate resumption, and the appearance of a positive reaction during treatment as a sign for more energetic measures, or for the adoption of another form of treatment, as the one in use is inefficient.

The bearing of the Wassermann reaction on treatment with salvarsan is considered later (pp. 224, 292).

Parasyphilitic Diseases

Not the least important outcome of Wassermann's discovery is the new field which it has opened in the investigation of, what are known as, the "parasyphilitic" diseases. That tabes and general paralysis occur in syphilitics is a fact which has long been recognised by clinicians. The relationship of the late nervous degenerations to the early infection has, however, been a subject of endless controversy and indefinite speculation. The theory of a premature decay of the neurone, due to a toxic influence which had already ceased to act some ten or fifteen years previously, is founded on evidence of a very imperfect character. Some observers have suggested that the original syphilitic infection exercised a debilitating effect on the body, thus preparing the way for an invasion of other organisms or for toxines whose action is manifested by degeneration of the central nervous system. This latter hypothesis finds support in the evidence that an active progressive inflam-

matory process does occur in the brain in cases of acute general paralysis. The presence of a positive Wassermann reaction, however, in such cases, constitutes a definite biological bond between the early syphilitic infection and the later nervous manifestations.

That a positive reaction is given by the serum in general paralysis was first demonstrated by Wassermann and Plaut,[35] who found that of 41 cases examined, 32 were positive. Subsequent experience in the method of carrying out the reaction, and the greater accuracy consequent on a knowledge of the sources of fallacy, have established the fact that a positive reaction may be obtained with the blood-serum of almost every case of general paralysis. Plaut [36] examined 200 cases in Kraeplin's clinique and found a positive reaction with the blood in 99·5 per cent., and with the cerebro-spinal fluid in 95 per cent. Lesser [37] examined 62 cases and found them all positive. The whole 139 cases examined by Boas [38] gave a positive result. From the material at our disposal upwards of 150 cases have been examined (*v.* Gilmour), and a positive reaction has been obtained with the serum in 96 per cent. We have further examined 30 cases of locomotor ataxy, and 72 per cent. of these gave a positive serum reaction. The comparatively small proportion of tabetics giving a positive reaction may be attributed to the fact that this condition is more chronic, and more liable to intercurrent periods of arrest than general paralysis. Boas [38] also calls attention to a very significant fact, namely, that while in untreated tabes the reaction was almost always present, in cases treated with antisyphilitic drugs (mercury, potassium iodide or arsenic) it was absent in more than half. He examined 43 cases ; 17 of these had not been treated, and each gave a positive reaction ; of 26 treated cases 11 were positive and 15 negative. It is to be noted that in certain cases of general paralysis the serum may react negatively while the cerebro-spinal fluid gives a positive reaction. Thus, Gilmour has observed (unpublished) four cases of general paralysis in which the serum reacted negatively on repeated examination ; but the cerebro-spinal fluid (tested simultaneously with the serum) gave a marked positive result. Two of the cases died, and the anatomical findings confirmed the diagnosis of general paralysis.

The occurrence of a positive reaction with the cerebro-spinal fluid and its significance, and the diagnostic value of the occurrence of a negatively reacting serum together with

a positive cerebro-spinal fluid, are discussed later (*v.* p. 146). In no other manifestation of syphilis is a positive Wassermann reaction a more constant feature than in general paralysis; and it is only in the early active period of syphilitic infection, when skin and throat lesions are present, that an equally high proportion of positive results can be obtained. This fact has an extremely important bearing on the problem of the etiology of this so-called parasyphilitic condition. It at once suggests the question as to whether general paralysis and also tabes are not expressions of the activity of the syphilitic virus itself.

There is abundant evidence in the literature to suggest that localisation of syphilis in the nervous system is, in some instances, dependent on the particular strain of spirochaete. Some infections lead to degeneration of bone and visceral organs, while others attack the central nervous system. Almost every city asylum has records of cases of tabes and general paralysis occurring in husband and wife. Cases are also on record where parents and children suffered from tabes and general paralysis. Brosius [39] mentions the case of seven glass-blowers, who all developed chancre of the lip from the same blowpipe. It was possible ten years after the infection to trace five of them, and of these, two were tabetic and two were suffering from general paralysis. Erb [40] was able to observe the later history of five cases infected from one source, all of whom developed tabes or general paralysis. Lavellé [41] publishes the details of five cases, all infected from the same source. Three of these died from general paralysis, one from syphilitic meningitis, and one from syphilitic dementia.

In the case of other infections, there is sufficient evidence to show that the toxic products of the organism may vary not only in their degree of virulence, but also in their affinity for different structures. Certain epidemics of enteric fever are characterised by a tendency to haemorrhagic oozing from the bowel, while in others the cases manifest signs of general toxaemia, with cardiac failure due to degeneration of the heart muscle. In scarlet fever the tendency for nephritis to occur in those infected from the same source has also been observed. The toxins which are produced as the result of any infection probably vary, not only in virulence, but also in their affinities for different structures, and the preponderance of certain affinities may be characteristic of certain strains. These analogies support the view that general paralysis and

tabes may depend to some extent on an infection by a particular strain of spirochaete. It must, however, be borne in mind that similarity of temperament and habit, together with a common environment, may exercise a not unimportant influence in the development of conjugal and family cases of parasyphilitic disease.

What other evidence is there then that the syphilitic virus is present in these diseases? As has already been pointed out, in many of the late manifestations of syphilis the spirochaetes have been demonstrated, although in very small numbers. Paucity in numbers and difficulty in staining may explain the hitherto fruitless attempts to find the organism in parasyphilitic lesions.

Recent research in experimental trypanosomiasis has been productive of very suggestive results which may ultimately throw much light on the etiology of tabes and general paralysis. It has now been accepted as a fact that sleeping sickness and trypanosome fever are different phases of the same disease. The infection with trypanosomes is conveyed by biting flies (*glossina palpalis*, etc.), which carry the parasite from one host to another. The various stages of the disease cannot be sharply demarcated. But there is a first or prodromal stage in which the patient suffers from recurrent attacks of fever with constitutional disturbance. The lymph glands are swollen, and the trypanosomes are demonstrable in the blood and in the juice of lymph glands during these attacks. Periods of well-being occur, during which a perfect accommodation may be supposed to exist between the host and the invading parasite. Severe constitutional symptoms resembling malaria, and accompanied by exanthemata of varying appearances, may supervene months or years after the original infection, and during these attacks the organisms are easily recovered from the blood and lymph glands. Another period of quiescence may precede the onset of sleeping sickness, which begins insidiously with indefinite nervous symptoms. Disinclination to work, exhaustion on slight exertion, and perhaps transient oedema of the face, are the earliest recognisable signs. Facial tremor and staccato speech are not infrequent, and psychical disturbance of a melancholic or maniacal character may be present. Even at this stage there may be periods of remission, followed later by more pronounced mental deterioration, accompanied by emotional disturbance and perhaps epileptiform seizures. In

the great majority of cases, if not in all, the disease progresses to a fatal issue ; the appetite, which was at first good, gradually diminishes, and the patient, falling into long periods of sleep, loses flesh, develops decubitus, muscular contracture and abscesses, and dies perhaps as the result of secondary infections, to which his weakness and decubitus have exposed him. This short account serves to show the close similarity which exists between the course of events in sleeping sickness and in general paralysis. In early trypanosomiasis, as in early syphilis, there is a polyadenitis, with the corresponding organisms, present in the glands in both conditions. The striking similarity which exists between the anatomical lesions of late trypanosomiasis and those of parasyphilitic disease has been pointed out by Mott[42], who has shown that the histological lesions in the brain in general paralysis can be distinguished from those in sleeping sickness only by minor differences. The perivascular exudate of lymphocytes and plasma cells, which was thought to be pathognomonic of general paralysis, is now recognised to be a constant feature of late trypanosome infection. Of great interest also in this connection is the fact that in *mal-du-coit*, a venereal infection in horses due to trypanosomes, an ataxic paraplegia may occur, with posterior root degeneration and sclerosis of the posterior columns of the cord. Further, Spielmeyer,[29] by experimental trypanosome infection of dogs, has produced optic atrophy and a lesion of the posterior columns resembling that seen in locomotor ataxy.

Reviewed shortly, the points of resemblance between trypanosome and syphilitic disease, including the parasyphilitic conditions, are as follows—

1. In trypanosomiasis the infecting agent is a protozoon, and this is also the case in syphilis ; both diseases may be transmitted to animals.

2. In experimental trypanosomiasis in dogs, lesions in the central nervous system have been produced, resembling those of locomotor ataxy.

3. There is a considerable similarity as regards early lymph-gland involvement, early febrile and constitutional disturbance with exanthemata, periods of latency, and late involvement of the central nervous system.

4. The cellular characters of the lesions are similar in both cases ; lymphocytes and plasma cells play a prominent part in the reaction. The perivascular infiltration in the brain in general paralysis closely resembles that seen in sleeping sickness.

The failure to demonstrate the organism of syphilis in general paralysis constitutes the missing link in the chain of comparison, though the similarity of the anatomical changes in sleeping sickness and general paralysis, and the association of the former with the presence of trypanosomes in the cerebro-spinal fluid, strongly suggests the possibility that the syphilitic virus is an active agent in the production of parasyphilitic disease.

A further point in favour of the view that parasyphilitic conditions are really due to the activity of the spirochaete pallida is the resistance to reinfection with syphilis shown by tabetics and general paralytics. A second infection with syphilis is extremely rare under any circumstances, and no case of a hard chancre occurring in a tabetic or general paralytic has ever been reported. Krafft-Ebing [43] failed to inoculate with the virus of a typical hard chancre nine cases of general paralysis, which gave no history and showed no signs of syphilis. Such observations have naturally led to the conclusion that one attack of syphilis confers a permanent immunity. It is more likely, however, that the patient still harbours the virus, because Neisser has shown that as long as living parasites are present there is a relative degree of resistance to reinoculation, whereas after thorough treatment of experimental syphilis and trypanosomiasis resulting in complete sterilisation, it is possible to reinfect the animal almost immediately.

It has been found that the administration of arsenophenylglycin and salvarsan in general paralysis is followed in a certain proportion of cases by the conversion of a positive serum reaction into a negative reaction. As in the case of late tertiary lesions, the conversion of a positive into a negative reaction is less frequent than in early cases. The occurrence of this phenomenon, however, strongly supports the view that an active syphilitic process is the cause of general paralysis.

The term "acquired immunity," as applied to syphilis, signifies a condition of equilibrium between the host and the spirochaetes of the original infection, whereby an inoculation with fresh material does not produce the usual manifestations. The existence of latent periods in disease is not peculiar to syphilis. Thus, trauma, an attack of measles, or of influenza are not the causes of tubercular infection; they are conditions which give rise to disturbed relations

between the patient and the organisms which have already been present for some time without causing an active lesion. We have made similar observations in the case of cerebro-spinal fever, where a blow on the head appeared to be the determining factor in the production of the acute manifestations of the disease. Sir Hector Cameron [44] calls attention to the occurrence of such latent periods, extending from twenty to thirty years, in cases of cancer, and also to the recrudescence of pyogenic disease in the abdomen after periods of well-being. In the case of the latent period occurring between the syphilitic and parasyphilitic symptoms, it is not too much to suppose that the *contagium vivum* is still present in a state of accommodation with its host. With reference to the occurrence of inherited general paralysis, it is doubtful whether, accurately speaking, such a condition is possible. It is more reasonable to suppose that the so-called inherited general paralysis is the result of syphilis acquired *in utero*. In many of the reported cases it is stated that there was evidence of congenital syphilis ; and in those cases in which there was no evidence, it is probable that the organism was present in a state of accommodation with its host, so far as the ordinary manifestations of syphilis were concerned. In any case it is unlikely that the neuron-degeneration acquired in one generation as a result of syphilitic infection could be transmitted to the offspring in the absence of the pathogenic agent.

It has frequently been urged that the failure to respond to antisyphilitic treatment is evidence in favour of the view that tabes and general paralysis are not true syphilitic diseases. Authoritative opinion is by no means unanimous as to the inefficacy of antisyphilitic treatment. Besides, it must be recognised that the virus may have become serum-resistant or drug-resistant, a condition well-known in experimental trypanosomiasis, although recent experiments of Margulies [45] make this unlikely in the case of the spirochaete. If drug-resistance can be developed, there is reason to believe that this will occur more readily in the tissues of one individual than another (Browning [27]). It is to be remembered, however, that the therapeutic action of antisyphilitic drugs is due probably in all cases to interaction between the tissues and the drug in the first place ; the failure of the drug in chronic infections may well be due to the tissues having lost the power of reacting with the drug. On the other hand, it may be the case that antisyphilitic treatment is disappointing in such

conditions because of the site of the lesion. In sleeping sickness it is possible by means of certain arsenical preparations to remove the trypanosomes from the blood, though not from the cerebro-spinal fluid. It has been found also that potassium iodide does not pass from the blood-stream into the cerebro-spinal fluid. In bacterial infections the antibodies concerned in the process of immunisation cannot be detected in the cerebro-spinal fluid, and on the strength of this fact the intra-spinal method of serum treatment in cerebro-spinal fever has been resorted to with great success (Mackenzie and Martin, Flexner, Kolle). It is possible, then, that in general paralysis, the site and not the nature of the lesion is largely responsible for the failure of treatment.

The administration of Ehrlich's drug, salvarsan (dioxydiamido-arsenobenzol), in tabes and general paralysis has caused an undoubted improvement in a certain proportion of cases, and it has been shown by Sicard and Bloch [52] that after intravenous injection of this drug arsenic is found in the cerebro-spinal fluid. Such improvement, taken along with the natural remissions that sometimes occur, is strong evidence in favour of the view that those diseases are due to the action of a virus still harboured by the patient, and that this virus is the organism of syphilis.

Congenital Syphilis

One of the most interesting results of Schaudinn's discovery was the observation of the extent to which the tissues of congenital syphilitics are invaded by the spirochaetes. In abortions the parasites are present in enormous numbers, more especially on the mucous surfaces and in the liver and supra-renal glands. In infants who die in the first weeks or months of life, the organisms may still be found in considerable numbers in those internal organs which show pathological changes, or in the skin if an exanthem be present. The spirochaetes are as a rule very scanty in those subjects of congenital syphilis which have survived the first years of life. Organisms have been found, however, in cases where the first evidence of congenital infection manifested itself in the twelfth year.

It was only to be expected, under these circumstances, that the serum reaction, characteristic of acquired syphilis, would also be present in the congenital form of the disease. The earlier investigations on the subject, while showing that the serum of those cases with obvious signs of congenital infec-

tion did give a positive reaction, threw but little light on the more obscure problems associated with this aspect of syphilis. Of great interest and importance are the questions bearing on the relation of syphilis in the parents to that in the offspring, whether the reaction with the blood of the child at birth possesses significance from the prognostic point of view, or whether children who manifest no evidence of disease are the subjects of a latent infection. On these particular points most important observations have been made by Thomsen and Boas [46, 38]. Their investigations include an examination of (1) new-born children and their mothers, (2) children up to two years of age, (3) older children and adults with evidence of congenital syphilis. In the first place, cases were examined to determine whether the blood of new-born children of syphilitic mothers gave a positive reaction, and whether the reaction, positive or negative, could be taken as a guide for prognosis. The blood was taken at birth from the placental end of the cord, and the cord itself was examined for pathological changes in the vessels characteristic of syphilis. At the same time, the blood of the mother was tested. Of 44 children examined, which either themselves showed syphilitic lesions or were born of syphilitic mothers, 20 reacted positively and 24 negatively. In 4 of the positive cases the reaction disappeared completely, and did not return in the course of subsequent observation extending over three to nine months, nor did these cases develop any evidence of syphilitic infection. Boas believes that in those 4 cases, the positive reaction was due to the passage of syphilitic reaction substances from the blood of the mother into that of the foetus. According to this view the foetuses were never infected; we must not, however, overlook the possibility that a mild infection may have passed into a latent condition. The other 16 cases which gave a positive reaction at birth either showed clinical evidence of syphilis within three months or died in the interval, the post-mortem examination showing characteristic changes in the organs. One child showed no signs beyond wasting, a condition which improved on treatment with calomel. In one case the mother had contracted syphilis twelve years previously, and in the meantime had given birth to several healthy children. Of the 24 cases whose blood was negative at birth, 17 remained so, and the children remained healthy during a period of observation extending over several months. Other five cases developed symptoms of infection,

and in one case the blood was positive two and a half months before the symptoms appeared. In two cases the children died without manifesting any clinical evidence of syphilis, and with a negative serum reaction, yet post-mortem examination showed advanced change in the internal organs. This anomaly is explained on the supposition (Roemer [47]) that the reacting substances, like antibodies, are not actively produced in the tissues of the newly born. With regard to the examination of the umbilical cord, it was found that a positive serum reaction might be present in the child where the cord was apparently healthy, and cord changes could be present without a positive serum reaction. One child with a normal cord and negative serum subsequently developed signs of infection. In every case where the cord was affected clinical evidence of disease was present in the child or appeared at a later date. The results of these observations lead only to general conclusions, viz. (1) the large majority of cases with a positive reaction of the blood at birth subsequently develop symptoms of disease and (2) the large majority of those with a negative reaction remain healthy. Examination of the blood from the mothers showed that (1) the mother may be positive and the child negative, (2) the mother may be negative and the child positive, (3) the mother and the child may be positive, and (4) the mother and the child may be negative. Of the 17 mothers whose children, as already mentioned, showed no symptoms during three months' observation, 10 gave a negative reaction and 7 a positive reaction. On the other hand, of 35 mothers whose children showed signs of syphilis, 26 reacted positively and 9 negatively. The general conclusion is that, although the result of examination of the mother does not warrant an absolutely definite prognosis for the child, when the mother is negative the outlook is more hopeful, and when the mother is positive the outlook is less hopeful.

Only 11 of the mothers with syphilitic children were aware of having had symptoms indicative of infection. The case of the mothers with a positive reaction and no history of syphilis might be explained on the assumption that the child was infected from the sperm, and that the reacting substances had passed from the tissues of the foetus to the blood of the mother. This, however, is unlikely as the positive reaction of these mothers did not disappear during an extended period of observation.

As regards older children, Thomsen and Boas found that 27 children up to two years of age with signs of congenital syphilis, all gave positive reactions. They further found that of 27 older children and adults with manifestations of congenital syphilis, each gave a positive reaction. The specific affections in these latter cases included interstitial keratitis, osteitis, choroiditis, and juvenile general paralysis.

Some interesting facts bearing on this aspect of the subject were recently elicited by the examination of a whole family, in some members of which there was evidence of congenital syphilis (Gilmour and Pollock—unpublished). The father suffered from locomotor ataxy and died in an asylum. The mother had always enjoyed good health, and had never shown any symptoms suggestive of syphilis. Her blood serum gave a positive reaction. With regard to the children, (1) the eldest, now $10\frac{3}{4}$ years, suffered when 7 years of age from an eye affection, which yielded to antisyphilitic treatment. At the age of 10 there was a recurrence, which in the absence of treatment became worse, until blindness set in. She is now mentally affected. The Wassermann reaction is positive. (2) The second child is 7 years of age, and shows evidence of eye affection in a comparatively mild degree. The condition is yielding to mercurial treatment. The Wassermann reaction is positive. (3) The third child, 6 years of age, is well, and the serum reaction is negative. (4) The fourth child is 5 years of age, is also in good health, and has a negative serum reaction. It is further important to note that up till the age of 7 years, the two elder children were regarded as healthy.

These observations confirm the view that apparently healthy mothers who give birth to syphilitic children, are to be regarded as latent syphilitics.

We have examined the serum of 17 cases of congenital syphilis varying in age from 3 months to 13 years. These cases presented the external signs of disease, and in each a positive reaction was obtained. We are able to confirm the opinion of Boas, who states that in congenital syphilis the reaction is very constant and, in general, strong as compared with that found in acquired syphilis. Accordingly, *a negative reaction in a case presenting active lesions is strongly against the latter being due to congenital syphilis.*

Two cases of paroxysmal haemoglobinuria in children (referred to already on p. 108) have been examined, and in each case the blood serum gave a positive reaction. In neither

case were there any of the physical stigmata characteristic of congenital syphilis. The blood of the mother of one child, 4 years old, gave a positive reaction, although she had never had any symptoms of syphilis. There was a history of abortions in the case of the mother of the second child, who was 8 years of age.

As in the case of late syphilis and general paralysis, the positive serum reaction in congenital syphilis becomes negative as a *result of treatment* only in a small proportion of cases. This applies to mercurial treatment and also to treatment with salvarsan (*v.* p. 193). The serum reaction remains positive in the majority of those congenital cases where the injection of salvarsan has produced immediate disappearance of the symptoms (Wechselmann [48]).

Syphilis and Congenital Mental Deficiency

With the exception of cretinism, mongolism, amaurotic idiocy, and juvenile general paralysis, it may be said that the only classification of congenital mental deficiency is one based on the degree of mental defect.

Idiocy, imbecility, and mental debility are terms employed to distinguish somewhat arbitrary classes of varying grades of mental deficiency, and these terms have no etiological significance. Even the neurological distinctions based on the varieties of motor affection in idiots and imbeciles with cerebral palsy, do not offer a satisfactory basis for a scientific classification of such cases.

A classification or interpretation on anatomical grounds must also in many cases prove unsatisfactory, inasmuch as the condition found at the time of death can only represent the end product of the processes originally responsible for the disease. For example, in two cases of general paralysis described by Alzheimer,[49] in addition to the anatomical changes characteristic of the paralysis, there were also small nodules present in the brain, representing areas of retarded development. These areas, from their site and extent, seemed to be due to an earlier disease of the blood-vessels, which was undoubtedly syphilitic in origin, but the lesion at the time of death was so old as to render its character impossible of recognition, had the occurrence of general paralysis not suggested the specific origin of the condition.

Thus, the part played by syphilis as a factor in congenital mental disease has until recently been estimated only by anato-

mical and clinical evidence. It is a remarkable fact that the evidence adduced by anatomy has, on the whole, scarcely confirmed the clinical conclusions, that is to say, the number of cases in which the nervous system is affected in children who die in the first weeks of life is small as compared with the percentage of those whose nervous systems show in the first year of life evidence of deficiency attributable to syphilis in the parents. This apparent discrepancy, however, finds its explanation in the fact that those children who die early, die as the result of a very acute affection; whereas the nervous symptoms would appear to be the result of a more chronic infection. For example, Dr. Manson has examined in the Western Asylums' Research Institute, Glasgow, six cases of congenital syphilis, two of these were premature births, and the others died in the first few days or weeks after birth. In none of the cases was the nervous system affected, but in every case advanced degenerative changes of the lungs or liver or both were found, and spirochaetes were easily demonstrable in the affected organs in all. On the other hand, a clinical investigation of 230 cases of idiocy by Heubner [50] showed that about a fourth of these were the children of syphilitic parents.

It must, however, be recognised that syphilis as an etiological factor is responsible for the most varied forms of mental and nervous disease. For example, in the case of Little's disease, the symptoms were referred by Little himself to meningeal haemorrhage incidental to accidents during labour. It has, however, been demonstrated that such haemorrhages have, in many instances, a syphilitic basis; the rôle of syphilis in this disease is now generally admitted; and in the case of allied conditions of cerebral paralysis in children, among the various etiological factors, syphilis occupies a place of primary importance. It is obvious, also, that so far as anatomical processes are concerned, the manifestations of changes in the young must differ widely from those in the adult. In the adult, the syphilitic processes are of the nature of gummatous infiltrations, endarteritis, meningitis, or meningo-encephalitis. In the young, growing and plastic brain there is always, in addition to the destruction of developed elements, an interference with, or obstruction of the process of development.

The part played by syphilis in cases where the nervous disturbance is purely psychical is, at present, difficult to determine, and this field of research can only be explored by the application of the biological test. In the same way, those

cases in which it may be supposed that syphilis has acted as a predisposing factor by reducing the resistance of certain nerve tracts, are still somewhat indefinite. This effect of syphilis as well as its influence in causing conditions such as the hereditary ataxic and muscular dystrophies, are largely matters of clinical impression and speculation. Whether syphilis is responsible to any marked extent for mental enfeeblement and retarded development without obvious evidence of mental disease, is also a problem which can only be solved by the widest application of the biological test to parents of mentally affected and ill-developed children and to families of recognised syphilitics.

Dean [51] has examined a series of cases of congenital mental deficiency, and the result of his observations was to reveal the presence of syphilis in a large number of cases in which, some years ago, it would never have been suspected. The series examined numbered 330 and included 287 cases of simple idiocy of all grades; the remaining cases were the subjects of congenital spastic diplegia, hydrocephalus, epilepsy, mongolism, deaf mutism and progressive muscular atrophy. Out of the total 330, 51 gave a positive serum reaction, that is 15·4 per cent. Among the 51 positive cases there were 13 which presented other evidence pointing to syphilitic infection, and in the whole series there were 2 cases with somatic evidence of syphilis, which gave a negative reaction. Of the 287 cases of simple idiocy 44 gave a positive reaction. The positive cases which showed evidence of a local lesion included one which was subject to epileptiform convulsions, one with strabismus and nystagmus, one with a right sided hemiplegia, one with spastic diplegia, one which was a deaf mute, and two which were aphasic.

Plaut's [36] observations in Kraeplin's clinique bearing on this subject, have yielded results of great importance and interest. He has found that in cases of imbecility with motor paralysis, syphilis would seem to play a part which hitherto has been unsuspected. In idiocy and imbecility without motor disturbances, positive reactions were obtained in a proportion of cases, and in some cases of mental weakness or deficiency which could scarcely be characterised as insane, the serum reacted positively. An investigation of the material of this class was undertaken by Dr. Chislett [19] at Woodilee Mental Hospital. In all, 28 cases were examined, and although the number is small, the results are significant. In 11 out of the

28 a positive result was obtained. Of the 11 positive cases 4 showed signs of congenital syphilis, 5 showed no such signs, and 2 were cases of juvenile general paralysis. The 9 positive cases, apart from the general paralytics, included cases of idiocy and imbecility, imbecility with epilepsy, and imbecility with cerebral palsy. The two cases of general paralysis appear to have enjoyed ordinary health till a few months prior to their coming into hospital; they are sufficiently interesting to justify special mention.

Case XV.—M.B., aged 8 years, female: illness extending over three months. This child had been at school, and was considered by her mother to be ordinarily intelligent. There is no history of syphilis in the family, although the father was a habitual drunkard and a social wastrel. On examination the child was found to be well nourished and developed. The mother said that during the previous three months she had grown very stout. The pupils reacted sluggishly to light. The knee-jerks were absent. There was a fine tremor of the lips, and difficulty in pronunciation. She was very emotional, and wept on the slightest provocation. She was dirty in her habits.

Both the blood and cerebro-spinal fluid gave a positive reaction.

Case XVI.—A.C., aged 13 years, female. She was quite healthy, according to her mother, until six months before coming into hospital, but had in that period lost interest in her work. She became deaf in one ear, and developed keratitis in one eye. On examination she was found to be depressed, although there was said to be emotional excitement at times. The pupils reacted sluggishly to light, and the knee-jerk was absent on one side. Two members of the family died in infancy from unknown causes, and there were two premature births. There was no other evidence suggestive of syphilis in the family.

In this case both the blood and cerebro-spinal fluid gave a positive reaction.

Examination of a whole family.—A whole family, the father of which suffered from general paralysis, was examined. The father acknowledged syphilis, and his serum gave a positive reaction. The mother, to her knowledge, had never shown the symptoms of primary or secondary syphilis, but eight years after marriage had a tertiary ulcer on the left leg. There were ten pregnancies. Two children were born prematurely,

and two died in infancy. The blood sera of the remaining six were examined. The eldest, a boy of 16, was described by his mother as very nervous and stupid when a schoolboy. At the time of examination the boy showed no signs of syphilis and appeared healthy and only of slightly less than average intelligence; his serum reacted positively. A girl of 12 was deaf in one ear, but otherwise normal; her serum was positive. A girl of 10 had a negative serum. A boy of 8, with rhinitis and conjunctivitis, had a positive reaction; and the two youngest children, of 6 and 4 respectively, had negative sera.

The interesting facts appear, that children who suffer from mental disease of congenital origin, but show no other sign of syphilis, may give a positive reaction; and children whose parents are syphilitic may have a positive reaction, although at the time of examination they appear to be in ordinary health, and show no signs of previous disease.

The significance of the results which have been detailed is that syphilis plays a larger part in congenital mental affection than is generally supposed. It is important, both for the classification of mental diseases and for the elucidation of the etiological basis of such maladies, that the serum reaction should be determined in a large series of cases. It would appear that the terms "racial degeneration" or "stigmata of degeneration," when applied to cases of congenital mental disease, may convey a false impression, especially when such cases are of syphilitic origin and are due to specific infection of the individual. Congenital syphilis outside the nervous system produces degeneration only in the sense that an infection leads to destruction of structural elements and consequent loss or perversion of function. In the same way, it is not too much to say that nervous disease associated with congenital syphilis is the expression of anatomical changes that are due to an intra-uterine infection with the spirochaete pallida. The changes which such a degeneration produces in the young, plastic brain are likely to be permanent, and hopeless from the point of view of individual therapy. If medical science is prepared to urge prophylactic measures for the prevention of such diseases, it is well that it should be definitely established and recognised that a considerable proportion of cases of congenital mental deficiency are of infectious origin, and that the infection is syphilis.

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CHAPTER VIII

THE CEREBRO-SPINAL FLUID

THE OCCURRENCE OF A POSITIVE WASSERMANN REACTION WITH THE CEREBRO-SPINAL FLUID—SUBSTANCES CONCERNED IN THE PRODUCTION OF THE WASSERMANN REACTION—CHEMICAL METHODS OF EXAMINING THE CEREBRO-SPINAL FLUID: COMPARISION WITH THE WASSERMANN TEST—COMPARISON OF THE WASSERMANN TEST AND CYTOLOGICAL EXAMINATION—THE SIGNIFICANCE OF THE WASSERMANN REACTION IN NERVOUS DISEASE

The Occurrence of a Positive Wassermann Reaction with the Cerebro-Spinal Fluid

IN diseases of the central nervous system, examination of the spinal fluid has in recent years become more or less a routine practice. The technique employed in performing spinal puncture has been described on page 20. The normal spinal fluid is clear, slightly alkaline and has a specific gravity of 1·006 to 1·008. It contains a very small amount of protein material and practically no corpuscular elements. There are traces of sodium and potassium salts in the form of chlorides, carbonates and phosphates, and there is a reducing substance, which is generally believed to be glucose. With regard to the presence of gases in the spinal fluid, Mott [1] has recently found that it contains varying small amounts of oxygen and nitrogen, and that by boiling *in vacuo* an average amount of 10 per cent. by volume of carbon dioxide can be determined. It is unlikely that the fluid is the product merely of a process of transudation from the blood or lymph vessels, because, while it contains only 0·02 per cent. of protein material, the blood-plasma and lymph contain 7 per cent. and 4·5 per cent. of protein material respectively. The anti-substances which are present as a rule in the blood as a result of bacterial infection cannot be demonstrated in any appreciable amount in the cerebro-spinal fluid. This holds good even in those infections which primarily involve the central nervous system. In

cerebro-spinal fever the specific immune body which is present in the blood cannot be detected in the spinal fluid (Mackenzie and Martin [2]) and the same holds good as regards the specific agglutinin and opsonin in this disease (Davis [22], Macgregor [3]). Bacteriolytic and haemolytic complement are also absent from the normal spinal fluid and from the spinal fluid in cerebro-spinal fever, so far as can be made out by the usual methods of demonstrating these properties of serum. Drugs which are administered by the mouth or subcutaneously only in exceptional cases reach the spinal fluid (*v.* p. 119). The toxin of tetanus which is believed to travel from the original site of infection by the perineural lymphatics, and to affect ultimately the anterior cornual cells, cannot as a rule be demonstrated in the spinal fluid. Of great interest and importance are the recent observations of Schmorl [4] on certain properties of the spinal fluid, and on the difference which can be demonstrated, in some instances, between the spinal fluid and the intraventricular fluid. It is a well recognised fact that in cases of jaundice the bile pigment is present in considerable amount in exudates in the serous sacs, such as the peritoneal and pleural cavities ; it is also present, though in a distinctly less degree, in the cerebro-spinal fluid. Schmorl has found that, in the majority of cases, the intraventricular fluid does not contain a trace of bile pigment even in those cases in which it is present in the spinal fluid. He records the results of the examination of eleven cases, ten being adults, and one a case of icterus neonatorum. In six of the adult cases the icterus had lasted from 10 to 20 days and in the others for months. In eight cases, including the child, the intraventricular fluid was absolutely clear and did not contain a trace of bile pigment ; in one case, in which the jaundice had been present for 8 months, there was a faint yellow colour, but no bile reaction, and in two cases the intraventricular fluid was markedly coloured, more so than the spinal fluid. In one case of diabetes mellitus Schmorl found reducing substance in considerable amount in the spinal fluid, which, by the same method, was not demonstrable in the intraventricular fluid. Seven cases of general paralysis were examined for the Wassermann reaction. In each case both the blood and spinal fluid gave positive results during life. It was found post-mortem that while the spinal fluid was positive in each, the intraventricular fluid tested simultaneously was positive only in one. Schmorl suggests, as a result of these observations, that the intraventricular

cavities are closed off from the sub-arachnoid space and that the foramen of Magendie and the foramina of Luschka should be regarded as artefacts. This conclusion he founds more particularly on the evidence afforded by the jaundice cases, pointing out that in the case of general paralysis with negatively reacting intraventricular fluid, it is possible that the chronic inflammatory processes in the meninges might have produced a closure of the foramina which are supposed to afford a communication between the ventricular cavities and the sub-arachnoid space. He suggests further that the presence in the intraventricular fluid of bile pigment and of the substances concerned in the production of the Wassermann reaction, is associated with degenerative changes in the choroid plexus. In one of the cases of jaundice (due to cirrhosis of the liver) with bile pigment in the intraventricular fluid, the patient died from an intercurrent tubercular meningitis with extensive necrosis of the epithelium of the choroid plexus. In another case with bile stained intraventricular fluid, the patient died from phosphorus poisoning, and the epithelium of the choroid plexus was fatty and degenerate, and extensively desquamated. In the third case of long standing jaundice with slight yellow discolouration of the intraventricular fluid, there was considerable inflammatory infiltration of the plexus, with isolated patches denuded of epithelium. In the single case of general paralysis in which a positive Wassermann reaction was obtained with the intraventricular as well as with the spinal fluid, the choroid plexus presented evidence of extensive degeneration. To sum up Schmorl's results, then, in the cases where the intraventricular fluid contained no bile pigment and did not give a positive Wassermann reaction, the epithelium of the choroid plexus was intact, and where the fluid did contain bile pigment or substances which produced a positive Wassermann reaction, the epithelium of the choroid plexus was degenerate. In further confirmation of the contention that the presence in the intraventricular fluid of substances which circulate in the blood is due to disease of the choroid plexus or intraventricular ependyma he cites the two following observations. In one case a gummatous mass in the brain had extended towards the ventricular cavities and the granulating process involved part of the choroid plexus, which had become infiltrated and denuded of its epithelium. In this case the blood and intraventricular fluid gave a positive reaction, while the spinal

fluid gave a negative reaction. In the other case (nonsyphilitic) an abscess, about the size of a hen's egg, was situated in the right hemisphere, and was separated from the right ventricle by a layer of tissue only 2 mm. thick. The ventricular wall in the neighbourhood of the abscess was injected and oedematous. Microscopic examination showed that the epithelium of the ependyma was partly necrotic and partly desquamated, and the sub-epithelial tissue was infiltrated with masses of polymorphonucleated cells. The choroid plexus was not affected. In this case the intraventricular fluid contained a considerable amount of albumin while the spinal fluid contained only a trace. These observations are of importance as indicating what local conditions determine the presence in the spinal and the ventricular fluid of substances which circulate in the blood. We have had an opportunity of making a comparative examination, for the Wassermann reaction, of the intraventricular fluid, and the spinal fluid in four cases of general paralysis. In two of these the intraventricular fluid was negative and the spinal fluid was positive. In the other two the fluid from both sources reacted positively. In one of the latter, however, the reaction of the intraventricular fluid was weak compared with that obtained with the spinal fluid from the same case. In both cases in which the intraventricular fluid gave a positive reaction, the choroid plexus showed evidence of degeneration, whereas in the other two cases degenerative changes were not observed (unpublished observations—Mackenzie and Morton).

Wassermann and Plaut [5, 6] were the first to examine the cerebro-spinal fluid of general paralytics for the syphilis reaction. They examined the fluid from advanced cases whose blood reacted positively and in a certain proportion were able to determine a positive result. Out of 54 samples of spinal fluid from undoubted cases, 41 were positive, 5 negative and 8 doubtful. Neisser, Bruck and Schucht [7] examined 8 cases and obtained a positive reaction in 4. Marie and Levaditi [8] obtained a positive reaction in 29 out of 39 cases, Morgenroth and Stertz [9] in 8 out of 8, Michaelis [10] in 18 out of 20, Fränkel-Heiden [11] in 11 out of 14, Marie, Levaditi and Yamanouchi [12] in 28 out of 30, Stertz [13] in 40 out of 45, and Henderson Smith and Candler [14] in 59 out of 64. In our first series we obtained positive results in 40 out of 66 cases examined. Some of these, however, were examined when we were working without the advantages which are derived from the improved technique

now followed. In a few cases at first we used only 0·05 c.cm. of the unheated fluid for each tube in the test. Later we used 0·1 c.cm., but this amount is also too small to give a positive reaction in some cases; it is better to use 0·4 c.cm. as recommended by Henderson Smith and Candler [14], and the fluid should always be unheated. We use now invariably the lecithin-cholesterin method (*v.* Chap. IV.), and the results obtained are eminently satisfactory. Morton (unpublished) has recently examined the cerebro-spinal fluid from 30 cases of general paralysis at various stages by this method, and in every instance has obtained a positive result. He has examined 20 control cases including fluids from cases of tubercular meningitis, cerebro-spinal fever, epilepsy, and dementia praecox, and in every case a negative result has been obtained. The method adopted varies somewhat from that used for the blood serum, in that a much larger quantity of the spinal fluid is used in the test. The procedure is as follows—two test tubes (4 in. by ½ in.) are taken, and into each there are poured three and a half cubic centimetres of spinal fluid. Half a cubic centimetre of the alcoholic lecithin solution is poured carefully with a pipette on to the surface of one, and an equal amount of the lecithin-cholesterin solution is poured on to the surface of the other; the fluids are mixed slowly by rotating the tubes. Two series of tubes are now taken, and half a cubic centimetre of the lecithin and spinal fluid mixture is placed in each tube of the one series, and half a cubic centimetre of the lecithin-cholesterin and spinal fluid mixture is placed in each tube of the second series. Increasing amounts of complement are added to each series, and the mixture is incubated for 1½ hours at 37° C. The sensitised corpuscles (1 c.c.) are then added, and after a further incubation for 1¼ hours at 37° C. the results are read. A difference, two or three doses more of complement absorbed with lecithin-cholesterin than with lecithin, is in favour of the fluid being positive, and a more marked difference (five doses or more) is conclusive. It will be seen that by this method almost half a cubic centimetre of spinal fluid is used for each tube in the test. A control experiment should be made to estimate the amount of complement absorbed by the spinal fluid alone. The amounts of complement absorbed by emulsions in salt solution of lecithin and lecithin-cholesterin respectively, should also be determined. These control mixtures of lecithin and lecithin-cholesterin are prepared by using normal saline solution instead of spinal

fluid in the proportion of one part of the alcoholic solution to seven of saline. The spinal fluid should be tested as soon as possible after withdrawal, it should be centrifugalised to free it from corpuscular elements, and it should not be heated before use. This method gives more delicate results than any other of which we have had experience. By adopting it the fallacies which arise as a result of variations in the individual properties of the extracts and haemolytic complement are obviated. There is no disadvantage in using the large amount of spinal fluid required; Morton had found that the unheated fluids from cases of tubercular meningitis and cerebro-spinal fever, which contained a considerable amount of protein material, did not deviate an appreciable amount of complement either by themselves or in the presence of lecithin and lecithin-cholesterin.

This method of examining the spinal fluid should be applied in cases of locomotor ataxy, where the substances which produce a Wassermann reaction would seem to be present in greater dilution than in the fluid from general paralytics. We have not had an opportunity of examining the fluid from cases of locomotor ataxy since the introduction of this method, but in an earlier series of six cases, the spinal fluid reacted positively only in two. In these cases only 0·1 c.c. of spinal fluid was used for each tube.

Substances concerned in the Production of the Wassermann Reaction

We have already discussed the theoretical problems involved in a study of this phenomenon (*v.* p. 88). There seemed at one time to be some doubt as to whether the substance in the blood serum or cerebro-spinal fluid which determined a positive Wassermann reaction was of the nature of a lipoid (Levaditi and Yamanouchi, Mott, Pighini) or of a protein (Sachs, Noguchi). Morton carried out a series of examinations of cerebro-spinal fluids from various sources with a view to determining their lipoid content, and the possible relationship between the lipoid content and the Wassermann reaction. The spinal fluids were extracted with alcohol, and the lecithin content of the extract was estimated by its lytic effect on ox's red blood-corpuscles to which a suitable amount of cobra-venom had been previously added. It appeared that the lytic effect along with venom, of extracts of the various specimens had no relation to the production of a Wassermann reaction, or to the relative intensity

of the Wassermann reaction in a series of positive cases. Of course, it must be remembered that such alcoholic extracts contain not merely substances which are lytic with cobra-venom (lecithin), but also antilytic bodies (cholesterin). And Pighini [15] has found comparatively large amounts of cholesterin in the cerebro-spinal fluid of cases of general paralysis, epilepsy and dementia praecox, especially in the acute phase. Morton found it impossible to extract with alcohol the substances in the fluid (" antibody ") which were concerned in the syphilis reaction. On the other hand, when a quantity of a cerebro-spinal fluid which gives a marked positive reaction is mixed with an equal amount of a saturated solution of ammonium sulphate, a precipitate is formed. The precipitate obtained by centrifugalising and removing the clear fluid, when dissolved in an amount of normal saline solution equal to the original volume of cerebro-spinal fluid, was found to contain substances which produced a positive Wassermann reaction, but did not inhibit complement by itself. It would thus appear that the substance concerned is of the nature of a globulin or associated with a particular variety of globulin, as Noguchi [16] suggests. Cerebro-spinal fluids from cases of tubercular meningitis and cerebro-spinal fever were found to have a high globulin content, but the globulin when precipitated and redissolved did not give a positive Wassermann reaction, and did not inhibit complement by itself. The results of an experiment demonstrating these points are shown in table I.

Specimens of unheated spinal fluid were taken from a case of general paralysis and from two cases of severe epilepsy and prepared for examination in the following manner.

Fluid A.—To 6 c.c. of spinal fluid from the case of general paralysis 0·6 c.c. of an alcoholic extract of fresh ox liver was added by floating it on to the surface ; a dense emulsion was formed by gradually mixing the two fluids. Of this mixture (Fluid A) 0·5 c.c. was placed in a series of tubes.

Fluid B.—10 c.c. of the spinal fluid from the general paralytic were precipitated by mixing with 10 c.c. of a saturated solution of ammonium sulphate. This mixture was centrifugalised and the supernatant fluid removed. The precipitate was then dissolved in 10 c.c. of normal saline solution. To 6 c.c. of this solution 0·6 c.c. of the same extract of fresh ox liver was added, and a dense emulsion produced by mixing. Of this mixture (Fluid B) 0·5 c.c. was added to a series of tubes.

Fluid C.—To 6 c.c. of the pooled specimen of spinal fluids from two cases of epilepsy, 0·6 c.c. of the alcoholic extract was added. The mixture was prepared as in the case of Fluid A, and of this 0·5 c.c. was placed in each of a series of tubes.

Fluid D.—30 c.c. of the pooled fluids from the cases of epilepsy were mixed with 30 c.c. of saturated solution of ammonium sulphate. This mixture was centrifugalised and after removing the supernatant fluid, 10 c.c. of normal saline solution were added to the precipitate. In this way the globulin in the saline solution was concentrated to three times the amount present in the spinal fluid. This procedure brought the amount of globulin present in the normal saline solution approximately to the same concentration as that in Fluid B. To 6 c.c. of this solution 0·6 c.c. of the liver extract was added and mixed so as to form a turbid emulsion, and of this (Fluid D) 0·5 c.c. was added to each of a series of tubes. To the tubes in each series increasing amounts of complement were added.

TABLE I

	Guinea-pig's Complement.				
	0·025 c.c.	0·05 c.c.	0·1 c.c.	0·15 c.c.	0·2 c.c.
Fluid A—0·5 c.c.	No lysis	No lysis	No lysis	Trace of lysis	Lysis complete
Fluid B—0·5 c.c.	No lysis	No lysis	No lysis	No lysis	Lysis complete
Fluid C—0·5 c.c.	Lysis complete	Lysis complete	Lysis complete	Lysis complete	Lysis complete
Fluid D—0·5 c.c.	Lysis complete	Lysis complete	Lysis complete	Lysis complete	Lysis complete

CONTROLS.

(1) Controls were made by placing in each of two tubes 0·5 c.c. of the spinal fluid, and of the globulin solution respectively, just as they were before the addition of the extract. To the two tubes of each series 0·01 c.c. and 0·02 c.c. of complement were added. Lysis was complete in both series with 0·02 c.c. of complement.

(2) An emulsion of one part of extract with ten parts of normal saline solution was made, and 0·5 c.c. of this along with 0·015 c.c. of complement caused complete lysis.

(3) The complement dose was 0·005 c.c.

Further light is thrown on the nature of the reaction by the following experiment: 10 c.c. of spinal fluid from an

advanced case of general paralysis were placed in a test tube and 1 c.c. of an alcoholic extract of ox liver was floated on to the surface of the spinal fluid. Mixing by rotation of the tube produced a dense precipitate which could be brought down by centrifugalising. The clear supernatant fluid was now removed, and the precipitate made up to the original volume of spinal fluid by the addition of normal saline solution. The clear fluid obtained by centrifugalising did not inhibit the action of haemolytic complement, whereas the mixture of saline solution and precipitate did. It was found that the alcoholic extract of liver had precipitated the proteid content of the spinal fluid. Again, the alcoholic extract of liver possessed the power of causing lysis along with cobra-venom to this extent, that of a mixture of one part of extract with ten parts of normal saline solution 0·015 c.cm. sufficed to produce complete lysis of 1 c.cm. of a 5 per cent. suspension of ox's red blood-corpuscles with cobra-venom (1 c.cm. of a $\frac{1}{1000}$ solution of cobra-venom to 10 c.cm. of 5 per cent. ox blood suspension). It was found that 1 c.cm. of the clear supernatant fluid obtained after centrifugalising the mixture of liver extract and cerebro-spinal fluid did not lyse 1 c.cm. of a 5 per cent. suspension of ox's red blood-corpuscles with cobra-venom. On the other hand, the mixture of the precipitate and normal saline solution caused complete haemolysis along with cobra-venom in an amount equal to 0·015 c.cm. for 1 c.cm. of ox's red blood-corpuscles.

It was found also that the globulin precipitated by half saturation with ammonium sulphate, and separated by centrifugalising, when re-dissolved in an amount of normal saline solution equal to the original spinal fluid, could be again precipitated by the addition of a tenth of its volume of alcoholic extract of ox's liver. Here again the precipitate deviated haemolytic complement, and produced lysis along with cobra-venom, whereas the clear fluid did neither.

EXPERIMENTS

(*a*) Ten cubic centimetres of spinal fluid from a case of general paralysis were mixed with 10 c.c. of a saturated solution of ammonium sulphate. The mixture was centrifugalised and the precipitate dissolved in 10 c.c. of normal saline solution. Care must be taken to remove the ammonium sulphate as thoroughly as possible from the precipitate by pressing between filter papers, because the ammonium sulphate even in small amounts exercises an anti-complement effect.

(*b*) To the 10 c.c. of solution of precipitate in normal saline 1 c.c. of alcoholic extract of ox liver was added. This was done by floating the extract on to the top of the fluid in a test tube. By gradual rotation of the tube and slow mixing a dense emulsion was formed from which a precipitate separated out on centrifugalising. The supernatant fluid was put into a clean test tube to be subsequently tested as Fluid A. To the precipitate obtained by centrifugalising, 10 c.c. of normal saline solution were added, and this was subsequently tested as Fluid B.

These two fluids were tested and compared (1) as regards their power of inhibiting the action of haemolytic complement (Wassermann reaction)—tables II and III, and (2) as regards their power of causing haemolysis of ox's red blood corpuscles along with cobra-venom—tables IV and V.

TABLE II

Fluid A.	Complement (Guinea-pig).	5 per cent. suspension of Ox Blood sensitised with Homologous Immune Body.	Result.
0·5 c.c. . . .	0·025 c.c.	1 c.c.	Lysis complete
0·5 c.c. . . .	0·05 c.c.	1 c.c.	,, ,,
0·5 c.c. . . .	0·075 c.c.	1 c.c.	,, ,,
0·5 c.c. . . .	0·1 c.c.	1 c.c.	,, ,,
0·5 c.c. . . .	0·15 c.c.	1 c.c.	,, ,,
0·5 c.c. . . .	0·2 c.c.	1 c.c.	,, ,,

TABLE III

Fluid B.	Complement (Guinea-pig).	5 per cent. suspension of Ox Blood sensitised with Homologous Immune Body.	Result.
0·5 c.c. . . .	0·025 c.c.	1 c.c.	No lysis
0·5 c.c. . . .	0·05 c.c.	1 c.c.	,,
0·5 c.c. . . .	0·075 c.c.	1 c.c.	,,
0·5 c.c. . . .	0·1 c.c.	1 c.c.	,,
0·5 c.c. . . .	0·15 c.c.	1 c.c.	,,
0·5 c.c. . . .	0·2 c.c.	1 c.c.	Trace of lysis

Controls for Tables II and III.—(*a*) Dose of complement = 0·01 c.c. (*b*) 0·5 c.c. of the solution of the ammonium sulphate precipitate in normal saline solution did not deviate more than two doses of complement by itself, that is, haemolysis was complete with 0·03 c.c. of complement. (*c*) An emulsion of one part of alcoholic extract of liver with ten parts of normal saline solution was made, and it was found that 0·5 c.c. of this emulsion did not deviate more than one dose of complement, that is, haemolysis was complete with 0·02 c.c. of complement.

TABLE IV

Fluid A.	5 per cent. suspension of Ox Blood with Cobra Venom, 0·1 mg.	Result.
0·01 c.c.	1 c.c.	No lysis
0·015 c.c.	1 c.c.	,,
0·03 c.c.	1 c.c.	,,
0·06 c.c.	1 c.c.	,,
0·1 c.c.	1 c.c.	,,
0·2 c.c.	1 c.c.	,,
0·3 c.c.	1 c.c.	,,

TABLE V

Fluid B.	5 per cent. suspension of Ox Blood with Cobra Venom, 0·1 mg.	Result.
0·01 c.c.	1 c.c.	Trace of lysis
0·015 c.c.	1 c.c.	Complete lysis
0·03 c.c.	1 c.c.	,, ,,
0·06 c.c.	1 c.c.	,, ,,
0·1 c.c.	1 c.c.	,, ,,
0·2 c.c.	1 c.c.	,, ,,
0·3 c.c.	1 c.c.	,, ,,

Controls for Tables IV and V.—(*a*) Of an emulsion made up of one part of alcoholic extract of liver with ten parts of normal saline solution, 0·015 c.c. sufficed to produce complete lysis of 1 c.c. of the ox corpuscle suspension and cobra-venom. (*b*) The amounts of cerebro-spinal fluid used in the experiment did not have any lytic power along with cobra venom.

The conclusions which may be drawn from these experiments are :—

1. The property of giving a Wassermann reaction is related to the presence of a substance associated with the globulin fraction of the cerebro-spinal fluid (which is precipitated on half saturating with ammonium sulphate). This substance reacts with lipoid bodies such as are present in an alcoholic extract of liver. On the other hand, a cerebro-spinal fluid may contain abundant globulin and yet react negatively in the syphilis test.

2. The specific substances (" syphilitic antibody ") in the cerebro-spinal fluid can be precipitated by a suitable amount of a suitable alcoholic extract of normal ox liver.

3. In the removal of the specific substances (" syphilitic antibody ") by precipitation with alcoholic extract of liver,

the substances in the extract which possess the power of causing haemolysis along with cobra-venom (lecithins) also pass into the precipitate.

4. The lipoid substances which are precipitated from the alcoholic extract of liver by syphilitic cerebro-spinal fluid do not lose their power of activating cobra-venom.

It has been found that the separation of the protein content of the spinal fluid and the lipoid content of the liver extract can be effected only when the fluid from very advanced cases of general paralysis is employed. It has also been found that only particular alcoholic extracts produce a precipitate dense enough to admit of complete separation by centrifugalising (Mackenzie and Morton, unpublished observations).

Chemical Methods of examining the Cerebro-Spinal Fluid: Comparison with the Wassermann Test

It has long been recognised that the cerebro-spinal fluid in cases of general paralysis contains an abnormal amount of protein substance. To this increase in protein content considerable importance has been attached from the diagnostic point of view, and various methods have been devised for determining the presence or absence of this condition. Nonne and Apelt,[17] and Ross and Jones [18] introduced the method of precipitation by means of half saturation with ammonium sulphate. This procedure gives an indication of the globulin content of the spinal fluid, although it fails to differentiate between an increase in globulin due to general paralysis and an increase due to other diseases, such as tubercular meningitis. Noguchi [16] has also introduced a procedure which gives very delicate results, so far as indicating the presence of protein material is concerned. According to this method one or two parts of spinal fluid are mixed with five parts of a 10 per cent. dilution of butyric acid in physiological salt solution, and the mixture is boiled for a few seconds over a flame. One part of normal NaOH solution is then added quickly to the heated mixture and the whole is again boiled for a few seconds. The actual quantities recommended are 0·1 c.cm. or 0·2 c.cm. of spinal fluid, 0·5 c.cm. of the butyric acid solution, and 0·1 c.cm. of normal NaOH solution. It is necessary to have the spinal fluid absolutely free from blood. The presence of an increased protein content of the spinal fluid is indicated by the occurrence of a granular or flocculent precipitate which gradually settles on standing. The rapidity with which the

precipitate falls is proportional to the amount of protein present, and Noguchi considers that a positive result has been obtained when the precipitate settles within 2 hours. This method, however, simply gives an indication of the protein content of the fluid examined, and does not afford a basis for differentiation between the various conditions in which a high protein content of the cerebro-spinal fluid is present. Noguchi himself does not claim any more for the method. Morton [19] has examined the protein content of the spinal fluid of cases suffering from various diseases. The method he adopted consisted in taking equal parts of cerebro-spinal fluid and 96 per cent. alcohol, and noting the density of the precipitate formed on mixing. He found that the results were practically parallel with those obtained by following the methods of Nonne and Apelt and of Noguchi. Taking a series of cases examined at the same time and with the same reagents, it was seen that there was no definite relation between the protein content of the spinal fluid and the occurrence or intensity of the Wassermann reaction. In the following table (VI) the figures indicating the density of the alcohol precipitate refer only to the relative density of the various

TABLE VI

Cerebro-spinal Fluid.	Alcohol Precipitate.	Wassermann Reaction, Doses of Complement Absorbed.
No. 21	1·0	30
„ 22	1·4	40—
„ 23	0·8	10
„ 24	1·0	20
„ 25	0·6	10
„ 26	1·2	10
„ 27	0·8	7
„ 28	0·8	24
„ 29	1·2	12
„ 30	0·3	30
„ 31	0·8	12
„ 32	1·2	40
„ 33	1·0	24
„ 34	0·4	40
No. 35	0·2	0
„ 36	0·1	0
„ 37	0·2	0
„ 38	0·2	0
„ 39	0·2	0
„ 40	1·0	0

specimens based on the adoption of an arbitrary standard. The figures dealing with the Wassermann reaction represent the absolute amounts in haemolytic doses of complement absorbed by the mixture of cerebro-spinal fluid and liver extract. (In these tests 0·1 c.c. of unheated cerebro-spinal fluid with 0·6 c.c. of turbid emulsion of alcoholic ox liver extract was employed and *all the fluids were examined on the same day.*)

Cases Nos. 21–34 suffered from general paralysis. Nos. 35–40 were cases of epilepsy and dementia praecox.

On reviewing the results recorded in this table, it is obvious that there is a great variation in the amount of precipitable substances in different cerebro-spinal fluids. On the whole, the precipitate is more abundant in cases of general paralysis, although there are instances, e.g. No. 40, where the amount of precipitate was considerable, yet the Wassermann reaction was negative.

When one compares the amounts of precipitate in the various cases with the intensity of the Wassermann reaction, as estimated by the number of doses of haemolytic complement which have been absorbed, one finds that there is no direct relationship. A fluid such as No. 26 yields a precipitate with alcohol represented by 1·2, and in the Wassermann reaction absorbs ten doses of haemolytic complement, whereas No. 34 yields a precipitate represented by 0·4, and in the Wassermann test deviates over forty doses of complement. Again, Nos. 27 and 28 contain each an amount of precipitable substance represented by 0·8, while in the Wassermann test No. 27 absorbs seven doses of complement and No. 28 absorbs twenty-four doses. Thus there is no relationship between the intensity of the Wassermann reaction and the content of the fluid in substances precipitable by alcohol.

The following table (VII) shows the results of the examination of a series of cases in which the various precipitation methods are compared with each other and with the Wassermann reaction, the tests of all the fluids being carried out on the same day.

Here again it is to be noted that while the precipitation methods show parallel results, they do not correspond with the results obtained in the Wassermann test. In case No. 50, where the patient was suffering from an acute katatonic stupor the precipitation methods all gave positive results, and the Wassermann reaction was negative. The blood serum in this case was also negative to the Wassermann test. In

TABLE VII

Case.	Disease.	Wassermann Reaction.	Noguchi Test.	Precipitate with Alcohol.	Half Saturation with Ammonium Sulphate.
No. 50	Dementia praecox .	—	+	1·2	1·4
„ 51	Epilepsy	—	—	0·1	0
„ 52	„	—	—	0·1	0
„ 53	Dementia praecox .	—	—	0·2	0·1
„ 54	General paralysis .	+	—	0·2	0·1
„ 55	„ „ .	+	+	0·8	0·6
„ 56	„ „ .	+	+	0·8	0·6
„ 57	„ „ .	+	+	0·4	0·3
„ 58	„ „ .	+	—	0·2	0·1
„ 59	„ „ .	+	+	1·6	1·2
„ 60	Dementia praecox .	—	—	0·2	0·1

cases 54 and 58, suffering from general paralysis, the precipitation tests were practically negative, and the Wassermann test was positive in each case. These results show quite definitely that a reliable conclusion as to the syphilitic nature of the case can only be obtained from the Wassermann test.

Comparison of the Wassermann Test and Cytological Examination

In the differential diagnosis of diseases of the central nervous system, importance has been attached to the results of cytological examination of the spinal fluid. While it is the case that general paralysis is, as a rule, associated with the presence of a high cellular content in the spinal fluid (Hamilton Marr [21]) there are other diseases apart from the well-recognised infections of the central nervous system in which a high cellular content is also present. For example, in the case of katatonic stupor, No. 50 in Table VII, where the protein content of the fluid was high and the Wassermann reaction was negative, about 350 cells of a mononuclear type were present in each cubic centimetre. Plaut [6] made a cytological examination of the spinal fluids from 56 cases of nervous disease, which he considered free from syphilis. He regards the result of a cytological examination as negative when the number of cells in 1 c.cm. is less than six, as doubtful when less than ten, and as positive when more than ten. In 48 out of the 56 cases the cytological examination gave a negative result, in 4 it was doubtful, and in 4 it was positive. The

spinal fluid of the 4 cases in which the cytological result was positive gave a negative Wassermann reaction. These cases included one of pachymeningitis, one of epilepsy, one which had been anaesthetised by the intraspinal injection of cocaine, and a case in which the diagnosis was uncertain. Cases of tubercular meningitis with lymphocytosis of the spinal fluid and a negative Wassermann reaction were also observed.

In the next place, a series of recognised syphilitics in different stages of the disease were examined by Plaut. The blood serum and the spinal fluid were tested for the Wassermann reaction and a cytological examination of the spinal fluid was also made. In a series of ten cases with symptoms of the secondary stage, the serum was positive in each and the spinal fluid was negative in all of the cases examined (the spinal fluid of one case was not examined). Cytological examination showed a positive result (over 10 cells in 1 c.cm.) in three cases and in one case there were 77 cells in 1 c.cm. of the fluid. A doubtful result (6–8 cells) was obtained in four cases, and a negative result (2–3 cells) was obtained in three. Twelve cases in the latent tertiary period were examined in the same way. In eleven the spinal fluid gave a negative Wassermann reaction; in the twelfth it was not examined. The blood serum was examined in eleven cases and was positive in nine. In two cases the cytological examination gave a positive result (22 and 23 cells in 1 c.cm.), in one case the result was doubtful (6 cells in 1 c.cm.) and in nine cases it was negative. Boas and Lind [20] have recently examined a series of cases of syphilis where there was no clinical evidence of implication of the nervous system. Although the blood serum gave a positive reaction in almost every instance, the spinal fluid was negative in every case. The spinal fluid, however, gave the precipitation reaction and contained an excess of lymphocytes in a few cases.

In general paralysis it has been found that in the great majority of cases both a positive Wassermann reaction and a positive cytological result are obtained. A few cases, however, have been encountered in which the results were divergent; a positive cytological result was found by Plaut in eight cases where the spinal fluid gave a negative Wassermann reaction; in each of these the blood serum gave a positive reaction. Plaut, however, considered it possible that these cases might be suffering from cerebral syphilis and not from general paralysis. In eight cases he found that the spinal fluid reacted positively in the Wassermann test,

without there being an accompanying lymphocytosis. Five of these cases were in the very early stages of general paralysis, and in two other cases the disease was possibly cerebral syphilis and not general paralysis. Corresponding results were obtained in a few cases of locomotor ataxy which were examined. A positive Wassermann reaction was associated with lymphocytosis in most cases, although three cases were met with in which the cells were few in number and the Wassermann reaction strongly positive, and in one case there was a lymphocytosis with a negative Wassermann reaction. We have recently had an opportunity of examining two cases of lead poisoning with cerebral symptoms. The Wassermann reaction was negative with both the blood and spinal fluid. The spinal fluid, however, gave positive precipitation tests and positive cytological tests.

Of special interest and importance were the results obtained by Plaut in cases of cerebral syphilis. He examined 21 cases of cerebral syphilis of congenital origin and only in one instance was the cytological result negative; in 3 cases the result was doubtful (5 to 9 cells in 1 c.cm.); in the remaining 17 cases there was a distinct lymphocytosis, the number of cells in one instance representing 424 in 1 c.cm. Only one spinal fluid gave a strong positive Wassermann reaction, three gave a weak reaction and the remaining 17 were negative. The blood serum was positive in every case. The four cases which gave positive Wassermann reactions showed a lymphocytosis of varying degree. The fluid which reacted strongly contained 17 cells in 1 c.cm., while the three which reacted weakly contained 10, 103, and 345 in 1 c.cm. respectively. These cases show definitely that lymphocytosis of the spinal fluid in syphilis and the presence in the fluid of substances which give a positive Wassermann reaction are independent manifestations.

The Significance of the Wassermann Reaction in Nervous Disease

If, in a case of nervous disease, a positive reaction is obtained with the blood serum, the disease may be syphilitic in character, or it may be a non-syphilitic disease occurring in the latent stage of syphilis or concomitantly with a syphilitic lesion outside the nervous system. If, in addition to a positive blood reaction, the cerebro-spinal fluid is positive, then one is justified in diagnosing a syphilitic affection of the central nervous system. The nature of the syphilitic affection must

be determined by further clinical evidence, although, speaking generally, it is only in general paralysis and locomotor ataxy that the spinal fluid as a rule gives a positive Wassermann reaction. Some difference of opinion exists as to whether the reaction with the spinal fluid is more intense than it is with the blood serum from the same case. Marie, Levaditi and Yamanouchi,[12] also Raviart, Breton and Petit (*v.* Plaut [6]) consider that in general paralysis the reaction is more marked with the spinal fluid. Plaut and Boas, on the other hand, hold the opposite view. To decide this question is difficult. The blood serum must be heated for half an hour at 55°–57° C. to deprive it of the deviating properties which are sometimes present in the sera of non-syphilitics (*v.* p. 80). It is doubtful whether such heating produces a fluid which is exactly comparable with the spinal fluid. We have examined a series of cases, estimating simultaneously in terms of haemolytic doses the comparative amounts of complement absorbed by the spinal fluid and the heated serum from the same patients. The results of such examinations showed that in some cases the spinal fluid, and in others the blood serum, produced the greater deviation. In two cases of general paralysis Gilmour found that the spinal fluid gave a positive result (0·1 c.c. of unheated fluid being employed in the test), whereas the blood serum after half an hour's exposure to a temperature of 55° C. gave a negative result on repeated examination. The presence of syphilitic reaction substances in the blood may be due in cases of general paralysis, to two concomitant processes; in the first place, to the disturbance which manifests itself in the sub-arachnoid space and adjacent tissue, and in the second place, to processes occurring outside the nervous system, such as give rise to a positive reaction with the blood serum in latent syphilis, or to an active syphilitic process in another part of the body. On the other hand, the reaction substances may be due solely to the disease in the central nervous system. Where there is a concomitant production of reaction substances outside the central nervous system, the blood serum might be expected to react more strongly than the cerebro-spinal fluid. Whereas if the production of reaction substances were confined to the subarachnoid space, the cerebro-spinal fluid might be expected to give a stronger reaction than the blood serum, and in some cases to react positively where the blood serum reacted negatively. This view is supported by Schmorl's case in which the occurrence of a positive Wassermann

reaction with the intraventricular fluid and the blood alone was to be ascribed to the fact that the gummatous process in the brain had extended to the choroid plexus, and was shut off from any direct association with the structures bathed by the spinal fluid. The question is one of great complexity, but these considerations would help to explain why it is that in cerebral syphilis the spinal fluid gives a positive Wassermann reaction less frequently than in general paralysis. It would be only in those cases where the syphilitic process involved the structures immediately in contact with the spinal fluid that a positive reaction would be expected.

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APPENDIX TO PART I

The biological syphilis reaction of Wassermann-Neisser-Bruck, its method and its relation to clinical medicine, are systematically dealt with in the following works:—

1. Boas, *Die Wassermannsche Reaktion*, etc., Berlin, 1911.
2. Bruck, *Die Serodiagnose der Syphilis*, Berlin, 1910; also in *Beiträge zur Pathologie u. Therapie der Syphilis*, Berlin, 1911.
3. Citron, in Kraus and Levaditi's *Handbuch d. Technik u. Methodik d. Immunitätsforschung*, Jena, 1909, Bd. ii.
4. Davis, *A System of Syphilis*, Oxford, 1910, vol. iii.
5. Harrison, *A System of Syphilis*, Oxford, 1910, vol. vi.
6. Levaditi and Roché, *La Syphilis*, Paris, 1910.
7. Morgenroth and Halberstaedter, "Komplementbindung als Serodiagnostische Methode," in *Die Deutsche Klinik*, Berlin, 1909.
8. Mulzer, *Praktische Anleitung zur Syphilisdiagnose auf biologischem Wege*, Berlin, 1910.
9. Noguchi, *Serum Diagnosis of Syphilis*, Philadelphia, 1910.
10. Plaut, *Die Wassermannsche Reaktion in ihrer Anwendung auf d. Psychiatrie*, Jena, 1909.
11. Sachs and Altmann, in Kolle-Wassermann's *Handbuch d. Pathogenen Mikroorganismen*, Jena, Ergänzungs-bd. ii, 1909.

PART II

THE TREATMENT OF SYPHILIS WITH SALVARSAN

(DIOXYDIAMIDOARSENOBENZOL—EHRLICH)

CHAPTER I

THE EXPERIMENTAL BASIS OF TREATMENT WITH SALVARSAN. EHRLICH AND HATA'S WORK ON THE CHEMOTHERAPY OF EXPERIMENTAL SPIRILLOSES

RELAPSING FEVER—FOWL SPIRILLOSIS—SYPHILIS IN RABBITS —THE QUESTION OF THE DEVELOPMENT OF RESISTANT STRAINS OF SPIRILLA (SPIROCHAETES)

SYPHILITIC disease was treated effectively long before the discovery of the spirochaete pallida. In the majority of cases the internal or external administration of mercury has been found to produce comparatively rapid, if only temporary, disappearance of the signs of disease, and there is little doubt that in a considerable proportion of cases complete cure has been effected as the result of energetic treatment. Thorough treatment with mercury involves courses of inunction, intramuscular injection, and oral administration extending over, at least, a period of two years. Although a comparatively small minority still questions the specific antisyphilitic action of mercury, general experience testifies to its value, and Neisser's experimental work proves conclusively its curative effect on infections with the spirochaete pallida. There are those also who, while not denying its efficiency in primary and secondary manifestations, still contend that its action only renders the infection latent. It is suggested that in such cases the symptoms reappear in what is known as the tertiary form and that the disease would have spent itself had it been allowed to take its natural course. Mercury has even been held responsible by some for the chronic degenerative and cachectic conditions in syphilitic subjects. We mention this in order to suggest how unwarrantable may be conclusions based on a hasty correlation of phenomena. Experimental therapy and clinical experience testify amply to the great effi-

ciency of mercury. The disease may become latent under the influence of the drug, and manifest itself at a subsequent period; this, however, is a well-known characteristic of protozoal infections, for example, malaria and trypanosomiasis. In certain cases the symptoms may be intensified by mercurial treatment. This phenomenon is also recognised as characteristic of protozoal infections. A specific remedy when administered in a small dose may stimulate the activity of the infecting organism. Spirilla and trypanosomes are increased in the blood of animals by the administration of very small doses of the specifics which exercise a curative effect in large doses. The fact that under the influence of mercury symptoms are occasionally intensified, and that evidence of disease disappears only to reappear later, does not prejudice the general conclusion that mercury is a powerful antisyphilitic remedy. The general disadvantages associated with the use of the drug are the digestive disturbances and uncertainty of dosage arising from oral administration, the discomfort, uncleanliness, and inaccuracy of the inunction method, and the pain of the injection method. But allowing for all these drawbacks and for the length of time prescribed for treatment, mercury must still be regarded as one of the most useful agents in scientific therapy. In particular cases, however, mercury proves to be a most unsuitable remedy, thus not infrequently idiosyncrasy shows itself in one of two ways, (1) the patient is hypersensitive to the drug and shows toxic symptoms with doses too small to influence the infection, and (2) even the largest doses administered over long periods, fail to cause more than temporary disappearance of the symptoms.

The administration of arsenic in protozoal infections has been for long a recognised method of treatment. Fowler's solution has been given internally in the cachectic stages of malaria with much benefit. The organic preparation of arsenic known as atoxyl (the sodium salt of para-amidophenylarsenic acid) was introduced by Thomas of the Liverpool School of Tropical Medicine, in the treatment of trypanosomiasis, and although this drug did not produce the results which were anticipated, it formed the starting point of important advances in the preparation of organic compounds of arsenic and their use in protozoal diseases. On the strength of Schaudinn's discovery that syphilis was due to a spirochaete and his view regarding the close biological relationship between spirochaetes and trypanosomes, Uhlenhuth was led to employ atoxyl in the

treatment of experimental spirillosis in fowls. These experiments proved beyond doubt the curative and protective properties of atoxyl in this form of spirillar infection. Similar therapeutic results were obtained by Uhlenhuth, Neisser, and Metchnikoff in syphilitic infections in animals. Following these experiments the drug was administered in cases of syphilis in the human subject, where it was found to have a very beneficial effect more especially in the malignant forms of the disease. Subsequent experience, however, made it manifest that the toxic properties of atoxyl rendered its administration undesirable in the human subject. Digestive disturbances, nephritis and especially optic atrophy, could be traced to its use even in small doses, and as a therapeutic agent it has gradually become discredited. The use of arsacetin (the sodium salt of acetyl-para-amidophenylarsenic acid) a derivative of atoxyl originally synthetised by Ehrlich and Bertheim, has also been discontinued on account of its toxic effects on human tissues.

The contributions made by Ehrlich to this department of scientific therapy are based on the recognition by himself and Bertheim of the chemical constitution of atoxyl. Proceeding on the basis of this knowledge, he was able to prepare closely related compounds and compare their effects in the treatment of trypanosome infections. The researches of Browning and Röhl on the therapeutic influence of these drugs in experimental trypanosomiasis, and on the development of specific resistant strains when non-curative doses were administered, showed that the toxic and parasiticidal effects of the substances depended on minute differences in their chemical constitution. The relatively great toxicity of these compounds for human tissues was the next problem which Ehrlich had to consider, and a most important advance in this direction was made when it was found that the unsaturated trivalent arsenic in arsenobenzol and arsenophenylglycin has a greater parasiticidal power relatively to its toxic action on the tissues of the host, than pentavalent arsenic compounds such as atoxyl and arsacetin. In dioxydiamidoarsenobenzol a substance was discovered which approaches the ideal in chemotherapy, a drug which possesses a maximum toxicity for the invading parasite, and a minimum toxicity for the organs of the body; for the chemistry of this compound *v.* p. 165.

In the published account of their experiments, Ehrlich and Hata [1] describe an extensive series of observations which prove conclusively the absolute curative effect of " salvarsan "

(dioxydiamidoarsenobenzol) in experimental spirillosis, its relatively feeble toxicity for the tissues of the infected animals and its great superiority over other allied substances which possess spirillicidal properties. In this work the authors give in detail the results which have a bearing on the therapeutic properties of salvarsan and its special merits as a curative agent. In the chemotherapy of experimental trypanosomiasis and spirillosis several hundred substances have been subjected to examination. Ehrlich points out that such of these substances as possess curative properties belong to the three following groups :—

1. The arsenical compounds ; including arsenious acid, atoxyl, and the more recent derivatives of phenylarsenic acid, namely, arsacetin, also arsenophenylglycin and salvarsan.

2. Certain azo-dyes of the benzidin group, for example, trypan-red, trypan-blue and trypan-violet.

3. Certain basic triphenylmethane dyes, such as parafuchsin, methylviolet and pyronin.

The experiments conducted by Hata included the observation of the effects of a large variety of substances in the spirillar infections, viz. relapsing fever, fowl spirillosis and syphilis.

Relapsing Fever

Preliminary observations were made to determine the natural course of the infection in untreated mice and the extent to which the disease could be modified by variations in the quantity and the virulence of the infective material with which the animals were inoculated. The therapeutic effect of about 200 aniline dyes was tested. Only a few of them were found to possess spirillicidal properties. It was found, for example, that in the test-tube methylene blue in a dilution of 1 : 6,000,000 inhibited the movements of the spirilla. When, however, the drug was injected into an infected mouse, 500 times that amount of methylene blue, estimated according to the body weight of the animal, failed to produce sterilisation ; so with the other dyes examined, the explanation being that when these substances are introduced into the animal body the tissues of the latter have a greater affinity for the dye than the spirilla have. In the test-tube, on the other hand, the whole of the dye is available for acting on the spirilla.

Of the arsenical compounds tested, Hata refers to the results obtained with six prior to the use of salvarsan. Atoxyl, arsacetin and arsenophenylglycin, which were found very effec-

tive in the treatment of trypanosomiasis in mice, did not give satisfactory results in the case of infections with the spirilla of relapsing fever. Arsenophenol, tetrachlorarsenophenol and tetrabromarsenophenol, when repeated in the largest doses tolerated, effected a cure. Dichlorphenolarsenic acid also cured the infection, but the toxic effect of this drug was such as to render its use impossible. The experiments with salvarsan demonstrated its great superiority over other arsenical compounds. This superiority consisted in the facts that the curative dose represented only a small fraction of the maximum tolerated dose, and that its administration was unaccompanied by any visible toxic disturbances.

Salvarsan, as it is obtained commercially in small sealed vacuum-tubes, is the di-hydrochloride of dioxydiamidoarsenobenzol (dioxydiamidoarsenobenzol is referred to by Ehrlich and Hata as No. 592, and its di-hydrochloride as No. 606). For purposes of injection, however, the substances are alike, as it is the soluble di-sodium salt which is injected, the solution being prepared by adding a suitable amount of caustic soda. The nature of the drug and its preparation for injection are referred to more fully on pages 165–170. Test-tube experiments failed to show the spirillicidal properties of the drug. Spirilla were killed in a dilution of No. 592 of 1 : 4,000 ; but it was found that the amount of alkali present was of itself sufficient to inhibit the movement of the parasites. In a dilution of 1 : 10,000 movement was not inhibited, but if the mixture of spirilla and drug was injected into a mouse, the animal did not become infected, whereas a mixture of spirilla with the equivalent amount of alkali, but without the drug, did give rise to infection.

The following table (I) shows the toxicity of the preparation :—

TABLE I

Animal.	Method of Administration.	Maximum tolerated Dose.
Mouse . .	Subcutaneous . . .	1 : 300 per 20 gramme
Mouse . .	Intravenous	1 : 350 „ 20 „
Rat . . .	Subcutaneous . . .	0·2 gramme per kilogramme
Fowl . .	Intramuscular . . .	0·25 „ „ „
Fowl . .	Intravenous	0·08 „ „ „
Rabbit . .	Intravenous	0·1 „ „ „
Rabbit . .	Subcutaneous. . . .	0·15 „ „ „

The maximum tolerated dose having been determined, it was necessary to find out the limits within which a cure could be effected. As the result of a very extensive series of experiments Hata arrived at the results shown in the following table (II). The animal was regarded as cured when during a period of two months daily examination of the blood failed to show the presence of spirilla. The correctness of this criterion of cure was demonstrated by the fact that on reinoculation such animals developed a characteristic infection ; whereas animals which had not been cured in the first instance, but in which the infection had merely become latent, showed a marked degree of immunity to the reinoculation.

TABLE II

RESULTS OF EXPERIMENTS WITH DIOXYDIAMIDOARSENOBENZOL

Dose.	Complete Cure obtained after		
	One injection.	Two injections.	Three injections.
1 : 600 . .	100 per cent.	—	—
1 : 700 . .	100 ,,	—	—
1 : 800 . .	100 ,,	—	—
1 : 1,000 . .	75 ,,	100 per cent.	100 per cent.
1 : 1,500 . .	18 ,,	75 ,,	100 ,,
1 : 2,000 . .	16 ,,	66 ,,	100 ,,
1 : 3,000 . .	0 ,,	0 ,,	33 ,,

By 1 : 600 is meant the injection into a mouse weighing 20 grammes of 1 c.c. of a dilution of 1 in 600 of the drug, etc.

By these experiments it was shown that complete sterilisation of an infected mouse could be attained by—

A single injection of 1 : 800.
Two injections of 1 : 1,000.
Three injections of 1 : 1,500.

It is pointed out that more than three injections do not improve the results and that in some animals signs of hypersensitiveness were noticed on giving repeated doses.

A correlation of the results which have just been referred to indicates the proportion of the curative dose to the tolerated dose of the drug :—

Single injection 300 : 800, that is 1 : 2·7.
Two injections 300 : 1,000 ,, ,, 1 : 3·3.
Three injections 300 : 1,500–2,000, that is 1 : 5–1 : 7.

The curative properties of salvarsan in relapsing fever infections in mice were thus definitely established. The therapeutic dose is only a fraction of the tolerated dose, and there are no accessory phenomena of a toxic character.

Further experiment demonstrated the ameliorating influence of small doses in virulent infections. It was also ascertained that the injection of 1 : 400 subcutaneously 24 hours prior to the injection of spirilla, prevented infection in two out of three mice. When, however, inoculation with spirilla was delayed until the third day after treatment, infection took place in all three, though in a mild form. The preventive action of the drug is thus very slight in mice.

Experiments carried out on rats corroborated on the whole the results obtained with mice.

Spirillosis of Fowls

In his experiments on fowl spirillosis, Hata followed the methods which he adopted in the case of relapsing fever in mice. He corroborated the findings of Levaditi, McIntosh, Uhlenhuth and others as to the spirillicidal action of atoxyl in this infection. Arsacetin, arsenophenylglycin and other arsenical compounds were also found to be curative in amounts which represented only a fraction of the tolerated dose. Salvarsan, however, proved greatly superior to any of the other substances tested. The results with intramuscular injections of these drugs are given in table III :—

TABLE III

	Curative Dose.	Maximum Tolerated Dose.	Ratio.
Atoxyl	0·03 gramme	0·06 gramme	1 : 2
Arsacetin	0·03 „	0·1 „	1 : 3·3
Arsenophenylglycin . . .	0·12 „	0·4 „	1 : 3·3
Dioxydiamidoarsenobenzol (Salvarsan)	0·0035 „	0·2 „	1 : 58

On the second day after infection an injection of 0·0035 gramme per kilogramme of body weight is sufficient to produce absolute cure. The tolerated dose was found to be 0·2 gramme. The proportion of curative dose to tolerated dose is thus 1 : 58. It was observed that in the case of simultaneous injection of spirilla and drug 0·0025 gramme per kilogramme sufficed to prevent the occurrence of infection.

The protective powers of the drug are also more pronounced in fowl spirillosis than in relapsing fever. An intramuscular injection of 0·07 gramme rendered the animal refractory for thirty days ; on inoculation thirty-five days after the protective injection a mild infection resulted. The protective action was absent at the end of fifty days. It is suggested that as a result of intramuscular injection a depôt of the substance is formed in the coagulated and necrotic tissue, and that this is responsible for the temporary refractory state of the animal. Of great interest and perhaps of considerable importance is the observation that while an intramuscular injection of 0·07 gramme per kilogramme had a protective effect lasting thirty days, a fowl treated intravenously with 0·03 gramme showed practically no resistance to infection on the sixth day after the injection.

Syphilis in Rabbits

Relapsing fever and fowl spirillosis are infections which are eminently suited for experiments in chemo-therapy. A certain uniformity of infection can be produced, and this can be varied as occasion demands. Further, the number of spirilla in the blood as indicated by microscopic examination, can be taken as evidence of the state of infection. Experimental syphilis, on the other hand, cannot be rendered uniform, and the variation in individual animals as regards natural resistance is very considerable. The spirilla in syphilis are for the most part in the connective tissue of the host and not in the blood ; thus the most certain means of observing the course of the disease in the other forms of spirillosis, i.e. microscopic examination of the blood, cannot be applied in experimental syphilis.

Rabbits can be infected with the spirochaete pallida (1) in the cornea and (2) in the scrotum. Corneal syphilis is produced by inserting a small piece of syphilitic corneal material into the anterior chamber of the eye. In five to eight weeks the cornea becomes cloudy and opaque, and evidence of vascularisation appears. Occasionally, the corneal condition disappears in the natural course of the infection, but in most cases the infiltration is present for four or six months after the inoculation. Hata treated his cases when the corneal opacity and vascularisation were far advanced. The result of treatment is seen from the following protocol. A rabbit inoculated in the right cornea showed a very advanced affection in two and a

half months; the cornea was absolutely opaque, extremely vascular, and had a yellowish-grey colour. An intravenous injection of 0·04 gramme of salvarsan per kilogramme was given. In ten days the opacity had partially disappeared and the pupil could be seen. In a month the cornea had become transparent, and only a slight pin-point scar could be observed; no vessels could be detected. Hata points out, however, that the syphilitic keratitis of rabbits is not well adapted for such experiments. The tissue cannot be examined from time to time for spirochaetes, and the only evidence of the course of the disease is the general appearance of the cornea. Moreover, the disease does not advance in a progressive manner, but is interrupted by remissions. Spontaneous cure may also take place. Scrotal infection is obtained by inserting a small piece of infected cornea into a subcutaneous pocket formed on the under aspect of the scrotum by removing a small portion of the thin soft skin. In ten to fourteen days a small nodule appears, and in six or eight weeks an indurated ulcer about the size of a shilling may be present. It is usually crusted over with a moist scab, the removal of which shows a dirty necrotic surface with bleeding points. This condition resembles the human hard chancre, contains abundant spirochaetes, and may last for months. Regular examination for spirochaetes can be made, and an observation of the course of the infection is thus to some extent possible. This is done by inserting a sterile needle into the indurated mass, and pressing out a drop of serous bloody fluid, which can easily be examined for spirochaetes with the aid of dark ground illumination. Hata began the treatment of infected animals when the chancre had attained a considerable size, when it still showed evidence of continued growth with no signs of natural retrogression, and when abundant spirochaetes were present. It was found that a single injection of a sufficiently large dose of salvarsan effected a complete and immediate disappearance of the organisms, with healing of the chancre in two or three weeks. On the day following a single injection of 0·015 gramme per kilogramme, spirochaetes could not be detected. After a dose of 0·01 gramme to 0·005 gramme very few organisms were present on the second day. The minimal dose necessary to produce complete cure is between 0·01 gramme and 0·015 gramme per kilogramme. The tolerated dose by intravenous injection is 0·1 gramme. The curative dose of salvarsan in syphilis of rabbits is thus only a seventh to a tenth of the tolerated dose.

The Question of the Development of Resistant Strains of Spirilla (Spirochaetes)

In the literature dealing with the treatment of syphilis, reference has been made repeatedly to the possibility of certain refractory infections being due to the development of resistant strains of spirochaetes, that is to say, the spirochaetes gradually becoming accustomed to the mercury or arsenic. There is no experimental evidence to substantiate the accuracy of such an explanation, although the occurrence of resistant strains in the treatment of experimental trypanosomiasis is a well recognised fact. As has been shown by Browning, Röhl, and others, the administration of non-curative doses of aniline dyes and arsenical preparations in experimental trypanosomiasis gives rise to a strain of organisms resistant to the specific type of substance administered. It was naturally a matter of interest and importance to determine whether in the case of salvarsan, resistant strains of trypanosomes and spirilla could be developed. This has been done by Margulies[2], who carried out her investigations—(1) in mice infected with the trypanosomes of nagana, dourine, and mal de caderas; (2) in fowls infected with the spirilla of fowl spirillosis; (3) in mice infected with the spirilla of relapsing fever; (4) in rabbits infected with scrotal syphilis.

It was found that in the case of the trypanosome infections the micro-organisms readily became resistant to small doses of salvarsan, and as a result of the continued administration of increasing amounts, the maximum tolerated dose could be given without producing sterilisation. Reference has been made above (p. 156) to the fact that the large series of trypanocidal substances fall into three groups. It has been found that resistance developed to a particular member of a group involves resistance to every other member of the group. As was to be expected, the strains resistant to salvarsan proved to be resistant also to atoxyl, arsacetin and arsenophenylglycin. The usual method pursued in developing a resistant strain consists in the administration of a very small dose of the curative substance to an infected animal. The parasites are then injected into a second animal, and a slightly larger dose of the drug is given. This process is continued till the infecting organisms can withstand a larger amount than the curative dose. The resistant property once developed is maintained through succeeding passages without administration of the drug.

Margulies modified the method so as to produce conditions which resembled more the method of treatment in human infections. In the trypanosome infections very small doses were given, and these were gradually increased in the same animal until a resistant strain was developed in from five to nine passages. A beginning was made with a dose of 1 : 12,000 of salvarsan, that is, a tenth of the smallest curative dose, and this was gradually increased to 1 : 300. As a result of the administration of 1 : 300 no change in the shape, activity or staining reaction of the trypanosomes was noticed.

Spirillar infections have not the same definite course as trypanosome infections, and in addition there is always in spirillar infections the possibility of spontaneous cure. They do not, therefore, afford the same favourable conditions for the production of resistant strains. Hata had found that in fowl spirillosis 0·0005 gramme per kilogramme of salvarsan effected a cure, and Margulies beginning with extremely small doses was able to increase the dose to 0·007 gramme, that is, fourteen times the curative dose, without sterilising the animal. This would seem to indicate a certain degree of resistance, but the tolerated dose for fowls is 0·2 gramme, so that there was no question of the development of a strain which would produce an infection incurable by the drug. In addition, it was found that although a certain degree of drug resistance had been developed, so that 0·007 gramme did not produce sterilisation, the spirilla had at the same time lost their pathogenic properties to a large extent.

Experiments with the spirilla of relapsing fever failed to produce any evidence of the possibility of developing a strain resistant to salvarsan.

Rabbits infected with scrotal syphilis were treated with amounts of salvarsan equal to a tenth or a fifteenth of the curative dose. Injections were repeated every two or three days, but after the first three injections the chancres began to soften and heal. The repeated administration of small amounts did not produce any concomitant disturbances such as might be attributed to the development of hypersensitiveness to the drug.

Thus the experimental evidence is entirely against the probability of the development of arsenic resistant syphilitic spirochaetes as the result of prolonged treatment with non-sterilising doses of salvarsan.

The experiments of Margulies are also of very great importance,

as they indicate that repeated injections of salvarsan are not likely to give rise to constitutional disturbances of an anaphylactic character.

REFERENCES.

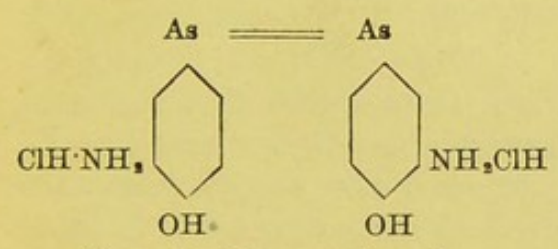
[1] Ehrlich and Hata, *Die Experimentelle Therapie der Spirillosen*, Berlin, 1910, *v.* also Ehrlich, *Zitschr. f. Immunitätsforsch.*, Ref., 1910, p. 1123.

[2] Margulies, "Die Behandlungder Syphilis" (*Königsberg Versammlung*), Leipzig, 1910, p. 930.

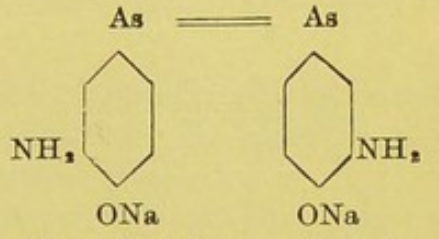
CHAPTER II

CHEMISTRY, ADMINISTRATION AND EFFECTS OF SALVARSAN

CHEMISTRY OF SALVARSAN—METHODS OF PREPARING THE DRUG FOR INJECTION—METHODS OF INJECTION—CONTRA-INDICATIONS—TREATMENT OF THE PATIENT BEFORE AND AFTER INTRAVENOUS INJECTION—GENERAL EFFECTS OF TREATMENT—JARISCH-HERXHEIMER REACTION—ELIMINATION OF THE DRUG.



Dioxydiamidoarsenobenzol di-hydrochloride (Salvarsan).

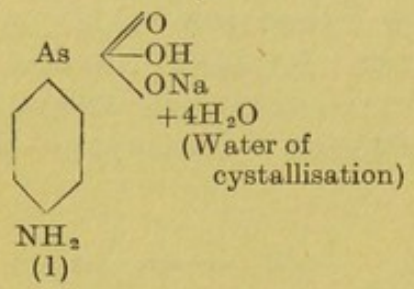


Dioxydiamidoarsenobenzol. disodium salt (alkaline solution)

SALVARSAN, as has already been pointed out, is the di-hydrochloride of dioxydiamidoarsenobenzol. Dioxydiamidoarsenobenzol is a yellow powder, which is easily oxidised and must be kept in vacuum tubes. It is insoluble in water, but dissolves readily on the addition of sodium hydrate. The di-hydrochloride is easily oxidised to poisonous compounds, and is accordingly preserved in sealed tubes *in vacuo* or containing inert gas; it is soluble in water and readily soluble in warm normal saline solution; the solution is strongly acid in reaction. If the acidity is neutralised by the addition of caustic soda solution the insoluble base (dioxydiamidoarsenobenzol) is precipitated. If only half of this amount of caustic soda is added, then the monohydrochloride of dioxydiamidoarsenobenzol is formed. If, in addition to the amount of caustic soda necessary to precipitate the base, a further quantity of

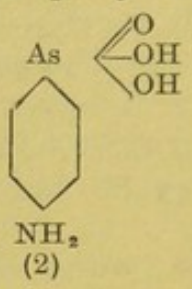
alkali is added, the hydrogen atoms of the phenol hydroxyls become replaced by Na, and the compound goes into solution as the disodium salt of dioxydiamidoarsenobenzol. These substances are examples of a very large series of synthetic organic preparations of arsenic, which have been prepared and tested by Ehrlich and his collaborators. The starting point in the preparation of these compounds is to be found, as we have already mentioned, in the recognition by Ehrlich and Bertheim of the constitution of atoxyl. They showed that atoxyl (formula 1) was not, as had been asserted previously, an anilido-derivative of arsenic acid, but that it was the sodium salt of para-amido-phenylarsenic acid (formula 2).

Atoxyl.



(1)

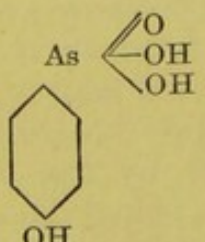
Para-amido-phenylarsenic acid.



(2)

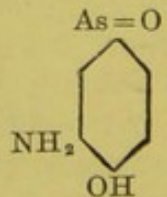
Para-amido-phenylarsenic acid is a very active chemical substance capable of forming numerous synthetic compounds. From it, for example, were prepared the acetyl-derivative (arsacetin) and the paraoxybenzaldehyde compound, both of which Browning found to be efficient therapeutic agents in the case of nagana infections in mice. By the process of diazotising, etc., para-amido-phenylarsenic acid becomes converted into para-oxyphenylarsenic acid (formula 3), and the latter by subsequent nitration and reduction gives rise to meta-amido-para-oxyphenylarsenious oxide (formula 4).

Para-oxyphenylarsenic acid.



(3)

Meta-amido-para-oxyphenylarsenious oxide.

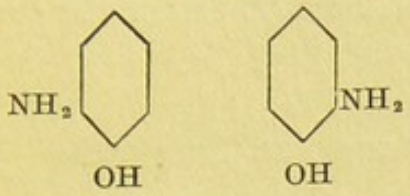


(4)

The condensation of two molecules of meta-amido-para-oxyphenylarsenious oxide leads to the formation of dioxydiamido-

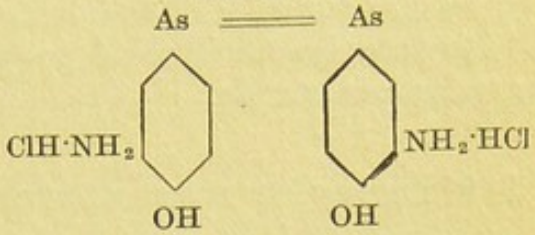
arsenobenzol (formula 5), the dihydrochloride of which is Salvarsan (formula 6).

Dioxydiamidoarsenobenzol.



(5)

Salvarsan.

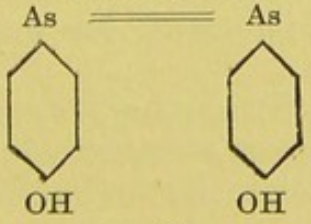


(6)

Thus, as Ehrlich points out, although the two substances, atoxyl and salvarsan, are related, that relationship is a distant one and forms no basis for the suspicion that the toxic effects of the former, e.g., amaurosis, will necessarily follow the administration of the latter. He emphasises the parasiticidal importance of the unsaturated trivalent arsenic (seen in formula 5 and formula 6) as compared with the pentavalent compounds (as in formula 3). The large doses of the latter necessary to produce the desired therapeutic effects are more often associated with toxic phenomena due to poisoning of the specialised nervous organs.

A point of importance, to which attention is drawn, is the *para*-position of the hydroxyl-group (OH-) as seen, for example, in arsenophenol (formula 7) and salvarsan. This position of the hydroxyl-group is correlated with a marked spirillicidal activity. Arsenophenol possesses marked spirillicidal properties ; but its use as a therapeutic agent is contra-indicated by the fact that it is difficult to procure in a pure form in large quantities, and it readily undergoes oxidation into poisonous compounds. The further introduction of the amido-group in the ortho-position to the hydroxyl-group was found to increase the therapeutic efficiency to a maximum.

Arsenophenol.



(7)

It is obvious that the main problem in the search for an ideal therapeutic agent was the preparation of an arsenical

substance which possessed strong parasiticidal properties and was at the same time divested of toxic action for the host. In salvarsan radicles are introduced which simultaneously (*a*) reduce the toxicity of the compound as a whole for the host, (*b*) increase the spirillicidal properties, and (*c*) render the compound more stable.

Methods of preparing the Drug for Injection

During the period immediately following the introduction of the drug several methods of preparation were suggested. These are now chiefly of historic interest, because it is generally accepted that both from the point of view of efficient treatment and of the comfort of the patient the intravenous injection of a dilute solution of the disodium salt is the best form of administration.

A proper appreciation of the results which are recorded in the literature can, however, be arrived at only after taking into consideration the several methods which have been employed. Many of the disadvantages attached to salvarsan treatment, and most of the inefficient results and so-called complications, are to be attributed to the modes of preparation and technique of treatment, which were employed in the earlier period.

We do not propose to record all the methods which have been devised and practised, but would refer only to such as give an indication of the general principles underlying the various modifications.

In every case, no matter how the drug is prepared for injection or how it is injected, the following precautions must be observed: (1) The capsule containing the drug should be examined carefully to see that it has not been damaged, thus allowing access of air to its contents. In the event of there being any sign of damage such as a crack, the sample must be discarded. (2) The drug must be prepared for injection *immediately* before use.

The Acid Solution.—When the drug is dissolved in warm water or warm physiological saline solution a strongly acid solution is obtained. In some instances this solution has been injected intramuscularly and in other cases intravenously. This is the form in which the drug is most irritating and also most toxic.

The Mono-Acid Solution.—When the strongly acid solution

referred to already has been completely neutralised by the addition of alkali, the base is formed, which precipitates from the solution. If half the amount of alkali necessary to produce complete neutralisation be added to the original acid solution a solution of the mono-acid compound will be formed. This solution has been given intramuscularly. It is also extremely irritating.

Neutral Suspension.—This, as has been shown, is the drug in the form of a precipitate of the base, prepared by adding to the original acid solution just sufficient alkali to neutralise it. This method of administering the drug was devised by Michaelis and Wechselmann, and has been employed by many others with slight modifications. The neutral suspension was injected subcutaneously and intramuscularly, and for some time was the form of administration most generally employed. It probably had the advantage of producing less local irritation and pain at the site of injection than the acid or alkaline solutions, but in many cases pain was severe and absorption was slow, and not infrequently there resulted encapsulation of masses of necrotic tissue containing considerable quantities of arsenic. The disadvantages and complications incident to this method of treatment are referred to later (*v.* p. 228).

Other Suspensions.—Suspensions in olive oil, almond oil, and liquid paraffin have also been injected subcutaneously and intramuscularly. These are said to be comparatively non-irritating. Their use is, however, subject to the disadvantages which apply to the neutral suspension. They cause local necrosis of the tissues, and only small amounts of the quantity injected gain access to the general circulation at a time. The injection of these suspensions has been given up, or ought to have been given up, in favour of the intravenous method.

Alkaline Solution of the Di-sodium Salt.—This is the form in which the drug should be administered. t is t he form in which it was used by Hata in his original experiments, and it was in this form that Ehrlich recommended its use in the first clinical observations. The method of preparation which we have adopted is as follows :—The glass ware used is carefully sterilised. The drug is placed in a 30 c.cm. stoppered cylinder containing about 75 small glass beads. To this are added about 15 c.cm sterile normal saline solution previously heated to 50° C. Thorough shaking brings the drug into solution. A 10 per cent. solution of caustic soda is now added drop by drop to the solution in the cylinder. A precipitate of the

base is first thrown down, and on further addition of caustic soda, aided by shaking, this is again brought into solution, the solution being strongly alkaline. The amount of caustic soda necessary is about 0·25 c.cm. of 10 per cent. solution for each 0·1 gramme of salvarsan, thus for 0·6 gramme of salvarsan 1·5 c.cm. of 10 per cent. solution of caustic soda would be required. A drop more of alkali than is just necessary to produce the clear solution should be added. Otherwise, on cooling, the diluted solution may show signs of a suspension falling out. Should the diluted solution show a precipitate, this can be redissolved by the addition of a drop of alkali. The drug now being in the form of a clear alkaline solution, is poured into a 200 c.cm. beaker, and the residue in the stoppered cylinder is washed out into the beaker with 3 or 4 c.cm. saline solution. It is advisable to pour the solution from the cylinder through fine silk, to remove any suspended matter. The drug is now in a solution of about 20 c.cm. and if an intramuscular injection is to be given this is the form to be preferred. Intramuscular injections should be given only in those cases where it is practically impossible to give the drug intravenously, as in the case of infants. For intravenous injection the solution of 20 c.cm. containing 0·6 gramme of salvarsan is diluted with 0·85 per cent. NaCl solution to 300 c.cm. and for this purpose another beaker of 400 c.cm. capacity is used, the fluid being thoroughly mixed by pouring it from the one beaker to the other. It will be seen that in this dilution of 300 c.cm. each 50 c.cm. contains 0·1 gramme of the drug, and the dose can be reckoned accordingly.

Dosage.—In the case of adult males 225 c.cm. to 250 c.cm., that is 0·45 gramme to 0·5 gramme of the drug, should be given. In the case of females 200 c.cm. is regarded as an average dose. Children according to size and age should get from 0·01 gramme to 0·1 gramme. In the case of weak and poorly-nourished adult patients, it is inadvisable to give more than 0·3 gramme. In diseases of the nervous system, such as general paralysis and locomotor ataxy, 0·3 gramme should not be exceeded. After an interval of about three weeks the injection should be repeated, because, although it is probable that in some cases a permanent cure is effected by a single injection, it is now considered advisable to give a second injection, and in some cases a third. This point is discussed more fully on page 291.

Methods of Injection

Injections have been given (1) intramuscularly, (2) subcutaneously, and (3) intravenously.

1. *Intramuscular Injections.*—The clear alkaline solution, the acid solutions, and the suspensions in oil, etc., as well as the neutral suspension have all been administered in this form. With due antiseptic precautions the injection was made in the gluteal region into the upper and outer quadrant of the muscles at a point on the line joining the anterior superior iliac spines. The needle was directed away from the vessels and nerve trunks which pass through the sciatic notch, and the injection made slowly, so as to avoid tearing of the tissue. In some cases the parts were massaged after injection, so as to cause a distribution of the material and prevent encapsulation.

2. *Subcutaneous Injections.*—This form of administration has been used chiefly for the neutral suspension. The suspension was injected either at the edge of the vertebral border of the scapula at its middle, or in other cases into the deeper tissue at the lower angle of the scapula.

3. *Intravenous Injections.*—For this form of administration the dilute clear alkaline solution described above is used. The apparatus * necessary for the injection comprises a cylindrical funnel of 300 c.cm. capacity, to the narrow lower end of which about 5 feet of rubber tubing is attached. This tubing is interrupted about eighteen inches above its attachment to the cylinder by a piece of glass tube to act as a window. At the other end of the tubing there is an arrangement whereby it can be easily fixed on to the needle used for venepuncture. The apparatus is easily sterilised ; the glass cylinder may be boiled, or it may be plugged with cotton wool at the upper and lower apertures and then placed in a hot air chamber, and the rubber tubing may be boiled. Immediately before use the tubing is fixed on to the glass cylinder and the apparatus is washed through with warm sterile saline solution. A screw clamp is placed on the tubing about three inches above the nozzle and the clamp is fixed, leaving the whole upper portion of the tube filled with saline and in addition about 10 c.cm. of saline solution in the cylinder. The salvarsan solution is now carefully poured into the cylinder so as to avoid the entrance of air bubbles into the tube. The skin in the region of the forearm and elbow has been care-

* The apparatus may be obtained from Messrs. Thomson, Skinner and Hamilton, 38, Sauchiehall Street, Glasgow.

fully sterilised. The arm is allowed to hang down for a few minutes, and a rubber tourniquet which can be quickly removed is placed on the arm above the elbow. A prominent vein on the flat surface of the arm, preferably below the elbow, is chosen,* and a " Record " needle of 1·0 mm. to 1·22 mm. external diameter is introduced into the lumen in the direction of the blood stream. The free flow of blood indicates that the needle has been properly inserted and one can easily recognise by the sense of touch whether the needle is resting freely in the lumen. The tourniquet should be removed immediately it is found that the needle has been properly introduced. The clamp on the tube is now released, and while the blood is flowing from the vein and the saline solution from the tubing the nozzle of the latter is fixed into the needle (preferably by an assistant, while the operator continues to hold the needle steady in the vein). The cylinder is then raised up so as to allow the solution to flow into the vein. If the needle has not been properly inserted this reveals itself by the appearance of a bulging at the side of the vein. In the event of this occurring, the needle should be removed at once, and another vein should be punctured. It would appear that in some cases the connective tissue surrounding the veins at the bend of the elbow is loose and gives comparatively little support to the vessels; thus in some instances, we have noticed an escape of fluid apparently at the point of entrance of the needle into the vein. Under these circumstances it would be advisable to select a vein considerably above or below the bend of the elbow. The use of a thinner needle (0·86 mm.) also diminishes the chance of haematoma-formation, but of course, lengthens the time necessary for the fluid to pass into the vein. Should the fluid appear to be falling too slowly in the cylinder this may be remedied by turning the needle in the vein, as the aperture may have become applied to the vein wall. If the method described above be accurately followed, the solution should find its way into the circulation in about seven to ten minutes without contamination and without the entrance of air bubbles. While in the case of adults the injection should always be given intravenously, in children and especially in infants, it may be impossible to carry out the intravenous method. In that case the injection should be given in the form of the clear alkaline solution intramuscularly in as small a bulk as possible. An attempt should be made, however, to

* In stout subjects a vein can frequently be felt when it cannot be seen.

administer the drug intravenously in every case, even should the procedure involve exposure of the vein.

Contra-Indications

It is inadvisable to give salvarsan to patients showing evidence of extensive disease of the central nervous system, or extensive disease of the circulatory system. The presence of localised areas of softening in the brain or spinal cord, of aneurysm or heart disease (especially myocarditis and affections of the coronary arteries), of severe nephritis, diabetes or ulceration of the stomach, should be regarded as definite contra-indications. The importance of recognising these warnings will be evident from our review of the complications which have attended the administration of the drug in such cases (*v.* chap. on fatalities, p. 263).

Treatment of the Patient before and after Intravenous Injection

Before injection the patient should have the treatment usually adopted before the administration of an anaesthetic. An aperient should be given the night before, and on the day of injection only light food should be taken. It is extremely important that the alimentary tract should be in a good condition. The presence of gastritis, for example, should be taken as an indication for postponing the injection. Within an hour or two after administration the arsenic is being excreted by the kidneys and bowels, and in the event of there being catarrh of the stomach, severe vomiting is almost certain to ensue. The patient should receive the injection in bed, or go to bed immediately afterwards. In a few hours there may be a rigor with a rise of temperature, varying from 101° F. to 103° F. Sickness and vomiting may also be present. Hot bottles should be put into the bed, and warm blankets put on the patient. For the vomiting a hot fomentation on the stomach and a drink of milk and soda water affords relief as a rule. Occasionally severe headache may be present in addition. These symptoms are, however, transitory, and after a night's sleep the patient is refreshed and feels well, as a rule. We have observed rigors and vomiting in only a small proportion of our cases, and those for the most part where the dose was over 0·5 gramme. Diarrhoea has been observed in a few of our cases; but it has not been severe and has passed off without special treatment. When some of the solution escapes into the loose

tissue surrounding the vein, it gives rise to severe pain and sometimes to a painful oedema which may last for some days. This may be treated by the application of hot fomentations sprinkled with laudanum. In a few cases venous thrombosis occurs as a result of injection. We have observed one such case. It is difficult to say what are the conditions which induce this result. With reference to the case of thrombosis under our care, another patient was injected with a portion of the same solution and no thrombosis occurred. It is attributed by some observers to the alkalinity of the solution injected. When thrombosis does occur the arm should be fixed in a splint and kept at rest, so as to avoid dislodgement. As a rule, patients suffer little or no discomfort from the treatment, and after two days' rest in bed with light diet on the first day, they may be allowed to resume their ordinary duties. When there is severe vomiting and sickness, it is advisable that the patient should stay longer in bed. Where there are local complications incident to faulty technique the after treatment must be modified accordingly.

General Effects of Treatment

The administration of salvarsan is followed as a rule by a sense of well-being on the part of the patient. Hoppe and Schreiber [1] ascribe this to a stimulating effect on the lecithin-metabolism. In any case, whatever be the explanation, patients who are anaemic, poorly nourished, and despondent, become in a very short time healthy, active and cheerful. In this change the mental element may play some part, but the increase in body-weight, and general improvement in every way, are evidence of a far-reaching change in the nutrition of the patient.

In some cases an arsenic rash in the form of an erythema follows injection. This may appear on the day of injection, or may be delayed for two or three days. To be distinguished from this toxic rash (which has been observed in nonsyphilitic cases injected with the drug) is the exanthem known as the Jarisch-Herxheimer Reaction.

Jarisch-Herxheimer Reaction

In the treatment of syphilitic cases in the early stages a phenomenon known as the Jarisch-Herxheimer Reaction has been noted by several observers. This reaction had already been observed in the course of mercurial treatment of syphilis and consists

in the development of a rash or the extension or intensification of a rash already present, as the result of treatment. Before the discovery of the spirochaete pallida or the Wassermann reaction, this phenomenon was often of considerable diagnostic importance. The reaction has been observed by Loeb [2] to occur two hours after the injection of salvarsan. It may last for two or three days and then, as a rule, it completely disappears. Any aggravation of syphilitic symptoms following the injection of salvarsan has been interpreted as a Jarisch-Herxheimer Reaction. The increase of the lancinating pains of locomotor ataxy which sometimes occurs as an immediate consequence of salvarsan treatment has been explained in this way. Truffi [3] by the administration of very small doses of salvarsan (0·025–0·05 gramme) observed the reaction in a very distinct form in a number of cases. Ehrlich believes that the phenomenon indicates failure of the injected dose to produce sterilisation. He considers that the amount of salvarsan injected has only served to stimulate the growth and activity of the organisms. This explanation coincides with Browning's observation in the treatment of experimental trypanosomiasis with methyl violet, where it was found that a fraction of the dose necessary to produce disappearance of the parasites actually led to an increase in their number. Iversen has made corroborating observations with small doses of salvarsan in cases of relapsing fever ; examination of the blood after the injection of small doses showed an increase in the number of spirilla. A corresponding phenomenon has been demonstrated in the case of certain bactericidal sera acting on the homologous organisms *in vitro* (Neisser, Mackenzie and Martin). Thus, for example, in plating out experiments with meningococci, as the result of the action of 0·5 c.cm. fresh antimeningococcus serum, only 10 colonies developed on a plate in the series where the control plate showed 1,000 colonies ; in the same series with 0·05 c.cm. of the same bactericidal serum a countless number of colonies grew. There is good reason to suppose that the occurrence of a Herxheimer reaction denotes a temporary stimulation of the infecting organisms to increased multiplication and activity. It is not quite certain, however, that an injection followed by this phenomenon has failed to produce a therapeutic effect. The reaction undoubtedly occurs in most cases before the salvarsan has had time to exercise its full influence, as is shown by the fact that ultimately all the symptoms may disappear without further treatment. In any case, there is no indication that

the injection should be repeated at a shorter interval than would be allowed to elapse in an ordinary case.

Elimination of the Drug

In view of the possible necessity of giving repeated doses of the drug, it is important to know how long the process of excretion lasts. Arsenic administered in the form of Fowler's solution is excreted by the kidneys in twenty-four to thirty-six hours. Given as atoxyl or arsacetin it is rapidly eliminated in the urine. In the case of arsenophenylglycin and salvarsan the arsenic is excreted partly in the urine and partly in the faeces. After subcutaneous injection of the former, arsenic is absent from the urine on the fourth day, whereas after a similar injection of salvarsan arsenic may still be detected in the urine on the tenth or twelfth day. There are, however, very great individual variations in the duration of excretion. Fischer and Hoppe found that in the case of general paralytics traces of arsenic were found in the urine on the twelfth day after injection, in the case of epileptics there was no arsenic to be detected on the fifth day. On the other hand, a case is reported of a patient who died five weeks after injection, and in the necrotic mass in the gluteal muscles a considerable quantity of arsenic was found. Fischer [4] calls attention to observations made by Loeb, who examined three cases ; in one case he found minute traces of arsenic in the urine in the eleventh and thirteenth weeks after injection ; in the second case, in the eighth and tenth weeks ; and in the third, which received only 0·15 gramme salvarsan, in the sixth and eighth weeks. On the strength of these observations Fischer believes that arsenic may be present in the body for three months after the intramuscular injection of an average dose of salvarsan. It is extremely doubtful, however, whether the potency of the drug extends over the whole period during which arsenic is being excreted. It is more probable that it is only during the period that excretion is taking place in considerable amounts that the therapeutic influence is being exercised. The urine from a series of our cases was examined by Miss B. Rutherford, who found that after intramuscular injection arsenic was present in fourteen out of sixteen cases on the tenth day. In six cases treated intravenously no arsenic was found in the urine on the fourth day (*v.* also Beveridge and Walker,[5] who found no marked difference in the rate of excretion after a second as compared with a first injection). This accords with Hata's

observations, already referred to, where it was found that intravenous injection in fowls conferred immunity to spirillosis for five days, whereas intramuscular injection conferred immunity for thirty days. It has also been found that in the human subject arsenic may be demonstrated in the urine two hours after injection, whether intravenous or intramuscular.

Although transient albuminuria has been noted by some observers, it is generally agreed that salvarsan does not exercise a deleterious effect on healthy kidneys. In one of our cases of general paralysis which received a gluteal injection, retention of urine occurred ; but this lasted only one day. Instances of acute nephritis supervening on a chronic affection as the result of salvarsan injection are recorded. The acute phase, however, passed off quickly. Where the nephritis is due to a syphilitic infection the administration of salvarsan is often attended with great benefit. We have treated a man with amyloid kidneys, whose blood gave a strongly positive syphilitic reaction, and the albumin fell from 0·2 per cent. by Esbach, to a trace in ten days. His general condition improved greatly and he felt better than he had done for years.

In the preceding pages we have noted the phenomena which have been described as commonly following treatment with salvarsan, and when they occur they should not give rise to any anxiety. Certain other complications, however, have been ascribed to the action of the drug, and have given rise to much controversy. Severe and extensive local necrosis has been observed in some instances ; in other cases the organs of special sense have become the seat of disease at an interval following treatment ; bladder and bowel complications and jaundice have been recorded ; and in a few cases death has followed the administration of the drug. The occurrence of these complications, and the various interpretations which may be put on them, are obviously matters of vital importance, accordingly we have dealt with them at length in special chapters (*v.* pp. 227, 263).

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[3] Truffi, *Biochemica y Therapica Sperimentale*, Vol. ii, Part 5.
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CHAPTER III

REVIEW OF THE LITERATURE ON THE TREATMENT OF HUMAN SYPHILIS, RELAPSING FEVER, FRAMBOESIA, AND OTHER DISEASES BY SALVARSAN

THE scope of the present volume does not admit of anything more than a short summary of the results which have been published since salvarsan was first used about two years ago. During this period the effect of the drug on about a million cases has been observed (*v.* p. 278), and continued experience has tended to confirm the earlier opinion that in salvarsan there had been discovered a substance which would produce a revolution in the treatment of syphilis.

The Early Observations of Alt, Schreiber, and Wechselmann

The clinical administration of the drug was first undertaken by Alt, who had previously carried out investigations on general paralytics treated with arsenophenylglycin. The results of his observations with salvarsan were published in March, 1910. The toxic effects of injection into dogs were first of all observed. Before treating patients, two of the medical assistants received intramuscular injections, which produced no disturbances beyond local swelling and pain, and these passed off in a few days. Twenty-three cases of general paralysis were injected, each receiving 0·3 gramme in alkaline solution. These were mostly cases which had not reacted to arsenophenylglycin. Some time after injection sixteen of these had still positive serum reactions, two had become negative, and other five showed some diminution in the deviating power of the serum. Alt concluded from this that the salvarsan had exercised a specific influence on the disease. He had already obtained similar results from the use of arsenophenylglycin. Following on these observations, and when it was definitely established that

0·3 gramme could be safely administered, Schreiber of Magdeburg injected twenty-seven cases of recent syphilis. The specific effect of a single injection was undeniable. Ulcerations healed within a few days, papular rashes disappeared within a week, and severe throat affections cleared up with an equal rapidity.

Wechselmann was one of the first to carry out trial observations with the drug. He injected it in cases of syphilitic pemphigus and in other advanced congenital conditions, in which, according to his experience, a fatal issue might be expected even with mercurial treatment. His first case was one of Little's Disease in a very weakly child, evidently in a hopeless condition. An injection of 0·03 gramme was given intramuscularly. There were no toxic symptoms, and when the child died fourteen days later, post-mortem examination revealed no changes in the organs which could be attributed to arsenical poisoning. Cases of pemphigus of the most severe type were treated. In every case there was a rapid recession of the signs of disease, but in some instances there was, in addition, rise of temperature, and marked anaemia, and in two cases there was a peculiar opisthotonus. While most of the children recovered, three died; there was no evidence in the organs of arsenical poisoning. Wechselmann suggests that as a result of the spirillicidal action of the drug, a quantity of endotoxine was set free, sufficient to account for the extraordinary symptoms in some cases and for the fatal issue in the three cases referred to above. This contention he supports by the fact that there was no evidence of such symptoms and no fatal issue when 0·015 gramme and 0·02 gramme were injected instead of 0·03 gramme. In the next place, Wechselmann chose for injection such cases as had proved refractory to the usual treatment. He cites the following instance as indicating the superiority of salvarsan to other forms of treatment.

The patient, 18 years of age, was admitted to hospital in June, 1906, suffering from malignant syphilis. Calomel injections and Zittmann treatment did not effect a disappearance of the symptoms. In October, there was still an exanthem on the body. There was an advancing ulcerative condition of the throat and larynx, and there were swellings on the bones. In the meantime, he had had inunction treatment, sajodin, calomel injections, arsenic, steam and sulphur baths, and potassium iodide. In February, 1907, the pain and difficulty in swallowing were so great that the throat had to be powdered with an anaesthetic. There was also an almost constant elevation of temperature. In March, a course of inunction treatment was carried out. The exanthem partly disappeared on the body, but that on the head was unaffected.

The uvula ulcerated away. In April, Zittmann and iodipin treatment were resorted to. In May, he received sublimate injections, and also atoxyl injections. The throat healed, leaving large scars, and he left the hospital in June, refusing further treatment. In the same month, however, he sought admission to another hospital owing to a renewed outbreak of skin symptoms. Here he received injections, ten of atoxyl and six of calomel. In November, he was treated by inunction, potassium iodide and iodipin in another hospital. In the summer of 1908 he had arsenic and calomel injections, potassium iodide and inunction treatment. In autumn, he had improved considerably, and at his own request left hospital. In November and December he had calomel treatment again, and from February till June, 1909, he was having inunction treatment, and from October till December calomel and inunctions. After a further course of treatment in the beginning of 1910, he came under Wechselmann's care in April. He was very weak and ill in appearance. There were ulcerations in the throat. On the inner aspect of the upper arm there was a large ulcer with serpiginous edges. Small ulcerating patches were present on other parts of the body, and bony thickenings were numerous. On April 13, he received an intramuscular injection of 0·45 gramme of salvarsan ; the temperature rose slightly, and the pain at the site of the injection was severe. During the succeeding days morphine had to be administered on account of the pain ; there was no other evidence of disturbance. On the 20th the ulcers showed evidence of healing. On May 9, he left the hospital, all the ulcers in the meantime having healed completely. Three weeks later he returned with a painful swelling at the lower end of the radius and a small ulcer on the hard palate. A further injection of 0·5 gramme of salvarsan produced an almost immediate disappearance of the symptoms. A subsequent erosion in the scar of an old ulcer was treated with another injection of 0·5 gramme. The lesions were now completely healed, and there was no evidence of toxic affection of the eyes, in spite of the fact that three doses of salvarsan had followed atoxyl injections. He was able to begin work again, and at the time of publication (November, 1910) was in good health. The Wassermann reaction became negative after the first two injections, and then in two months became positive. After the last injection it again became negative, and had remained so.

A detailed account of this case is given, because it is more conclusive than any statistical evidence could be of the therapeutic value of salvarsan. In this particular case all the resources of combined experience would seem to have been exhausted without avail. Four years of ineffectual treatment, with chronic skin and bone lesions afforded a good opportunity for deciding the efficiency of the drug and its superiority over other remedies.

Wechselmann says that in view of this result he felt justified in giving salvarsan an extended trial in ordinary cases of syphilis, and all the more so, because the use of the insoluble mercurial compounds, which he considers the most efficient of

other antisyphilitic remedies, had been so often attended by severe and even fatal intoxication. His observations have reference to the treatment of 1,250 cases in all the stages and exhibiting the most varied manifestations of syphilitic infection.

Primary Affections.—In the case of superficial erosions, healing was usually complete within 48 hours in Wechselmann's experience. Specific phimosis receded without operative interference. The hard chancres with thickened edges disappeared more slowly, probably because the specific curative agent found its way with difficulty into the comparatively bloodless sclerotic tissue surrounding the ulcer. On the strength of this assumption he suggests that where there is extensive induration the part should be exposed to the action of hot air so as to increase the local circulation. It is further suggested by Hallopeau that in addition to the ordinary injection, small local injections should be made round the primary ulcer. These suggestons are considered to be of practical importance because of Hoffmann's discovery that spirochaetes are present in the scars of healed chancres years after infection. But even in the case of extensive induration the process receded and the part appeared normal, as a rule, after a fortnight or three weeks. Primary affections of the lip disappeared rapidly, as also did the associated submaxillary glandular swellings. In the gangrenous form of primary affections the healing process was often astonishingly rapid. Where a hard chancre was associated with a soft sore, the salvarsan treatment exercised no influence on the latter.

Secondary Symptoms.—In the great majority of primary cases development of secondary symptoms did not occur. In a few, however, an exanthem did appear in spite of treatment. A typical case of this nature is reported, in which 0·5 gramme salvarsan was injected in the form of neutral suspension a month after the appearance of the chancre. Two days after injection a papular syphilide appeared, accompanied by a rise of temperature. The syphilide and chancre healed in a few days, and in a month the patient was well and the serum reaction negative. This appearance of the rash may be regarded as an example of Herxheimer's Reaction : the injection in the first instance probably stimulated the activity of the spirochaetes prior to the exercise of its spirillicidal properties (*v.* p. 174). As a rule the ordinary mucous patches disappeared in 24 hours. The otherwise refractory mucous lesions in the mouth which gave rise to persistent feelings of discom-

fort were found to be specially susceptible to treatment with salvarsan.

Broad condylomata in the genital region dried up and healed in a few days. Macular syphilides disappeared quickly and papular syphilides receded in ten days or a fortnight, although this particular form of skin affection required in some instances a second dose. The pustular and crusty syphilides, which were often very extensive, yielded very readily to treatment, even in cases which had proved refractory to mercurial remedies. Eruptions of the micropapular and lichenoid variety, which had not disappeared with mercury, healed in a short time.

Wechselmann, however, thinks that even more marvellous results were obtained in the case of the ulcerating *malignant forms of syphilis*, which affect the skin and mucous membranes. The following is one of the cases which he quotes as illustrating the effect of treatment on this particular type of infection—

A patient, 23 years of age, had been infected eight months previously. A month after the development of the chancre a rash appeared. Between November 28, 1909, and the middle of March, 1910, he received thirty-five injections of mercury salicylate and calomel. During the latter three months he suffered from pains in the knees. On May 5, 1910, when he came under Wechselmann's observation he was in a very enfeebled condition. His skin was pale, and he presented a haggard and wasted appearance. All over the body there were ulcers from a quarter to half an inch in diameter, penetrating deep into the subcutaneous tissue, and covered in some instances with dirty, crusty material. Here and there one could see evidence of healing in the form of irregular scars. There was a foul odour from the nose. The septum was perforated and the vomer was coming away in pieces. There was extensive ulceration in the nasal cavity. The throat was extensively involved, and on account of pain he had to be fed by tube. So weakly was his condition that it was a matter of doubt as to whether salvarsan treatment should be resorted to. Seeing, however, that iodipin injections had, if anything, aggravated the condition, an injection of 0·4 gramme of salvarsan was administered on May 21. There was no rise of temperature, and the local pain was moderately intense. Two or three days after injection, the general condition had improved. On May 25, there was evidence of beginning healing of the ulcers. On May 30, the ulcerations on the skin and mucous membranes had practically healed, and the necrotic loose tissue and degenerating bony material had become separated in the nasal cavities. The foul odour had disappeared and the general condition had undergone a great improvement. On June 7, he was able to feed himself, and was out of bed and going about. His body weight increased from 6 stone 7 lbs. on May 21, to 7 stone 9½ lbs. on June 20.

No less satisfactory were the results obtained in cases of periostitis, and more especially in such as were affected by the

severe pains incident to that condition. As an illustration of this Wechselmann records the following case—

> The patient was a sensitive, nervous individual and had suffered from pain in bones for five years. Every conceivable form of treatment had been resorted to. He had a swelling on the distal end of the ulna, and examination by Röntgen rays showed a marked periostitis with ulceration of the bone. Although the injection gave rise to immediate pain, he enjoyed the first night's rest he had had for five years. Another case recorded is that of a man who had contracted syphilis eight years previously. There were numerous painful nodular thickenings on the bones of the limbs and skull. So severe were the pains that he had recourse to 3 grammes of morphia daily. On the day after injection the pains had almost disappeared, and in a few days they were gone, and he voluntarily gave up the use of morphia.

Tertiary Lesions, Parasyphilis.—In tertiary conditions, including visceral lesions, eminently satisfactory results were obtained also. Testicular affections, epilepsy with a syphilitic basis, jaundice following syphilis, ulceration of the larynx and gummata of the brain, responded to treatment. With regard to tabes and general paralysis the results, as might have been expected, were not so remarkable. The permanent damage already done could not be repaired. Nevertheless, in the case of tabes, the more acute symptoms, such as shooting pains and gastric crisis in many instances, were beneficially influenced. In one case the patient had suffered for years from weakness of the muscles of deglutition, whereby the taking of food was rendered difficult. After an injection of salvarsan this feeling of weakness disappeared. In many cases a marked diminution of the ataxia was noted. In two cases trophic ulcers on the soles of the feet healed after treatment. In cases of general paralysis, slight improvement in certain manifestations of the disease was observed in some cases, more especially when the treatment was carried out in the early stages. On this aspect of the subject, Wechselmann prefers to await the results of more extended observation over a longer period before formulating a definite opinion.

We have referred at some length to Wechselmann's observations, because he has had an opportunity of applying the therapeutic test to very extensive material. The results which he obtained in the treatment of 1,250 cases were in most instances both immediate and satisfactory. He himself is convinced of the great superiority of salvarsan over any other anti-syphilitic remedy. In his latest publication he says he has observed 40 cases of recurrence among those already injected. The

ultimate effect of treatment cannot yet be definitely determined. It must be remembered that earlier cases received small doses, about 0·3 gramme, and that the great majority were treated by the injection of a neutral suspension of the drug, a method which experience had proved to be decidedly inferior to the intravenous method now more generally adopted.

A review of the publications of other observers shows that the opinions of Alt, Schreiber, and Wechselmann have been substantially confirmed.

General Clinical Results

Primary Stage

According to Dörr, Iversen, Schreiber, Zeissl and McIntosh, the initial chancres disappear more quickly than with mercury. In Neisser's cases the primary affections healed with great rapidity, as also did those observed by Bethmann. With Spatz the ulcers softened and healed within six days. Géronne and Huggenberg found in ten cases that the ulcers, each about half an inch in diameter, healed completely in ten days. According to Herxheimer the chancres heal as a rule in from eight to fourteen days. In some cases the induration would seem to have lasted longer. Spiethoff found that the rapidity of healing depended to some extent on the dosage. Cases of phimosis yielded to 0·3 gramme subcutaneously in neutral suspension in about three weeks without any local treatment; with a dose of 0·6 gramme similar cases yielded to treatment in a week. Michaelis and Jadassohn report the rapid effect of treatment on hard chancres even with extensive induration. Gennerich states that the healing depends to some extent on the amount of induration; where the chancre is superficial and more in the form of an erosion healing is rapid, but where the surrounding tissue is hard and infiltrated, healing may be delayed as long as three weeks. Pick, Halberstädter and Duhot found complete healing and disappearance of the ulcer in from five to fourteen days.

In some instances the response to treatment would seem to have been more tardy. Beneke and Schild found that chancres of the lip healed slowly; on the other hand, Wechselmann's cases of lip chancres healed rapidly. Stern states that with doses of 0·5 gramme to 0·7 gramme in neutral suspension healing was in some cases slow, and in some instances the local application of mercury was resorted to. In eight cases out of eighty reported by Stern the result was not more satisfactory

than it would presumably have been with mercury. Two cases, one with persistent nodosities of the skull, and the other with symptoms of meningo-myelitis each received 0·7 gramme in neutral suspension without showing any benefit whatever. Stern did not repeat the injection in any of these cases, and he always used the neutral suspension. Glück thinks that infections of the cervix are more resistant to treatment, and in nine such cases the average time of healing was eighteen days. Sieskind confirms the interesting observation of Wechselmann that in cases of combined hard and soft chancres the induration disappears and leaves the soft ulcer unaffected by the treatment.

The lymphadenitis which occurs in the proximity of the chancre is also favourably influenced by treatment. The swelling, however, does not as a rule entirely disappear with the disappearance of the ulcer. Where the lymphatic swelling assumes the proportions of a bubo, this recedes within a comparatively short time; thus Blumenfeld records a case in which within eight days the bubo had become reduced to a fifth of its initial size. But in most cases small chains of indurated glands may be felt weeks after all other symptoms have disappeared (Géronne and Huggenberg, Miekleys).

Secondary Stage

The manifestations of infection which characterise the so-called secondary stage would seem to be specially susceptible to the influence of treatment. Lesions of the skin and mucous membranes heal, as a rule, within a week. Spiethoff found that mucous plaques disappeared completely in two or three days. Géronne and Huggenberg saw the plaques in cases of severe specific angina disappear within a week. Grouven and Fränkel treated with immediate success cases of extensive erosion of the mucous membranes, in which mercurial treatment had failed. According to Miekleys the exudates on the tonsils disappear in a day or two, reminding one of the influence of anti-toxic serum on diphtheria. The severe inflammatory swellings of the tonsils recede, as a rule, in a week. The hoarseness which often accompanies syphilitic affection of the throat disappears generally in three to five days (Glück).

The roseolar and simple macular exanthemata of the secondary stage recede within a few days (Michaelis, Treupel, Iversen, Spiethoff and others). In some cases Miekleys found the roseola change its appearance and remain for weeks in a pigmented form with slight hyperplasia of the affected tissue.

Papular and pustular eruptions dry up and disappear in a few days, although in the case of papular affections slight pigmentation may remain (Dörr). Spiethoff reports a case where a papular crusty eruption healed completely in two days after the administration of 0·6 gramme in alkaline solution. Sieskind thinks that while the micro-papular and lichenoid syphilides respond well to treatment, the macro-papular syphilides are more or less refractory. Ulcerating and rupial eruptions heal very rapidly (Isaac, Brandenburg) and condylomata disappear as a rule within a week or ten days (Grouven, Fränkel). According to Volk and Lipschutz excellent results are obtained in cases of syphilitic paronychia. Palmar and plantar psoriasis of syphilitic origin disappears within ten days as a rule (Géronne), although resistant cases of this condition have been reported (Mulzer).

The generalised swelling of the lymphatic glands improves under treatment, although the glands may remain palpable for a considerable time after all other evidence of disease has gone (Hoffman, Lesser). The severe headaches of the secondary stage disappear often within 24 hours, and pains due to bone affections are equally amenable to treatment (Neisser, Halberstädter, Sieskind). The hard and hypertrophic papules are, according to Hoffmann and Wechselmann, the most resistant manifestations of the secondary stage.

While the records of the treatment of secondary symptoms show excellent results on the whole, there are some authors who have found that in certain cases the recovery was not more rapid than in the average case treated with mercury (Mulzer, Blaschko).

Tertiary Stage

Not infrequently the manifestations of syphilis which appear a year or two after infection are extremely refractory to treatment with mercury or iodides. It is perhaps in such cases that the most striking results have been obtained with salvarsan. Reference has already been made to some of the obdurate cases which Wechselmann treated with marked success (*v.* pp. 179, 182). Spiethoff reports a case of tertiary ulceration in the nose, in which the purulent secretion was visibly less on the second day after the injection of 0·6 gramme salvarsan in neutral suspension, and on the third day the ulcer had healed, leaving only some induration behind. Ledermann reported a case which

during four years had received a variety of forms of mercurial treatment for a large gummatous ulcer. Following an injection of 0·5 gramme salvarsan in neutral suspension the necrotic exudate was thrown off in two days, and in thirteen days half of the ulcer, which was about three inches in diameter, had healed over. Glück believes that the administration of salvarsan in three cases of severe laryngeal syphilis prevented a fatal issue. Herxheimer treated nine cases showing late syphilitic manifestations. A periostitis of the skull disappeared in a week, multiple gummatous ulcers of the hard and soft palate in ten days, and a large gumma of the testicle was reduced to half its original size in twelve days. A gumma of the tonsil disappeared in six days, and an extensive ulcerating lesion of the hard and soft palate, which had resisted intermittent treatment with mercury and iodides for seven years, was so influenced by treatment that in a week solid food could be taken without pain, and in three weeks only small remnants of the original lesion could be detected.

Meirowsky and Zeissl report cases in which the healing of ulcerating gummata and of perforating lesions of the palate occurred in a remarkably short time. Bone lesions are occasionally extremely painful after treatment, but complete cure follows very rapidly. Malignant ulcers of the skin, of the large and small gummatous varieties, heal up at once even in those cases where prolonged injection of insoluble mercurial preparations has proved of little or no avail. Freidländer treated with extraordinary success fifteen cases of advanced ulcerating processes in the mouth, throat and larynx. Weber reports a case of brain syphilis and syphilitic meningitis of a severe type which was completely cured as a result of treatment. In a few days the pupillary reactions which had been markedly affected became normal, and the speech affection and paresis along with mental disturbances also disappeared in a short time.

Affections of the Organs of Special Sense

In view of the fact that atoxyl has been found experimentally and clinically to exercise serious toxic influences on the optic nerve, and that arsacetin has been found experimentally to affect the auditory apparatus, great caution was at first exercised in the use of salvarsan in diseases of the eye and ear. Special attention has also been paid to the possible influence of the drug on these organs in their healthy state. We have

dealt very fully with this question in Chapter V. It is sufficient to state here that there is no evidence in the literature pointing to a toxic action of salvarsan on the organs of special sense. On the other hand, in acute syphilitic affections of the eye such as iritis, choroiditis, optic neuritis, and neuroretinitis, injection of salvarsan has had the most beneficial results (Wechselmann, Schanz).

Even in the more chronic forms of acquired syphilitic eye disease remarkable results have been recorded (Hirsch, *v.* also p. 236). With regard to syphilitic disease of the organ of hearing equally satisfactory results have been obtained. Deafness occurring in the early stages of syphilis in untreated cases and deafness occurring in cases which have been treated with mercury have been cured with salvarsan (Wechselmann, Benario, Beck). While the acute syphilitic affections of the eye and ear, or affections associated with a recently acquired syphilis, yield readily to treatment, it is to be noted that the complications of these organs in hereditary syphilis have proved much more refractory.

The Malignant Forms of Syphilis

It would seem as if the severe symptoms of those infections, which are characterised as malignant, were specially susceptible to the influence of salvarsan. Grouven, Hoffmann, Ledermann, Wechselmann, and Mulzer produce evidence confirmatory of this view. Two cases of malignant syphilis were treated by Dörr with immediate effect, and in one of these the symptoms were so grave as to justify in the first instance the most serious prognosis. Pick reports two cases of malignant syphilis with extensive involvement of the tonsils and pharynx in which all symptoms disappeared a few days after the injection. Sieskind in his record of the treatment of 375 cases of syphilis in all stages, expresses the opinion that the most remarkable results are obtained in the malignant forms of the disease. On the other hand, a few instances are on record where salvarsan has not proved effective. Meirowsky failed to produce any influence on a malignant case with the intravenous injection of 0·4 gramme. In three malignant cases Gennerich had recurrences after a single subcutaneous dose of the drug. These are, however, exceptional cases, and are insignificant in proportion to the large number which have been successfully treated, many of which had already proved refractory to mercury and iodides.

Hereditary Syphilis

Reference has already been made to Wechselmann's early observations on the treatment of congenital syphilis with salvarsan. Michaelis, Pick, and Spiethoff record remarkable success in their treatment of such cases. The usual dose for an infant is 0·03 gramme to 0·06 gramme, and the injection is given into the gluteal muscles. Wechselmann recommends an initial small dose of 0·02 gramme, because when larger doses are given in cases infected with large numbers of spirochaetes the toxic influence of the dead organisms may be serious. It must, however, be noted that congenital syphilis is much more refractory to treatment than acquired syphilis ; this, of course, has also been the experience with mercury. In the case of salvarsan, while some conditions are healed which have not yielded to mercury, still affections like interstitial keratitis respond slowly, and in some instances do not seem to improve at all. Ear complications have in some cases been benefited, and in others not.

Of general interest and of considerable theoretical importance are the cases recorded in which treatment of the mother produced immediate therapeutic effects on breast-fed children. Taege reports such a case, in which a mother with obvious signs of syphilis gave birth to a child, who, when ten days old, suffered from pemphigus and paronychia. On the day following the appearance of these symptoms in the child, the mother was treated with 0·3 gramme of salvarsan. On the third day after injection the condylomata from which the mother suffered began to dry up and recede, and at the same time the manifestations in the child began to disappear. A fortnight after the treatment of the mother the infant no longer showed evidence of the disease. Duhot describes a similar case. He is, however, of opinion that treatment of the mother does not suffice in itself, and recommends injection of the child in addition. Meirowsky treated successfully a pemphigoid eruption on a child with hereditary syphilis by injection of the serum of a syphilitic patient who had already been treated with salvarsan. Plaut and Scholz also record the beneficial influence of sera of injected patients on the symptoms of hereditary syphilis. Examination of the milk and of the serum in these cases failed to show the presence of arsenic, and it has been concluded that the therapeutic effect was due to the presence of antibodies in the fluid. This point, however, has not yet been definitely decided. It is interesting to note in this connection

that according to v. Zeissl, the milk of goats and asses which have received inunctions of mercury has been found to exercise a therapeutic influence on children suffering from congenital syphilis.

Parasyphilitic Diseases

As has been pointed out already, the first observations on parasyphilitic diseases were made by Alt. He had previously treated a large series of cases of general paralysis with arsenophenylglycin, and had found that this drug exercised a decided effect on the course of the disease. In a considerable proportion of the treated cases the Wassermann reaction became negative and remained so over a period of several months; as a result of treatment symptoms disappeared or were alleviated, and the disease appeared to be brought to a standstill. On employing salvarsan Alt found that the positive serum reactions became negative in a proportion of the cases treated, and he reported in a later communication the result of treatment in the case of a man who after the injection of 0·3 gramme in clear alkaline solution into the gluteal muscles, was able to resume the duties of a responsible office in the law courts. It cannot, however, be said that the highest expectations have been fulfilled in the treatment of parasyphilitic conditions with salvarsan. The symptoms which are most manifest in tabes and general paralysis have been found in many cases to undergo very distinct amelioration, while in other cases the condition has remained uninfluenced. In the early stages of general paralysis of the tabetic form, Alt found that there was often a marked improvement following treatment. This was most obvious in cases where the spinal cord was involved, with consequent shooting pains, girdle sensations and bladder involvement. Wechselmann also found that in tabetics with headache, intercostal neuralgia, and involvement of deglutition and urination, treatment with salvarsan was followed by considerable improvement. Treupel, Friedländer, and Michaelis state that in a certain proportion of their cases of syphilitic or parasyphilitic nervous affection, treatment resulted in the disappearance or amelioration of the most prominent symptoms. On the other hand, Glück treated two advanced cases of general paralysis without apparent benefit. Behring did not observe any beneficial effect from the treatment of six cases; and Meyer treated sixteen cases without obvious benefit. Willige treated twenty-four cases of general paralysis and cerebral syphilis, and

in seven he noticed a distinct amelioration of the symptoms, while in three cases the patients so far recovered as to be able to leave the institution. In each of the three latter cases the serum reaction, which had been positive prior to treatment, became negative. Two of the cases recovered so far that it was no longer possible to make a clinical diagnosis of general paralysis. The third case, which was of the tabetic type, while still showing signs of tabes, presented no evidence of mental disease, and the tabetic symptoms, although still present, had greatly improved. The first case received 0·8 gramme, 1·2 gramme and 0·45 gramme intramuscularly in clear alkaline solution, at intervals; the second case, 0·8 gramme and 1·2 gramme; and the third case one injection of 0·6 gramme.

The Effect of Treatment on the Spirochaetes

It has already been pointed out that in the treatment of experimental spirillosis in fowls and mice, the spirilla disappear in sixteen to thirty-six hours after the administration of a curative dose. According to Sieskind and Schreiber, a notable diminution in the number of organisms in the primary and secondary lesions of human syphilis may be observed twenty-four hours after the injection of salvarsan, and after two days they have usually disappeared, or if present exhibit a swollen appearance, and reduced activity in their movements. Spiethoff, examining the serum expressed from the base of the chancre, found that the spirochaetes disappeared in twenty-four to forty-eight hours. Scholz made a routine examination in thirty-two cases, and found that in fifteen of these spirochaetes could not be detected twenty hours after treatment, in twelve they disappeared after two to three days, and only in five cases could they be detected after the fourth day. Iversen punctured the glands in ten cases of secondary syphilitic lymphadenitis, and found that in three to five days after treatment with salvarsan the organisms could not be seen, whereas before treatment they were demonstrable in abundance. Hoffmann found that the spirochaetes as a rule disappeared from papules and mucous plaques in one to three days, although in some cases they were present even at the end of a week. Grouven observed a case in which spirochaetes were found two months after treatment. Herxheimer examined two severe cases of congenital syphilis which died four days after injection. In none of the internal organs, with the exception of the lungs, was it possible to demonstrate the presence of spirochaetes, and in

the lung the organisms presented a degenerate and broken-up appearance. Herxheimer attaches great significance to this observation, in view of the fact that in cases of this description spirochaetes are present as a rule in enormous numbers in the liver, spleen and suprarenals, as well as in the lungs and other internal organs.

The Effect of Treatment on the Wassermann Reaction

Primary, Secondary and Tertiary Stages

While there is an almost universal consensus of opinion as to the efficacy of salvarsan in removing the clinical manifestations of syphilis, the evidence regarding the influence of treatment on the serum reaction is somewhat conflicting. From the original communication of Wechselmann it would appear that if observations were continued long enough the serum would be found to become negative in almost every case. The period required for the conversion of a positive into a negative reaction would appear to depend on the strength of the reaction at the time of treatment. If the reaction were weak to begin with, it would become negative more quickly than if it were strong at the time of treatment. Zeissl, McDonagh and Pick record the conversion of a positive reaction into a negative one in all their cases. Gennerich states that the conversion is hastened by a second injection. Hoppe and Schreiber in their first communication record a negative reaction in 84·6 per cent. of their cases after treatment ; in a later communication, however, Schreiber puts the number of negative results at 50 per cent. According to Iversen a positive reaction becomes negative in from twenty to forty days and in some cases in from eight to ten days. Fränkel and Grouven found that in a proportion of their cases of tabes a positive reaction became negative in about three weeks ; on the other hand, in a considerable number of cases of early syphilis the serum reaction remained positive long after the manifestations of disease had disappeared. In Herxheimer's cases the blood reaction remained positive in 25 per cent., and Kromayer and Stern saw the conversion of a positive into a negative reaction in only 50 per cent. of their cases. In 76 cases reported by Behring, the reaction became negative in 26 after four or five weeks, while out of 77 cases treated by Géronne the reaction became negative in 37. After a second injection the reaction became negative in other nine. Neisser records in his first series a negative reaction after treatment in 10 per cent., in his second series the nega-

tive cases numbered 50 per cent. He points out that a negative reaction is more readily obtained when the cases are treated early. Miekley, Scholtz, Pick, Halberstädter, and Mulzer found a negative reaction after treatment only in a very small proportion of their cases.

Parasyphilis and Congenital Syphilis

Generally speaking, it is more difficult to convert a positively reacting serum into a negative one in the late than in the early stages of syphilis. In this respect parasyphilitic cases are to be classed with the other late conditions; thus in general paralysis only a small proportion of the treated cases have been found subsequently to give a negative serum reaction. Alt treated 121 cases of general paralysis with arsenophenylglycin, and found that of these there were 20 in which the blood serum became negative. In other 3 the reaction became distinctly weaker, so that in 33, or 27·3 per cent., treatment was followed by a distinct influence on the Wassermann reaction. In addition, he treated 18 cases with salvarsan. Each received 0·3 gramme intramuscularly. In two of these the blood reaction became negative, in two it became markedly weaker, and in other three it was distinctly weaker. Willige treated 21 cases of general paralysis with subcutaneous injections of salvarsan, and as a result the serum reaction became negative in six, although subsequently it became positive again in all. There was no relation between the improvement in the general condition of the patients and the change in the serum reaction. Treupel found also that in a certain proportion of his cases of general paralysis a positively reacting serum became negative after treatment. In congenital syphilis it has been found that the injection of salvarsan is followed by a change in the serum reaction in only a small proportion of cases. Mulzer says that the experience with salvarsan coincides with that in the case of mercury, after which only a few of the cases of congenital syphilis come to react negatively in the serum test. Favento treated three cases of congenital syphilis, and found that the serum reaction remained positive in two.

The discrepancies in the results of the various observers are to be explained by the following considerations: (1) The ease with which a positive serum reaction can be transformed into a negative one varies at different stages of the disease. Thus, in general, the earlier treatment is begun the greater is its efficiency. (2) The serum reaction does not become negative

until after the lapse of a variable period succeeding treatment. The length of this period depends both on the dosage and on the individual patient. (3) Discrepancies in some cases are also probably to be explained by the use of imperfectly controlled or "simplified" methods in performing the test.

Recurrences after Treatment with Salvarsan

The period during which salvarsan has been in use is still too short to admit of a definite conclusion as to the extent to which absolute cure is possible. The results of early observations cannot now be accepted as indicative of the limits of efficiency of the drug, inasmuch as the mode of treatment both as regards dosage and method of injection, has been considerably improved. At first the drug was administered in single doses of 0·3 gramme subcutaneously or intramuscularly. Now it is given intravenously in doses up to 0·6 gramme, and the results would appear to confirm the superiority of this method.

Wechselmann during the first three months of his experience with salvarsan did not meet with a recurrence. After six months he observed three recurrences out of a total of five hundred cases; he had not, however, a complete record of all his cases, so that there were probably other recurrences which did not present themselves. In the meantime, also, Wechselmann had given up the intramuscular method of injection of the clear alkaline solution for injection of a neutral suspension. Neisser, after four months' experience, had also observed several recurrences, which he attributed to the small dose employed. Géronne and Huggenberg had five cases of recurrence after three months. In three of these the symptoms had disappeared and the serum reaction became negative as the result of the single injection of salvarsan. The recurrence manifested itself in these three cases in the form of an erosion at the site of the primary chancre, with the presence of spirochaetes, and the reappearance of a positive serum reaction. Hoffmann had also several cases of recurrence and found that spirochaetes could be demonstrated in the lesion before the blood reaction again became positive. On the other hand, Boas points out that the blood reaction may in some cases become positive before the appearance of the symptoms of a recrudescence. Géronne in a later publication reports the occurrence of 14 cases of recrudescence out of a total of 80 cases. In 11 of these the recurrence took the form of an induration at the site of the primary chancre, with accompanying rash and sore throat.

Rille also observed a recurrence of the primary sore four weeks after complete healing. Schreiber observed 16 recurrences in about 300 cases. The majority of these had received 0·3 gramme to 0·4 gramme intramuscularly in alkaline solution. In one case a recurrence took place after an intramuscular injection of 0·7 gramme in alkaline solution. Schreiber had not observed a recurrence following the intravenous method of treatment. Grouven had three cases which received 0·3, 0·4, 0·5 gramme respectively (alkaline solution and neutral suspension), and in which the treatment failed to prevent the subsequent appearance of secondary symptoms. In two other instances he observed recurrence; one was a case of congenital syphilis which received 0·05 gramme, the other of acquired syphilis which received 0·3 gramme and 0·6 gramme. Friedländer also observed three cases, injected intramuscularly, in which early treatment failed to prevent the appearance of later symptoms.

In addition to these cases in which an injection of salvarsan has produced only a temporary remission, several cases are recorded which have proved refractory from the start. It must, however, be admitted that such cases are comparatively rare. The usual rapid reaction to the drug may not take place, as in a case with a serpiginous eruption reported by Grouven and Fränkel where the lesions healed only after three injections extending over a period of two months. Glück and Iversen reported cases in which syphilitic papules failed to respond to treatment within a month. Hoffmann saw a case with a severe papular syphilide on which salvarsan produced no effect, and in which mercurial inunctions employed in addition produced only slight effect. Pick had two cases with secondary papular rashes, which received each 0·4 gramme and 0·45 gramme, and in which the symptoms were still present four weeks after treatment. Spiethoff records five cases out of a total of sixty-four with secondary symptoms in which an injection of salvarsan failed to produce disappearance of the symptoms.

Various instances are recorded in which recurrences have manifested themselves in the form of lesions localised to particular organs, more especially the eye and the ear. Some observers are inclined to find in such occurrences evidence of a toxic influence of the drug on these organs. It is certain, however, that these disturbances are of the nature of a recrudescence consequent on the failure of the drug or its spirillicidal products to reach comparatively avascular tissues in which the spiro-

chaetes have been localised. This point is dealt with more fully where the toxic influences of the drug are referred to (*v.* p. 232).

Treatment of Diseases other than Syphilis

The preparation of salvarsan was the result of an extended series of investigations carried out by Ehrlich and his collaborators on the therapeutic influence of arsenical preparations on protozoal infections in general. Reference has already been made to the fact that the effect of salvarsan and other allied preparations was tested in experimental infections with trypanosomes, with the spirilla of relapsing fever and of fowl spirillosis, and with the spirochaete pallida. It is not surprising, then, that syphilis is not the only human disease in which salvarsan has been found to exercise a therapeutic influence.

Relapsing Fever

Iversen of St. Petersburg treated with remarkable success 52 cases of relapsing fever. The method employed at first consisted in the intramuscular injection of 0·3 gramme salvarsan in clear alkaline solution. Subsequently the intravenous injection of 0·3 gramme was resorted to, and this is the method which Iversen recommends. His conclusions are as follows: (1) An injection of salvarsan during the febrile attack suffices to produce a normal temperature within seven to fourteen hours and at most within twenty hours. A single injection prevented the recurrence of an attack in 92 per cent. of the cases. (2) The therapeutic dose for relapsing fever is from 0·2 gramme to 0·3 gramme given intravenously. (3) In from four to ten hours the spirilla disappear completely from the blood. (4) The temperature falls by crisis to normal and there is an immediate disappearance of all subjective symptoms. (5) The intramuscular injection gives rise to local swelling and pain, which disappear in a few days: the intravenous injection is painless and unassociated with disturbances of any kind; the therapeutic effect of the drug becomes apparent about four hours earlier by the intravenous than by the intramuscular method.

The observations of Iversen establish beyond doubt the efficacy of salvarsan in this disease. This type of infection is peculiarly suitable for testing the influence of treatment, inasmuch as the acute manifestations of disease are associated with the presence of large numbers of spirilla in the blood. The successful treatment of relapsing fever is a striking tribute to

the experimental method of investigating and combating disease. Even although forty years have elapsed since the discovery of the spirillum of Obermeyer, that discovery has only now been linked up with an effective form of treatment by the clear, rational and persistent application of laboratory methods.

Framboesia

A considerable diversity of opinion has existed as to the identity of yaws or framboesia as a specific disease. In many respects the symptoms and lesions resemble those of syphilis. The causal organisms, however, while resembling each other in appearance, can be distinguished. The number of cases treated with salvarsan is still small, but the reports which have been published indicate that for this disease also a specific remedy has been discovered.

Strong, in a communication on the subject, points out that prolonged treatment with potassium iodide serves in many cases to heal the sores. The first case treated with salvarsan had already been under the influence of potassium iodide for about two months, and at the same time the skin lesions were being treated locally with mercury. The treatment had, however, failed to influence the condition. An injection of 0·3 gramme of salvarsan, intramuscularly in alkaline solution, was given, and in three days a marked improvement was noted; in a week open sores on the skin had almost healed. In twelve days the sores had all healed, leaving cicatrices, which soon became quite soft. In other 25 cases the injection of salvarsan was resorted to with equally satisfactory results. At the time of writing the author had not had the cases sufficiently long under observation to be able to determine the permanent effect. At any rate, the temporary result was such as has not been attained by any previous form of treatment.

Confirmatory evidence has been furnished by Alston as to the influence of salvarsan on the lesions of yaws. He has found further that the serum of patients treated with salvarsan exercised a therapeutic effect when injected into other cases, and also that the serum from these serum-treated cases possessed therapeutic properties.

Castellani has treated 8 cases of framboesia, 6 of which were recent and 2 were chronic, of 10 to 15 years' standing. Each case received from 0·3 gramme to 0·4 gramme administered in the form of neutral suspension except in one instance, where it was given intramuscularly in clear alkaline solution. The

injection had no therapeutic effect on the chronic cases, but in the acute cases the healing was the most rapid and effective which Castellani has ever observed in this disease.

Salvarsan has been administered in other diseases than those mentioned, but it is not yet possible to form an opinion as to its efficacy outside that group of diseases which are the manifestations of spirillar infection. Haller injected the drug with good results in a case of small-pox, of a type which he considered would otherwise have terminated fatally. Gioseffi has employed it without apparent benefit in a case of leprosy. Reichmann has reported an unusual case of filarial infection which recovered after a single injection of salvarsan. Several forms of disease in which arsenical preparations are usually administered have been treated with salvarsan, but the results are not such as to warrant a definite conclusion. Wechselmann and Klemperer have employed it without effect in pernicious anaemia ; on the other hand, Byrom Bramwell reports two cases of pernicious anaemia, in which it would seem to have exercised a beneficial influence on the course of the disease. Maranon has obtained good results in typhus fever.

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CHAPTER IV

AUTHORS' OBSERVATIONS ON TREATMENT WITH SALVARSAN

PRIMARY CASES—EARLY SECONDARY CASES—LATE SECONDARY CASES REFRACTORY TO MERCURIAL TREATMENT—TERTIARY CASES—PREGNANCY IN SYPHILIS—CONGENITAL SYPHILIS—CASES WITHOUT MANIFEST SYMPTOMS (LATENT SYPHILIS)—PARASYPHILITIC DISEASES, GENERAL PARALYSIS OF THE INSANE, LOCOMOTOR ATAXY—NEURALGIA IN SYPHILITIC SUBJECTS—MENTAL DEPRESSION FOLLOWING SYPHILIS—NON-SYPHILITIC CASES—COMPLICATIONS OF TREATMENT—EFFECT ON THE WASSERMANN REACTION—MODE OF ACTION OF SALVARSAN, THERAPEUTIC EFFECT OF THE SERUM AFTER TREATMENT

IT is now almost eighteen months since Professor Ehrlich placed at our disposal a supply of salvarsan, and during the intervening period we have had an opportunity of testing its therapeutic value in the various forms and stages of syphilitic infection. We are indebted to several of our medical colleagues, who gave us an opportunity of treating suitable cases, and we would specially record our thanks to Colonel Gordon Hall, of the Military Hospital at Maryhill, for the arrangements whereby we were enabled to observe the effects of salvarsan on the recent cases of syphilis under his care.

To begin with, we injected an alkaline solution, prepared according to Alt's method, into the gluteal muscles. Subsequently this method was discarded in favour of the neutral suspension method. For the past ten months we have employed exclusively the intravenous method of treatment.

Primary Cases

We have treated 102 cases of recent syphilis. In 22 of these the injection was given when the only symptom of infec-

tion was the presence of the chancre. Of these 22 cases 2 were treated intramuscularly with the clear alkaline solution in doses of 0·3 gramme, 4 were treated with the neutral suspension in doses of 0·4 gramme. The remaining 16 were treated intravenously; of these 4 received 0·3 gramme, 10 received 0·4 gramme and 2 received 0·6 gramme. Secondary symptoms in the form of sore throat or rash did not appear in any of the 22 cases treated at the earliest stage. The chancre disappeared in from seven to sixteen days, and the patients remained well. Ten months have elapsed since five of them were treated (all intramuscularly, four with neutral suspension and one with clear alkaline solution) and there has been no evidence of recurrence; and in four out of the five the Wassermann reaction was negative six months after the first and only injection. The fifth case, where the Wassermann reaction was positive after six months, received an intravenous injection of 0·5 gramme, and his blood a month later gave a negative reaction. Of the 16 cases treated intravenously, the blood of 10 was examined a month after a second injection, and in 7 of these the reaction was negative. The time which has elapsed since these cases first came under observation is too short to admit of a complete record of their progress. The main fact in their condition is that the chancres have healed and the appearance of secondary symptoms has so far been obviated. A second injection is now given in every case as a matter of routine three weeks after the first injection.

Early Secondary Cases

We have treated 80 cases of early syphilis in which, in addition to the chancre, there were other evidences of infection, viz. headache, sore throat, and exanthemata. Of these 6 received intramuscular injection of the clear alkaline solution, 17 subscapular injection of the neutral suspension, and 57 were treated intravenously. With regard to dosage, of those who received clear alkaline intramuscular injections, 4 had 0·3 gramme, 1 had 0·4 gramme, and 1 had 0·45 gramme; of the 17 who had neutral suspensions, 8 had 0·4 gramme, 6 had 0·45 gramme, and 3 had 0·5 gramme; and of the 57 who were treated intravenously, 13 had 0·3 gramme, 23 had 0·4 gramme, 19 had 0·5 gramme, and 2 had 0·6 gramme. These amounts represent the dose given at the first injection. Cases to which two injections were given: of the 17 cases which received an injection of neutral suspension, 8 subsequently received intra-

venous injections of 0·45 gramme, and of the 57 who were treated for the first time intravenously, 44 received subsequent intravenous injections, the second injection containing on an average from 0·4 to 0·5 gramme of salvarsan. It is impossible to classify the cases according to the method of injection or to the amounts given, because the same patient was treated in some cases by two different methods, and the dosage varied with the weight and sex of the patient. The result of treatment in these 80 cases was an almost immediate disappearance of all the symptoms of disease after a single injection. In every case the chancre which was present healed within a fortnight and in some cases within five days. Macular and roseolar eruptions disappeared within ten days. Mucous patches in the mouth and ulcers in the throat healed within ten days. In three of the cases treated by subcutaneous injection of the neutral suspension (0·4 gramme) relapse occurred before the treatment was repeated. In one of these cases (a man) the relapse occurred in November, 1910, two months after injection and four months after the appearance of the chancre; it took the form of an iridocyclitis and choroiditis in one eye, and slight optic neuritis in the other eye. It was impossible to say whether a neuritis was also present in the eye with choroiditis. After a second injection on December 12, 1910, of 0·4 gramme in the form of a neutral suspension into the gluteal muscles, the iridocyclitis, choroiditis and neuritis disappeared within a week. Two months after the second injection a severe iridocyclitis developed in the left eye. We did not have an opportunity of seeing the patient at this time and he was given three injections of mercury. The eye condition gradually became quiescent. On July 4, 1911, he was seen by us. There was evidence that both the injections of salvarsan had been imperfectly absorbed. His blood gave a positive reaction. His eyes were examined by Dr. W. H. Manson, of the Glasgow Eye Infirmary, who considered the condition to be characteristic of syphilitic affection of the uveal tract. There was at this time considerable impairment of vision due to deposits in the vitreous humour. In the second case relapse occurred six weeks after injection, in the form of an ulcerating throat with a roseolar eruption. In this case the symptoms disappeared in a week after an intravenous injection of 0·4 gramme. This patient has now been well for six months, and her blood gives a negative Wassermann reaction. In the third case of relapse there were small ulcerating patches

on the tongue and buccal mucosa. These healed in four days after an intravenous injection of 0·5 gramme. Six months later the patient was in good health and his blood reacted negatively. It is to be noted that in each of these cases of relapse, the salvarsan was injected in the form of neutral suspension, which never became properly absorbed, and at the time of the relapse fluctuant masses about the size of a hen's egg were present at the site of injection.

As an illustration of the effect of salvarsan on the symptoms of the secondary stage of syphilitic infection, the record of the following case which was observed along with Dr. Haswell Wilson, may be cited—

The patient was exposed to infection in the beginning of October, 1910. Three weeks later there was a small scab on the dorsum of the penis, which developed into a large, septic, indolent ulcer, still present on November 29. The inguinal glands were large and tender, there was considerable periglandular oedema and on the right side there was fluctuation. Spirochaetes were not seen in a smear taken from the edge of the ulcer. On December 1, there were rose spots and papules, most marked on the face. On December 3, a pustular eruption resembling chicken-pox appeared. Pus was found to be present in the right inguinal bubo, but spirochaetes could not be detected. On December 8, an ulcer about half an inch in diameter was present between the pillars of the fauces on the right side; it was covered with a white slough. The temperature was 101° F. On December 15, there were punched-out ulcers on the scalp, face and back; they were circular, clean cut and deep and were quite painless. Skin which was apparently healthy would slough in a few hours, leaving a well demarcated ulcer. The other eruptions were extending and he was suffering from nocturnal headaches. On December 18 spirochaetes were found in the primary sore and in the skin lesions, and his blood gave a positive Wassermann reaction. On December 19, he received a subcutaneous injection of 0·6 gramme of salvarsan as neutral suspension. There was some stiffness and tenderness at the site of injection for a week, but otherwise there was no disturbance. On December 20, the throat lesions had disappeared, and after this date there was no new appearance of skin lesions. On December 22, the ulcers were beginning to fill up and present the appearance of healing wounds. The roseolar and papular eruptions had already disappeared. On December 26, all the ulcers except the largest

had healed, and these were now clean and healthy-looking and on a level with the skin. On December 31, all the skin lesions had healed; these included a deep ulcer on the tip of the nose extending down to the cartilage, and another deep ulcer on the forehead about the size of a shilling and penetrating to the pericranium. The original chancre was healed by January 4. On January 9, he received a second injection of 0·5 gramme salvarsan in a neutral suspension. On February 6, his Wassermann reaction was slightly positive, but he had no symptoms of disease, his general health was good, and his complexion, which was sallow during his illness, had now resumed its normal appearance; since then he has continued to feel perfectly well and has shown no lesions.

Late Secondary Cases Refractory to Mercurial Treatment

In those cases which have proved refractory to mercurial treatment the results have been very striking. Of such cases 32 have been treated, 7 by the neutral suspension method, in the first instance, and 25 intravenously only. In one case only was there a recrudescence; this was a woman with a severe ulcerating condition of the pharynx and larynx, who received a subcutaneous injection of 0·3 gramme in a neutral suspension. The original symptoms disappeared in a week, but there was a recurrence in six weeks. The symptoms in the other 31 cases disappeared after a single injection, and there have been no recurrences. All these cases, with one exception, have had a second injection after three to four weeks; the second injection has in every case with one exception been given intravenously, one patient having had both injections in the form of a neutral suspension subcutaneously. The symptoms in these patients were representative of the severe lesions of the skin and mucous membranes characteristic of such cases. In some there were deep ulcers involving the skin and subcutaneous tissues; in others the tonsils and soft palate and turbinate bones were involved in extensive necrotic degeneration. In some cases there were nodular thickenings of bones, with the usual pain associated with such conditions. In every case mercury had been administered in some form, and in some cases in various forms. In no case did the salvarsan fail to produce an immediate and complete remedial effect so far as the symptoms were concerned. In 18 of the cases the Wassermann reaction was negative one to three months after

the second injection, in 5 it was still positive after a similar interval, in the remaining 9 the blood reaction had not been recently determined. The main facts relative to some of these cases are of sufficient interest to warrant a more detailed reference.

Case I.—Male. Infected May, 1910; primary sore appeared June 24. Spirochaetes found in scraping from ulcer. Mercury was applied locally, and in addition calomel (1 grain) and Dover's powder were administered internally thrice daily. The sore healed. On October 12, 1910, there was a typical syphilitic sore throat with indurated, painless submaxillary glands. The mercurial treatment had been continued and the blood reaction was negative. On October 27, 0·6 gramme salvarsan was given subcutaneously in a neutral suspension, and in two days the throat symptoms had disappeared. There was pain at the site of injection for a week, but no constitutional disturbance. When the salvarsan was given, all other treatment was stopped. On February 17, 1911, an intravenous injection of 0·5 gramme was given. The Wassermann reaction was negative and there were no symptoms. (Case observed with Dr. Haswell Wilson.)

Case II.—Male. Infected August, 1909; chancre appeared in September, and in October there was a severe sore throat and generalised rash. Mercurial treatment was begun immediately after the rash appeared, in the form of calomel and Dover's powder thrice daily. The chancre healed in about a month, but the rash persisted for about two months in a very extensive form. The ulcerations in the throat healed on one side, but persisted on the other side for six months. The rash, although it disappeared to a considerable extent from the trunk, remained on the arms and legs for six months. In April, 1910, he had a course of atoxyl injections, with the result that the rash completely disappeared, and there remained in the throat only a very small ulcer on one tonsil. He gained weight and enjoyed good health till August, 1910, when his throat again became sore, and deep ulcerations developed in two days. A large ulcer appeared on the left arm immediately above the elbow, and another on the right leg about six inches below the knee. He had fever, and was confined to bed. The tongue became swollen, and on the edges there were small ulcerating patches. Mercurial treatment was begun immediately, and this was continued for about six weeks without apparent benefit. At the end of six weeks he received a second course of atoxyl injections. Following the administration

of atoxyl the throat symptoms gradually passed off, but the deep ulcerations on the arm and leg did not recede. In the meantime, a third deep ulcerating sore, with well defined margins, appeared on the side of the head above the right ear. Mercury and potassium iodide were continued after the atoxyl. In the middle of December the ulcers were still large and deep and showed no sign of healing. There were numerous small ulcerating patches on the tongue and buccal mucosa. He was pale and thin, and looked very miserable. His blood serum gave a positive reaction. On December 20, he received an intravenous injection of 0·5 gramme salvarsan. On December 24, the mucous membrane of the mouth showed no trace of ulceration and he said that he enjoyed a feeling of well-being such as he had not experienced since he contracted the disease. The deep ulcers on the leg, arm and scalp presented a clean and healthy appearance, and were gradually becoming filled with granulation tissue. On December 29, the ulcers on the leg and scalp had healed completely, while that on the arm had filled up to a level with the skin. On January 6, the ulcer on the arm which was originally about the size of a five shilling piece, had completely healed. The scar was soft, and there was scarcely any contraction of the tissue. He expressed himself as feeling in perfect health. On January 16, he received a second intravenous injection of 0·5 gramme. On March 10, his serum gave a negative reaction. He had added 1½ stones to his weight since the date of the first injection. The anaemic look had disappeared, and he was to all appearances perfectly well.

Case III.—Male. Infected July, 1908. The primary sore in this case was large and extended on to the skin on the dorsum of the penis. He had a suppurating bubo in the left inguinal region, which was opened and healed after three months. The appearance of the chancre was followed in a month by a generalised rash and slight sore throat. Mercurial treatment was begun immediately after the appearance of the rash, and in six weeks the chancre was healed and the rash had disappeared, with the exception of some " blotchy spots " on the face. A course of mercurial inunction was given, and simultaneously there was internal administration of mercury, but the spots did not disappear. The inunction treatment was continued for two months, and the internal treatment was continued almost regularly for two years. Six months after the first appearance of symptoms, and while the mercurial treat-

ment was proceeding, the scar on the site of the original chancre broke down into a fresh ulcer with an indurated base. Local treatment was adopted and the ulcer healed in three weeks. Immediately after the healing of the ulcer, a rash broke out on the trunk and small ulcers formed on the tongue and on the inside of the cheeks. He had severe headaches and pains in the limbs. After a week's rest in bed his condition began to improve, and he was given a mixture of mercury and potassium iodide. The symptoms gradually disappeared with the exception of the small ulcers in the mouth, and spots on the arms and legs, but for about a year these slight manifestations of disease remained in much the same state. In January, 1910, a large deep ulcer appeared below the right clavicle and another on the left forearm about three inches below the elbow. In spite of the local application of mercurial ointment and internal medication with mercury and iodides these ulcers did not heal. At times they would appear clean and raw-looking and the edges showed signs of healing, but this temporary improvement was followed by a subsequent breaking down and formation of slough. In September a third ulcer appeared on the left upper arm posteriorly, about three inches above the elbow. This ulcer resembled the others in appearance. When the patient was seen in the middle of October, 1910, he was of sallow complexion, but fairly well nourished. Although his condition caused him a great deal of worry and anxiety, there was no pain or discomfort except occasionally in the tongue after excessive smoking. He said that he did not take his food well and felt disinclined for work. He had been undergoing anti-syphilitic treatment more or less continuously for longer than two years. His blood gave a strongly positive reaction. On October 18, 1910, he received a subcutaneous injection of 0·5 gramme salvarsan in a neutral suspension at the lower end of the vertebral border of the left scapula. There was a feeling of tension at the site of injection which lasted about ten days, but otherwise there were no disturbing effects. Four days after injection the small ulcers in the mouth had completely disappeared and already the large ulcers on the arm and thorax showed evidence of commencing to heal. At the end of a week, they had filled up with granulation tissue almost to the skin level and the newly-formed skin had encroached on the ulcer about an eighth of an inch round the circumference. In sixteen days the ulcers had healed completely and the patient appeared to be quite well. On December 10, he had an intravenous injection of 0·45 gramme salvar-

san, which did not produce any disturbance. On March 10, he appeared to be in good health. His weight had increased since the first injection by 10 lbs. Instead of being sallow and anaemic he was now fresh and healthy-looking, and expressed himself as feeling in the best of health and spirits. His blood serum gave a negative reaction.

Case IV.—Male. Infected January, 1910. This patient suffered from a gonococcal infection in January, 1910. There was at that time no evidence of a chancre, but in the beginning of March he had an ulcerating throat and a generalised rash. The rash was apparently of a papular character, and at the end of three months of regular mercurial treatment, it had disappeared except for a few spots on the forehead. The throat, however, had never completely healed, and was at times extremely painful. In June, 1910, he had ten injections of soamin, and following this the rash disappeared completely, and the throat condition improved. In August the throat again became ulcerated, and spots appeared once more on the forehead, arms and legs. Mercurial treatment was again begun, but the ulceration in the throat extended to the nose, and was so painful that food was swallowed with difficulty. At the same time his voice became husky, and his hearing was affected. In addition to internal medication, mercurial inunction was employed; but after a trial of six weeks this did not appear to produce obvious abatement of the disease. When seen in the beginning of November he presented a miserable appearance. He was pale and anaemic and very thin. During the preceding six weeks, he had lost flesh to a considerable extent, and he was very much dejected and distressed about his condition. The papular eruption was still present on the forehead. The nose was swollen and on the left side red and slightly oedematous. A very extensive ulcerating surface occupied the site of the left tonsil and faucial pillars. The whole of the soft palate on the left side had sloughed away and the ulceration had extended into the nose leaving the cartilage exposed in parts. His blood gave a strongly positive Wassermann reaction. On November 1, he received a subcutaneous injection of 0·5 gramme salvarsan in a neutral suspension in the region of the lower end of the vertebral border of the scapula. There was considerable pain after the injection, but this disappeared in two days, and there were no other disturbing consequences. Three days after the injection the pain in the throat had completely disappeared; the greyish necrotic material which was present on the ulcerating surfaces

was also gone, so that they presented a healthy and active appearance. The swelling and oedema of the nose had likewise receded. His condition improved steadily and rapidly, and on December 3 there was no trace of ulceration left, and the papular eruption had disappeared from the face. His blood, however, still gave a positive reaction. On December 6, he received an intravenous injection of 0·4 gramme. His general health continued to improve and when he was seen at the end of January he felt perfectly well, was strong and robust in appearance, and had gained a stone in weight since the first injection. His blood serum was still slightly positive. He was seen again at the beginning of April, and was in good health. An examination of his blood at that date gave a negative result.

Case V.—The following case belongs also to the late secondary period and affords a striking example of the superiority of salvarsan to mercury. This patient was infected in January, 1910. He had a typical hard chancre followed by sore throat and rash. This disappeared in about a month with mercurial treatment. The mercury was continued regularly. In September he suffered from occasional lapses of memory with mental confusion and a slight paresis of the left side of the body was noticed. He had to give up work. The paresis became worse, and his swallowing became affected. During this time he was taking both mercury and potassium iodide. His blood serum gave a positive reaction. On October 20, he was given a subcutaneous injection of 0·45 gramme salvarsan in a neutral suspension in the scapular region. From the second day after injection the symptoms gradually disappeared, and in three weeks there was no trace of paresis and his memory appeared to be quite good. In January, 1911, he appeared to be in good health; he received a second injection of 0·4 gramme, on this occasion intravenously. There was no return of symptoms in the six months following the first injection, and during that period no mercury or iodide was given. His blood reaction was negative when tested at the end of six months after the first injection.

These five cases afford a very definite proof of the therapeutic value of salvarsan. Whether the results will be permanent is, of course, a matter for future observation, but the immediate effect is such as to warrant the opinion that it would be wrong not to treat such cases with salvarsan. It has been suggested by some observers that cases of this type are the most favourable for salvarsan treatment. There is, however, no reason to

believe that the drug is less effective in the earlier and milder forms of infection. The more striking results in the malignant and refractory cases are simply due to the fact that the change produced has been greater owing to the symptoms being more severe and of longer standing. In our experience the results have been as satisfactory in early mild cases as in those which have been prolonged and severe. It seems only rational to suppose that a drug which will remove symptoms in a severe case will have an equally beneficial effect on a mild case ; and a definite and lasting cure is undoubtedly most likely to be obtained when the disease is mild and when it is treated early in its course.

Tertiary Cases

We have treated 14 cases in this stage of the disease. These cases included four in which there were gummata, one with amyloid disease of the kidneys, one with periosteal nodosities, one with severe neuralgia, and one with a laryngeal affection. The gummata were situated in three instances below the knee, and were of the nature of large indolent ulcers. In one case the ulcer healed in a fortnight after an intramuscular injection of 0·5 gramme salvarsan in a clear alkaline solution. In the other two cases healing was complete in a month. The fourth case of gumma was that of a man thirty-two years of age, who had contracted syphilis eight years previously. The disease had involved the sinus cavities of the nose and had produced extensive destruction of the hard and soft palate. The bone was bare in two places. He was given a subcutaneous injection of 0·5 gramme salvarsan in a neutral suspension. In a week there was a notable difference in his condition, as the foul smell from the nasal cavities had almost disappeared. The ulceration which had been present on the soft palate, disappeared in a fortnight. There still remained, however, very large raw surfaces inside the nasal cavities and a small piece of necrotic bone was removed. A month after the first injection he received a second subcutaneous injection of 0·5 gramme in a neutral suspension. The healing process continued and the patient's bodily condition became greatly improved. A month after the second injection the ulceration had healed completely. His blood serum, which gave a positive result at first, was still positive a month after the second injection.

The patient with amyloid disease of the kidneys was forty-two years of age. He was supposed at one time to be the sub-

ject of tubercular infection of the lungs, but tubercle bacilli were never demonstrated. Four years previously (in 1907) he was treated also for disease of the kidneys. At that time the albumin in the urine registered over 7 in Esbach's tube. There was general oedema of the loose subcutaneous tissues, and he was weak, anaemic and unfit for work. The pulmonary symptoms of cough and spit cleared up after two months' rest, but the albumin did not fall below 4 by Esbach. Two years later the weakness and pains in the back and legs were still present, and the albuminuria registered between 3 and 4. In March, 1911, a Wassermann test was done, and his blood was found to react strongly positive. The albumin registered 2 in Esbach's tube. He was still anaemic and easily exhausted. He was given an intravenous injection of 0·3 gramme salvarsan. There was no disturbance of any kind following the injection. A week later he said that he felt stronger and more vigorous than he had done for years. A month later the improvement in his general condition had been maintained, and the albumin was at this time below 1 in Esbach's tube.

The patient with periosteal nodosities was a woman forty years of age, who was unaware of the fact that she was suffering from syphilis. Her blood serum gave a positive reaction. There was a small nodule on the right parietal eminence, and another in front of the right tibia about two inches below the anterior tubercle. These swellings had been present for about six months and at times had been very painful, more especially the one on the tibia. She was given 0·3 gramme salvarsan in a neutral suspension, injected into the soft tissue at the lower end of the vertebral border of the scapula. For two days there was a considerable amount of uneasiness at the site of injection. The periosteal thickenings gradually disappeared, and in a fortnight there was no trace of them left. Nine months have elapsed since she was treated, and in the meantime there have been no symptoms.

The case of neuralgia which was treated is of particular interest. She had suffered for nine months from severe shooting pains in the right side of the head. The pain was of a sharp stabbing character and appeared to have its centre of intensity in the right eye. The attacks came on at intervals of from six to ten days, and lasted from half an hour to two hours. The application of hot fomentations did not seem to give relief. After some months the attacks came on every three or four

days and lasted longer. They now appeared to be more frequent in the night time. Latterly they were of almost nightly occurrence and when she was seen on January 16, 1911, she had spent three days and three nights without sleep. During this period the pain had been very severe, and she said that it seemed as if her right eye were being wrenched out. She had not taken any food during these three days, because she had recognised that the taking of food had on previous occasions caused exacerbation of the pain. When she was first seen there was no suspicion of syphilis in her case, but on making a routine inquiry as to her personal and family history it was found that her first pregnancy had resulted in a miscarriage, and that a baby had died at three months covered with a rash. On examination the blood gave a positive Wassermann reaction. She was given an intragluteal injection of 0·5 gramme salvarsan in a clear alkaline solution. There was very considerable pain at the site of injection, which lasted for about five days. During the first twenty-four hours following the injection the neuralgic pains did not diminish in intensity to an appreciable degree, but on the second night after the injection she slept six hours. On the second day after the injection the pains came at intervals, and were not so intense as previously. They were no longer severe in the eye, but appeared to shoot up into the head in the parietal region. The sixth and seventh day and night after injection were entirely free from pain. On the morning of the eighth day she had a slight attack when she wakened at 6 a.m.; this passed off in about a quarter of an hour. Occasionally during the second week after treatment there were temporary seizures of pain of a shooting character, which went up into the head, but did not affect the eye. From the third till the eighth week she had no attacks. In the ninth week there were occasional sharp pains in the region of the upper jaw on the right side. These attacks lasted a few minutes and occurred at irregular intervals. Since the injection she had had about ten seizures. The pain on these occasions did not affect the eye or shoot up into the head. The attacks ceased without further treatment, and for the last eight months she has been quite well and free from pain. What the exact lesion is in this case it would be difficult to say. It is not impossible that it is of the nature of a syphilitic thickening of the meningeal tissue surrounding the ganglion or large trunks of the fifth nerve. Whatever was the origin of the pain, there is no doubt that it has been relieved by the injection.

The case with laryngeal affection is the least satisfactory which we have observed, owing apparently to the extreme susceptibility of the patient's subcutaneous tissue to the drug. On November 10, 1910, she received 0·6 gramme salvarsan as neutral suspension subcutaneously in the scapular region. The subsequent local pain was very severe and morphia had to be given for upwards of a week. A large swelling remained, and the overlying skin sloughed in the course of several months, leaving an ulcer which measured 2½ by 1½ inches. As might be expected, the therapeutic effect of the drug in this case was not marked.

Case of Pregnancy

This patient came under observation in the beginning of the sixth month of pregnancy. She was in the secondary stage and had been previously treated with mercury, but her blood still reacted positively. She was given an intravenous injection of 0·4 gramme salvarsan, and in five weeks the injection was repeated. The injections did not produce any general disturbance. At full time she was delivered of an apparently healthy child. Except for a white scar of firm fibrous tissue, the placenta appeared perfectly normal. The child is now six months old, and appears to enjoy perfect health.

Congenital Syphilis

Three cases of congenital syphilis have been treated. Two of them were infants of about three months, and the third was a boy of fifteen years. The infants had " snuffles " and general erythematous exanthems. In each case 0·02 gramme of salvarsan in the form of a neutral suspension was injected into the gluteal muscles. One of the children made a perfect recovery in a fortnight, and has now been free from evidence of disease for seven months, being well nourished and healthy looking. The other infant, living in very miserable surroundings and fed artificially, was in a poor state of nutrition. The rash, however, disappeared in about ten days, but returned in a month. There was no opportunity of giving this infant the nourishment and nursing which were the necessary adjuncts to medicinal treatment.

The history of disease in the boy goes back to 1901, when he was treated for an affection of the lower end of the femur which was then thought to be tuberculous. Six months after the first operation a sequestrum was removed from the site of the original trouble. In 1903 there was another operation in the same

region for a discharging sinus. On October 17, 1910, he was admitted to hospital with an ulcerating condition of the right ankle of a year's duration. There was some cicatricial tissue in the centre of the ulcerating area, and at the margin of the lesion there were ulcerating masses, which presented a nodular appearance. It was noted that the patient had Hutchinson's teeth, and a blood examination gave the syphilitic reaction. On November 2, 0·45 gramme salvarsan was injected in the form of a neutral suspension into the loose tissue at the vertebral border of the scapula. There was very considerable pain at the site of injection for some days, for which injections of morphia had to be given. On November 11, the ulceration had receded considerably and he was evidently improving. On November 27, some fresh spots of ulceration of the skin appeared, and the improvement originally noted in the large ulcer had ceased. On December 22 the blood serum still reacted positively and he was given an intravenous injection of 0·2 gramme salvarsan. On January 15, the skin lesions had healed completely. On January 24, the blood reaction was positive, and on January 26 he received another intravenous injection of 0·27 gramme. On March 6, the lesions were healed; the ankle which had previously been stiff showed very considerable freedom of movement, and the blood serum gave a negative reaction. It is to be noted that in this case the original injection of neutral suspension became encapsulated. The fluctuant mass at the site of injection was at one time about the size of a hen's egg. It receded gradually, however, without breaking through the skin. In the beginning of February it was about the size of a walnut, and was punctured. The material which escaped consisted of shreds of necrotic tissue. There was no pus. We are indebted to Dr. G. H. Edington for the history and clinical notes of this case.

Cases without Manifest Symptoms (Latent Syphilis)

When the blood serum gives a positive Wassermann reaction in a case with a history of syphilis, but with no other evidence of the disease, we have acted on the principle that although symptoms may be absent, the disease has not been cured. We have treated seven cases of this kind. Two were treated on the first occasion by the injection of 0·5 gramme salvarsan in a neutral suspension. In one the serum was negative after six weeks; in the other case the serum was still positive at the end of six weeks, and a second injection of 0·4 gramme

was given intravenously. Six weeks later the serum of this patient was negative also. The other five cases were all treated intravenously to begin with, each receiving 0·4 gramme. In one of them the blood was negative in a month. In other two the blood was negative six weeks after a second injection of 0·4 gramme. In the fourth case the blood was still positive six weeks after the second injection, that is, ten weeks after the first injection. This patient did not receive a third injection, as a longer interval than six weeks is probably necessary in some cases for the positive reaction to disappear. The fifth case has not been tested further.

Parasyphilitic Diseases

In marked contrast with the results recorded in the preceding pages is our experience with salvarsan in the treatment of parasyphilitic affections. We have not encountered a single case with the more acute manifestations of infection, such as lesions of the skin, mucous membranes, bones and eyes, which has not promptly yielded to treatment provided that absorption of the drug occurred. Even in some of those cases where certain of the lesions had existed for years, very rapid disappearance of the symptoms followed the injection. In the case of tabes and general paralysis, however, one is dealing with conditions in which irreparable damage has already occurred in highly specialised tissue. In the advanced stages of these diseases treatment of any kind is hopeless. We have also alluded in an earlier chapter to the fact that substances circulating in the blood are not as a rule present in an appreciable amount in the cerebro-spinal fluid; although as far as salvarsan is concerned, arsenic has been demonstrated in the spinal fluid after injection (*v.* p. 119). While making these reservations, however, it must be acknowledged that in a certain proportion of cases we have observed a very distinct improvement following the injection of salvarsan, and this applies to cases of tabes as well as to cases of general paralysis. To what extent such improvement may be maintained, or whether the progress of the disease can be definitely interrupted it is difficult to say, since, of course, remissions do occur in a proportion of untreated cases.

General Paralysis of the Insane

Of this condition 58 cases have been treated. The cases selected for injection were for the most part in the early second-

ary stage. In 12 cases out of 58 there has been a very noticeable improvement, and 6 of these have so far recovered that they have been discharged from the asylums in which they were treated. The improvement which has taken place has manifested itself in changes both in the mental and physical condition of the patients. The improvement has been maintained for three to five months after dismissal.

The following cases illustrate the benefits which accrue in some instances from treatment.

Case I.—Female, aged thirty-three years, was admitted to a mental hospital in January, 1910. She was married when she was twenty-five years of age and the first pregnancy occurred three years later and ended in abortion in the sixth month. At this time she was very ill, and her mental abnormality is said to date from the illness. Eighteen months after the first pregnancy she was delivered of a second premature child; and eighteen months later she had a full-time child which lived four and a half months. Eighteen months before admission to hospital she gave birth to a child, which is still alive. For some five or six years she had lost interest in her house-work, and latterly she had become very extravagant, buying all sorts of useless articles. When admitted to hospital she was anaemic and poorly nourished. The pupils were unequal and reacted sluggishly to light and on accommodation. The knee jerks were exaggerated, but there was no ankle clonus. Her speech was slightly slurred, and she pronounced difficult words with hesitation and deliberation. Her handwriting was very shaky. She was restless and excited, and talked incessantly about the large gifts she was about to bestow. She imagined she could play a great many musical instruments. Her mental condition was dominated by delusions of grandeur. During the first four and a half months of residence there were periods of exacerbation of the excitement, when she was very treacherous and overbearing in her conduct. She was at times filthy in her habits and language, and destroyed her clothing or other objects that came in her way. She manifested no interest in her family concerns and in general showed no sense of responsibility. She was subject to fleeting delusions about those amongst whom she lived. On May 15, 1910, her blood serum was examined and gave a positive Wassermann reaction. On May 17, she was given an injection of 0·3 gramme salvarsan in the form of a clear alkaline solution into the gluteal muscles. This was followed by considerable pain and some oedema

which lasted about a week; there was also a transient leucocytosis with rise of temperature. During the month following the injection there was a decided improvement in her general bodily condition. In that period her weight increased from 7 stone 9½ lb. to 9 stone 2 lb. Her mental condition did not, however, exhibit much change. She remained irritable and treacherous and subject to violent outbursts of temper, when her language was very obscene. She was given to self-decoration, and was very destructive of her clothing. In the second month after treatment her mental condition showed signs of improvement. She was less irritable, and her language was less objectionable. She ceased to be filthy in her habits and did not destroy her clothes. She continued, however, to decorate herself in a ridiculous fashion. During the succeeding weeks the improvement was maintained. Her blood, however, at the end of August still gave a positive reaction, and on September 1 she was given a second injection of salvarsan. On this occasion 0·3 gramme was injected in the form of a neutral suspension into the soft tissue at the vertebral border of the scapula. There was no disturbance associated with the injection. The note on her condition on September 11 shows that since the first injection her mental condition had undoubtedly improved greatly. She was at this time more neat and tidy and showed some interest in the work she was doing, although she lacked spontaneity. Her orientation was quite good, although she was facile and her attention distractible. There was still a suggestion of wantonness about her conduct generally. Her physical condition was very good, and her weight had now increased to 9 stone 10 lb. The note on her condition on December 19 shows that the improvement had been maintained. Although her mental attitude at that date was still apathetic, she was otherwise astonishingly well. She was taking her food well, and her habits are described as being beyond reproach. She was pleasant to talk to, though her ideation was still limited. There was no tendency to undue irritability, and she had not been heard to indulge recently in violent and objectionable language. She ceased to decorate herself at this time and there was no evidence of delusions of any kind. Her general bodily health was excellent, and her weight was now 10 stone 9½ lb. The blood serum still gave a positive Wassermann reaction on February 16, and on February 20 she was given another injection of 0·3 gramme salvarsan in a clear alkaline solution into the gluteal muscles.

At the beginning of April, 1911, she was very well, she was well nourished and her colour was good. The pupils were equal (although this condition varied from time to time), but reacted very sluggishly. Her articulation was normal, and her conversation rational. Her conduct was good and she was fairly active and industrious. At times she was slightly irritable, but there were none of the violent outbursts of language which characterised the earlier part of her illness. She dressed quite naturally and there was no evidence of delusions. Apart from the fact that she was somewhat facile and that her pupils were sluggish and varied at times in size, there was no evidence of mental or somatic disease. Her blood still gave a positive Wassermann reaction. Four months later the improvement was still maintained.

Case II.—Male, aged forty-four years, was admitted to a mental hospital in October, 1910. There was evidence of mental derangement dating back two years prior to admission. He had contracted syphilis twenty-five years before. When admitted to hospital he was sallow in complexion but moderately well nourished. His pupils were unequal and reacted sluggishly to light and on accommodation. There was a fine tremor of the tongue and articulation was imperfect. The knee reflexes were exaggerated, but clonus was not elicited. He was quiet in demeanour and betrayed no evidence of excitement. He had very exaggerated delusions of grandeur, imagining that he possessed very large amounts of money and property. He was untidy in his habits, and at times took his food with his fingers, or would put it into his pockets. His blood serum gave a positive Wassermann reaction. On December 21 he was given an intravenous injection of 0·3 gramme salvarsan. There was no disturbance of the nature of sickness or vomiting following the injection. A second injection of an equal amount was given on January 17, 1911. On February 1 his blood was again examined and gave a positive reaction. Since the first injection his bodily condition had greatly improved and his weight had increased from 9 stone 8 lb. to 10 stone 6 lb. The pupils were seen to vary in size from time to time, and reacted sluggishly. The articulation had not improved much, and he still imagined that he possessed great wealth, although he was more reticent about it and attempted to give a rational explanation of his possessions. On February 13 there was a distinct improvement noted in his mental condition. He said that the exaggerated ideas to which he had given expression

were "bluff," and would not admit the possession of any extraordinary wealth or property. His physical condition showed evidence of continued improvement. His articulation was slow but clear, and his gait was quite steady. On March 31, his blood serum gave a positive reaction, and he was given another intravenous injection of 0·3 gramme salvarsan. At the beginning of April, 1911, his general condition could be regarded as very satisfactory. The sallow complexion had given place to a more healthy and fresh appearance. His weight had increased to 10 stone 11 lb. He took his food well and in a clean, tidy manner. His speech, though slow, was clear and distinct. His pupils were equal and reacted fairly well to light and on accommodation, although this condition seemed to vary from time to time. His gait was steady, and his mental condition was fairly good. His conversation was perfectly rational, and his ideation clear and varied. He expressed himself well and took an intelligent interest in his immediate surroundings and in his family affairs. There was no trace of delusions. He realised his position perfectly and appeared to be quite capable of looking after himself. His condition was such that he was dismissed from hospital. His blood serum gave a slightly positive reaction. Three and a half months later the improvement in his condition was still maintained.

We have taken these two cases as typical of the course and degree of improvement which has followed the administration of salvarsan in 12 out of the 58 cases which we have treated. The improvement in these cases has been maintained over a period varying from three months to a year. There were other 2 cases in which temporary improvement lasting six weeks and two months was followed by relapse. It may be contended that these are but examples of the remissions which occur in some cases of general paralysis in the ordinary course of the disease. Be that as it may, there is no doubt that in these instances the remission has been induced by the treatment. Whether the improvement is only temporary it is as yet impossible to say, but the immediate results are sufficiently gratifying to justify a belief in the possible recovery of a certain proportion of these cases. It is doubtful, however, whether recovery in such cases would imply a complete restoration of the previous brain activity. But it is quite clear that in general paralysis the degree of depressed or abnormal function at any given time is no indication of the extent of irreparable damage. It has

been the custom for some observers to regard the progressive symptoms of general paralysis as the evidence of a gradual and final destruction of irreplaceable elements in the substance of the brain. It is obvious, however, from the fact that natural remissions occur, and that disappearance of symptoms can be induced as a result of treatment, that many of the signs of general paralysis are due to transient and removeable causes. The removal of the cause of those transient conditions would, therefore, mean the arrest of the disease. The ultimate result and success of treatment must depend on the stage at which intervention occurs. No organ of the body can be exposed to the influence of severe pathological disturbance without suffering some irreparable impairment of function. In the kidney, heart or liver, a certain degree of disordered function may be present for years without obtruding on the casual observer the manifestation of those disturbances which are arbitrarily known as disease. With the brain, however, it is otherwise. The mental expression is the most delicate evidence of organic action given by any tissue in the body, and any abnormality in this department of bodily activity is soon noticed. In the case of a diseased brain the observer is forced to notice what the mind can do, and also what it cannot do. If the treatment of general paralysis does not lead to a perfect restoration of function with renewal of previous initiative and mental activity, it may at least restore and maintain a condition compatible with the performance of the ordinary routine duties of life, a result which in itself would be valuable both to the patient and his relatives.

Locomotor Ataxy

We have treated seven cases of this condition. The chronic character of the disease and its invariable association with sclerosis of nerve structures, render its cure, in the later stages at least, absolutely impossible. These cases presented the classical symptoms of the disease, and in one of them there were, in addition, signs of commencing brain involvement, that is to say, he was the subject of tabetic general paralysis. This latter case is of interest inasmuch as the intramuscular injection of 0·4 gramme salvarsan in a clear alkaline solution was followed two days later by considerable mental disturbance. He became confused and excited, and imagined that some one wished to do him harm. His mental state before the

injection was one of apathy and indifference to his surroundings and responsibilities. This temporary disturbance following the injection lasted two days, and was followed by a condition of well-being such as he had not exhibited for months. Mentally he became more active and interested in his affairs. He had suffered considerably from shooting pains in the legs, and these disappeared completely and have not returned during the succeeding four months. His pupils before the injection were extremely small and almost fixed. A week after the injection they were considerably larger and showed a fair degree of mobility. He called attention spontaneously to the change which had occurred in his pupils. Three of the other six cases which were treated exhibited a certain degree of improvement. This was most marked as regards subjective symptoms. One patient had severe gastric pains and another had shooting pains in the legs. In both cases these symptoms disappeared after treatment and have been absent for seven months in one case and five months in the other. Here, again, it should be recognised that if substantial benefit is to follow treatment the cases should be seen early and injected immediately. In the later stages there is no doubt that the subjective symptoms may be considerably relieved, but in the earlier stages there is the possibility that the disease may be brought to a standstill.

Neuralgia in Syphilitic Subjects

We have observed two cases in which the injection of salvarsan was followed by the disappearance of severe lancinating pains in the legs in patients who were the subjects of syphilis of twenty years' standing. The pains resembled those which are characteristic of locomotor ataxy, but there were no ataxic symptoms. The knee reflexes were present in both cases, and in neither was there any affection of the eyes. In one case the blood reaction was slightly positive and in the other it was negative. Each received 0·4 gramme salvarsan intravenously. Before treatment the pains came on at intervals of a week or ten days, and the attacks were gradually becoming more frequent and more severe. In one case the pains have been absent for a month; in the other after seven weeks the pains recurred and proved refractory to a second injection. Of similar nature is the case with trigeminal neuralgia described on p. 211, in which the pains have been absent for eight months.

Mental Depression following Syphilis

We have treated with salvarsan two cases in which there was acute mental depression following syphilitic infection. In one case the secondary rash was still present, and the patient was under mercurial treatment. She imagined that her body was in a depraved and wasting condition and that there was no possible cure. She was restless and sleepless and did not take her food well. She had lost flesh considerably. A subcutaneous injection of 0·4 gramme salvarsan in the form of neutral suspension was followed by a rapid disappearance of the skin lesions, and the mental condition returned to normal within a month. In the second case, the infection was of eighteen months' duration. The evidence of syphilitic infection in the form of cutaneous eruption and sore throat had disappeared under the influence of mercury in the first three months of treatment. When she was seen fifteen months after the disappearance of the ordinary signs of the disease, she was very much depressed. She imagined that her health had been irretrievably ruined, that her "brain was leaving her," and that her skin and head were in a foul condition. She did not take her food well, and she had lost flesh during the previous two months. Her blood gave a positive reaction. A very noticeable improvement in her mental condition followed the subcutaneous injection of 0·3 gramme salvarsan in neutral suspension. This was repeated in two months, and at the end of four months from the date of the first injection she was almost quite well. Her blood reaction at this period was still slightly positive.

Non-Syphilitic Cases

We have treated twelve cases in which there was no history or evidence of syphilis. One of these was a female patient suffering from severe psoriasis. This patient was in a state of advanced dementia. Gilmour administered two doses of salvarsan subcutaneously in neutral suspension at an interval of two months. The psoriasis, which had proved refractory to all other forms of treatment, disappeared almost completely. The disappearance of the psoriasis was accompanied by a very marked improvement in the mental condition, so much so that the patient began to take an interest in her surroundings and to take part in the ward work, whereas previously she had been in a helpless condition mentally and unable to do anything for herself. Two cases of neurasthenia which were treated with 0·4 and 0·5

gramme respectively in the form of subcutaneous injections of neutral suspension, improved very rapidly. They both put on flesh quickly, and their bodily and mental condition returned to normal in about six weeks. One of these patients has now remained well for six months and the other for five. Another non-syphilitic case whose recovery followed the administration of salvarsan was that of a girl nineteen years old, who for a year had been suffering from recurrent attacks of violent excitement. These attacks occurred at intervals of a fortnight, and lasted for several days. She received a subcutaneous injection of 0·3 gramme in neutral suspension. One attack of excitement occurred a week after the injection, but she has been in a state of perfect health now for nine months. One cannot be certain, however, that in such a case the recovery was due to salvarsan. The other eight cases included various forms of mental disease, and in none of them was there evidence of improvement following treatment.

Complications of Treatment

Reference has been made to the results obtained in the treatment of 240 cases. In addition, 41 cases have been treated in which the time since injection is too short to admit of inclusion in the series. In this total of 281 cases the drug has been administered 442 times. The only serious complications following administration were: (1) the case of severe vomiting and collapse followed by jaundice (*v.* p. 260), (2) two cases of recrudescence in the form of iritis and choroiditis (syphilitic) following the injection of neutral suspension (*v.* pp. 202, 239), and (3) the extensive sloughing of the skin and subcutaneous tissues following the subcutaneous injection of neutral suspension (*v.* p. 213). Altogether almost 400 intravenous injections have been given without any untoward effect beyond that noted above (jaundice).

Effect on the Wassermann Reaction

It would appear to be possible by means of energetic treatment to produce a negatively reacting blood in almost every case of syphilis. This result is much more easily attained when the patient is treated early in the course of the disease. In the chronic stages of infection, and especially in congenital syphilis, locomotor ataxy and general paralysis, the conversion of a positively reacting serum into a negative one can only be attained slowly and by prolonged energetic treatment. It is doubtful in what proportion of cases the blood, once it becomes

negative, will remain so in the chronic manifestations. It should be the object of treatment in every case not only to dissipate the external and obvious lesions of the disease, but to produce a condition of the blood in which the Wassermann test is permanently negative. The criteria by which the results of treatment can be judged are (1) the continued absence of symptoms, and (2) the permanent negative reaction of the blood. With regard to the first of these, it cannot be too strongly emphasised that while the absence of symptoms even over a period of years is in itself a satisfactory condition, it is no proof that the disease is cured. Not only may such a case be liable to gross lesions at a later date, but while apparently in perfect health, the subject of a latent infection, in whom symptoms may not have been present for years, may transmit the disease to others. We have recently observed the case of a man, treated energetically with mercury for three months early in the secondary stage, who, after complete absence of symptoms for four years, transmitted the disease to his wife shortly after marriage. The blood in this man's case gave a positive reaction. With regard to the second criterion, the serum reaction, we would repeat here also that a negative result on a single examination, while a satisfactory indication of the progress of treatment, is not to be taken as evidence of a permanent cure. We have seen that the blood reaction may become negative only to become positive again after the treatment has ceased. The reappearance of a positive reaction is usually the earliest indication of impending lesions. This has been conclusively demonstrated in a large number of cases treated with mercury, and in many instances of recrudescence occurring after treatment with salvarsan. Those two criteria constitute the only available means of determining the probable effect of treatment. It would be obviously unwise in any given case to give an absolutely definite prognosis, but for all practical purposes the continued absence of symptoms, and a permanently negative reaction of the blood, are strong presumptive evidence that a cure has been effected.

Mode of Action of Salvarsan—Therapeutic Effect of the Serum after Treatment

Reference has been made (*v.* p. 189) to the observations of Taege, Duhot and others, in which the milk from a syphilitic mother treated with salvarsan would appear to exercise a therapeutic influence on the congenital infection in the child.

The amount of arsenic in the milk in such cases is so small that the effect has been attributed to the presence of antibodies produced as a result of the endotoxines liberated by the spirillicidal process. In support of this supposition is the fact recorded by Ehrlich * that salvarsan itself when administered by the mouth does not produce a disappearance of symptoms, even when given in considerable amount. Ehrlich further states that Marinesco, Plaut, and Michaelis have produced curative effects by the injection of serum from syphilitic patients treated with salvarsan. Corresponding results are recorded by Alston in the treatment of yaws (*v.* p. 197). We have made observations on this point, but have not been able to confirm these results. Blood was taken from three cases in the secondary stage of syphilis on the third day after receiving, in each case, an intravenous injection of 0·3 gramme salvarsan. The serum was separated and after heating for an hour at 57° C. was injected subcutaneously in doses of 20 c.c. into patients suffering from secondary symptoms. Two cases in the secondary stage received three injections, in each instance, at intervals of four days, without showing any signs of improvement, and a child with congenital syphilis received two injections of 15 c.c. without manifesting any evidence of therapeutic effect. Jesionek † has recorded the results of observations of two syphilitic children whose mothers received intravenous injections of 0·6 gramme and 0·5 gramme of salvarsan. In each case on the day following the treatment of the mother, a rash appeared on the child. The exanthem is stated to have been of a syphilitic character and not of the nature of a drug rash. The author did not find that the suckling children benefited by the milk of the treated mother. He was able in each case to prove the presence of arsenic in the milk, which was tested in quantities of 100 c.c. He injected a goat intravenously with 0·4 gramme salvarsan, and fed with the milk a child five years old, who was suffering from acquired syphilis. The symptoms disappeared in twelve days. A considerable quantity of arsenic was demonstrated in the milk. The evidence as to the production of syphilitic antibodies following the injection of salvarsan is thus, so far, to be regarded as inconclusive. Whatever be the mode of action of the drug, a remarkable feature in some instances is the almost immediate

* "Die Behandlung der Syphilis," *Königsberg Versammlung*, Leipzig, 1910, p. 885.

† *Münch. med. Wochenschr.*, 1911, p. 1169.

disappearance of pain after injection. We have recently observed a case in the secondary stage, where the sore throat was so severe as to prevent the swallowing of solid food. Within twelve hours after receiving the injection the pain had completely disappeared. The patient had been treated with mercury for three weeks prior to the salvarsan injection. Ehrlich * records a similar case in which the excessive pain due to a gumma of the tonsil disappeared within five hours after the injection. He suggests that in such cases the pain is due to products secreted by the spirochaetes and not directly to the anatomical lesions, and that the disappearance of pain is due to a neutralisation of these products by the salvarsan directly or indirectly.

In conclusion it may be said that nothing definite is known as to the mode of action of the drug. The therapeutic effect is undoubtedly due to a process in which the drug and the tissues of the host co-operate ; it is not merely internal antisepsis.

* *Königsberg Versammlung.*

CHAPTER V

THE ACCESSORY EFFECTS OF SALVARSAN TREATMENT

LOCAL EFFECTS OF INTRAMUSCULAR AND SUBCUTANEOUS ADMINISTRATION—COMPLICATIONS INVOLVING THE CRANIAL NERVES (NEURO-RECURRENCES)—ALIMENTARY AND URINARY BLADDER COMPLICATIONS—NEPHRITIS—JAUNDICE

IN an earlier chapter (*v.* p. 173) reference was made to the phenomena which are more commonly associated with the administration of salvarsan. These included rise of temperature, headache, sickness and vomiting and occasionally diarrhoea, rigors, transient erythemata, as well as local pain in those cases where intramuscular or subcutaneous injections had been given. It is proposed to deal now with the more serious sequelae which have been observed. These include the severe local reactions, which, however, since the universal adoption of the intravenous method, possess only an historic interest. The alleged toxic influence of salvarsan on the optic nerve and on the auditory nerve will be fully discussed, and reference will be made to the less frequent disturbances of the alimentary and urinary systems which follow salvarsan injection. An attempt will be made to distinguish between the complications due to the methods of administration and those which can be attributed to the drug itself, that is to say, between those complications which can be avoided by a proper technique, and those which are independent of technique. The cases which have been recorded as fatalities following treatment are dealt with in detail in a separate chapter (*v.* p. 263).

Local Effects of Intramuscular and Subcutaneous Administration

In the first place the local effects of intramuscular and subcutaneous injections must be considered. The local effects which have been noted include (1) pain, (2) necrosis of the tissue, (3) thrombosis of vessels, (4) occurrence of nervous derangements, and (5) retention of arsenic in the necrotic encapsulated masses.

Pain.—The severity of the pain depends on the method of preparation of the drug, on the amount injected and on the susceptibility of the individual patient. The most painful preparations are the clear solutions, but we have observed cases where the pain was extreme after injection of the neutral suspension. The degree of pain will depend also on the extent to which sensory nerves are involved in the zone of irritation surrounding the injected material. When shooting pains and sciatica have followed intragluteal injection, it is almost certain that the fibres of the sciatic nerve have been to some extent involved in the local reaction. It is not the acidity or the alkalinity alone which causes the pain and irritation. The drug itself in the form of a neutral suspension of the base possesses extremely irritating properties. The amount of material injected is also an important factor in the production of pain. If the amount be small it will become localised more readily. On the other hand, if a large amount be injected, the muscle fibres may become lacerated in the process, and some of the material will be certain to find its way a considerable distance from the original site of injection. This is proved by the fact that patients allowed up on the third or fourth day after injection may develop severe pains in the legs and swelling of the buttocks, such symptoms being most readily accounted for by the supposition that the movements of the muscles have dislodged the injected material from its original site.

Necrosis.—Hata observed in his experimental work that the intramuscular injection of the clear alkaline solution caused local necrosis of the tissues. It is now known that the intramuscular or subcutaneous injection of salvarsan in any form produces local necrosis. The necrotic tissue in many cases is small in amount and becomes quickly absorbed, but in a proportion of cases it becomes encapsulated, and large masses may persist for months. Encapsulation occurs

most frequently after the injection of the neutral suspension. We have observed several instances in which injections of the neutral suspension of the drug were not absorbed. In one case where the injection was given in the interscapular region, a large sloughing sore resulted, and this remained for weeks in an indolent condition. The action of the drug was unsatisfactory in this case (*v.* p. 213). In another case two succeeding injections of neutral suspension given at an interval of two months, remained unabsorbed for months; the necrotic masses did not involve the skin in this case, but severe recrudescences in the form of iridocyclitis and choroiditis took place (*v.* p. 202). A considerable number of cases with encapsulated masses of necrotic tissue have been examined macroscopically and microscopically, and it has been found that they all present more or less the same appearance. Martius [1] has recorded the details in a series of cases which came under his notice. There were three cases with necrosis in the gluteal muscles. The first was that of a child of eight months, who received a gluteal injection of 0·025 gramme in a weak alkaline solution. The child, who was suffering from a retroperitoneal malignant tumour, died fourteen days after the injection. The skin at the site of injection was normal. In the centre of the gluteal muscles there was a moderately firm mass measuring 3 × 3 × 1 cm., sharply demarcated from the surrounding tissues. Microscopically it was seen to be composed of totally necrotic tissue. In the centre there were small granular brown masses, but there was no nuclear staining. The fatty tissue was also necrotic, although there were in places droplets of fat which stained normally. The involved muscle tissue was necrotic; some small vessels were thrombosed and the walls of the vessels were necrosed. There was a zone of cellular infiltration round the necrotic mass. No bacteria could be found. The second case, that of a woman with brain tumour, died eleven days after receiving an intragluteal injection of 0·5 gramme in a neutral suspension. The necrotic mass measured 7 × 6 × 3 cm. and was sharply demarcated from the surrounding muscle tissue. It was of a reddish-brown colour with yellowish-grey spots. No nuclear structure could be detected, and the vessels in the necrotic mass were thrombosed. There was a dense wall of cellular infiltration round the mass, and the muscle fibres adjoining it contained a considerable amount of fat. No organisms were found. The third case was that of a girl who also had a brain tumour. She received an intragluteal

injection of 0·5 gramme in liquid paraffin on August 26, 1910, and on November 25, 1910, she was admitted to hospital with pain and swelling in the buttocks. An incision was made, and in the gluteal musculature there was found a large soft mass of necrotic tissue about twice the size of a goose's egg, and in the centre of this soft material there was a reddish-brown mass of firm consistence. This firm mass on microscopic examination presented the appearance of necrotic tissue similar to that seen in the preceding two cases. The wall of the cavity consisted of a very cellular granulation tissue containing traces of muscle fibres. Thrombosed vessels could also be seen. No organisms were found. In the puriform and necrotic material arsenic in considerable amount was detected. The importance of this case consists in the fact that even after three months the necrotic material, which was apparently sterile, had not become absorbed, and that even at the end of that time arsenic was still present at the site of injection. Although in the majority of cases the necrotic mass is not infected, yet in some instances micro-organisms have been observed, and in one of Martius' cases the abscess formation was associated with the presence of septic thrombosis and embolism, leading to a fatal result. This was the case of a man with commencing general paralysis, who received an intragluteal injection of 0·5 gramme salvarsan in neutral suspension. For a few days after the injection he complained of pain in the buttock and hip joint; this improved, however, to such an extent that he was able to get up and walk about. On the sixth day after treatment there was a hard non-fluctuant tender mass at the site of injection; there was also at this period slight bladder disturbance. On the fourteenth day the mass was still hard, non-fluctuant, and tender. The left leg was slightly swollen and oedematous. He died suddenly with symptoms of cardiac and pulmonary complication. Post-mortem examination revealed the presence of an abscess about the size of the fist in the left buttock. The contents consisted of thick greenish-yellow pus with small masses of necrotic tissue. The abscess formation had penetrated the gluteal muscles and at one part had exposed the iliac bone. Venous thrombosis extending into the left iliac vein was present, and there was a pulmonary embolism. Microscopic examination revealed a condition similar to that which has already been described and cultures of streptococcus longus were obtained from the contents of the abscess.

Thrombosis of Vessels.—The concentrated injection material irrespective of the manner in which it is prepared, causes necrosis of every tissue with which it comes into immediate contact. The vessels become thrombosed and, as we have seen, this process may extend into larger branches and give rise to serious consequences.

Implication of Nerves.—The nerves which lie within the area of necrosis are also involved in the destructive process and those which lie in the zone of reaction are irritated. There can be no doubt that many of the disturbing phenomena which have been described as following intramuscular and subcutaneous injection must be attributed to the direct local influence on the nerves. Severe sciatic pains, shooting pains in the legs, paresis of the peroneal muscles, have been described, and these affections are undoubtedly due in many instances to direct implication of the nerves in the local process. The occurrence of retention of urine, tenesmus and obstinate constipation have been cited as evidence of the neurotropic action of salvarsan. It is more likely, however, as Martius points out, that these complications are not infrequently due to the local action of the injection in the gluteal region. The bladder and lower bowel are supplied by the pudendal plexus, and measurements carried out on the dead body by Martius show that this plexus comes within the possible area of necrosis and irritation, to the same extent as the sciatic nerve.

Retention of Arsenic in the Necrotic Tissues.—The fact that arsenic is still present in the necrotic tissue at the site of injection weeks and months after administration constitutes another serious objection to the intramuscular or subcutaneous method of treatment. It is obvious that by this method it is impossible to estimate how much of the drug is actually absorbed into the system. It was supposed that the intramuscular method had this advantage that it left a depôt from which the arsenic exercised a continuous therapeutic effect. It is very doubtful, however, whether the arsenic still present some weeks after injection is in a form capable of exerting an efficient antisyphilitic action. It is a significant fact that a large number of the syphilitic recrudescences which have been recorded, have occurred at a period when the necrotic material at the site of injection has still remained unabsorbed. The drug is known to be very unstable and its potency as a spirillicidal agent may become impaired in a short space of time.

Another point which suggests itself is that in the event of the drug undergoing decomposition, its derivates might in some instances exercise a toxic effect on the patient. It is probable, however, that absorption from a necrotic mass in which the vessels are thrombosed is extremely slow, and there is no definite evidence from recorded experience that such toxic effects have been observed in any considerable number of instances.

These considerations indicate very definitely the disadvantages associated with the earlier methods of treatment. By the intravenous method there ought to be no local disturbance at the site of injection. By this method also the dosage can be accurately regulated, and the drug is enabled to exercise its therapeutic power in great concentration on the disease in the tissues as a whole.

Complications involving the Cranial Nerves (Neuro-Recurrences)

Contrast between the Action of Salvarsan and of Atoxyl on the Eye

Although atoxyl or soamin was found to possess very definite antisyphilitic properties, its use had to be abandoned on account of its toxic influence on nerves. About a hundred cases have already appeared in the literature recording the occurrence of blindness as a result of the injection of this drug. On this account very strict warning was given by Ehrlich when he supplied salvarsan for therapeutic purposes that special cognisance should be taken of the influence exercised by its administration on the optic nerve in particular, as well as on the other cranial nerves. Schanz,[2] of Dresden, has made a special investigation of the influence of salvarsan in diseases of the eye, and of the effect of salvarsan, as compared with atoxyl, on the optic nerve, and has come to the conclusion that so far as the eye is concerned, salvarsan does not possess neurotoxic properties. He points out that atoxyl neuritis and syphilitic neuritis are very distinct and different affections. Atoxyl atrophy, according to Birch-Hirschfeld and Köster (cited by Schanz), who have collected forty-five clinical examples and have also studied the condition experimentally, presents a characteristic course and appearance. The disease once it begins progresses gradually to blindness. The structures first affected are probably situated at some distance from the disc, since impairment of vision invariably pre-

cedes by a considerable interval the appearances of degeneration which can be recognised on clinical examination. As a rule, a few months elapse before ophthalmoscopic evidence of disease presents itself. The first signs are usually a greyish discolouration of the papilla with narrowing of the vessels. These changes are as a rule most marked on the nasal side. In some cases the centre of the field of vision remains unimpaired longer than any other part. The colour test which generally gives a very early indication of visual impairment in the usual forms of optic atrophy, does not reveal any change in the early stages of atoxyl atrophy. Another difference between the ordinary forms of optic atrophy and that due to atoxyl is seen in the pupillary phenomenon. In the usual form of syphilitic atrophy the pupillary reaction to light is absent or markedly impaired, while in the case of atoxyl atrophy, the reaction to light may be normal even in cases of almost complete amaurosis. There is thus, according to Schanz, a well defined means of differentiating between affections due to the toxic influence of the organic preparations of arsenic and those which supervene in the natural course of syphilitic disease. Atoxyl amaurosis sets in a few weeks or at the latest six or seven months after treatment. There are now tens of thousands of patients whose treatment with salvarsan was undertaken more than six months ago, and there is only one case on record where the evidence would suggest the likelihood of a neurotoxic effect of the drug. This case has been repeatedly cited as proof of the danger of salvarsan ; for that reason we consider it advisable to give a full account of the circumstances. The patient, who was treated by Finger of Vienna, was twenty-two years of age, and was the subject of a malignant form of syphilis ; she had been under treatment for over a year before receiving on July 6, 1910, a subcutaneous injection of 0·4 gramme salvarsan in a neutral emulsion. On October 5, 1910, that is, three months after injection, she complained of defective vision. The field of vision was curtailed on both sides ; there was an inequality of the pupils and a commencing optic atrophy. Of great importance, however, is the nature of the earlier treatment which had been carried out in this case :

1. Between March 16, 1909, and May 10, 1909—30 injections of arsacetin.

2. Between August 11, 1909, and September 19, 1909—28 injections of enesol.

3. Between October 25, 1909, and November 8, 1909—9 injections of enesol.

4. Between November 18, 1909, and December 22, 1909—18 injections of enesol.

5. Between March 8, 1910, and March 28, 1910—14 injections of enesol.

For more than a year prior to the injection of salvarsan this patient had been under the influence of organic preparations of arsenic. Ehrlich, in discussing the case, points out that in all probability the earlier arsenical treatment was the determining factor in the occurrence of the optic atrophy.

The study of atoxyl amaurosis has shown that the administration of that drug produces a functional hypersensibility of the eye, and that the retina acquires an increased affinity for other arsenical preparations. This increased avidity of the tissues of the organism for arsenic is well seen in some cases when a course of atoxyl has been followed by a course of arsacetin (Ehrlich[3]). In the tropics it has been found that cases treated with atoxyl exhibited a hypersensitiveness to arsacetin, which, judging from control cases, they would not have shown had atoxyl been given in the second course instead of arsacetin. Thus the hypersensitiveness produced by atoxyl may not manifest itself on continuing the atoxyl itself, but may first become obvious when another arsenical preparation different from atoxyl is administered. On the strength of these observations Ehrlich has given repeated warning, that before treatment with salvarsan is undertaken careful inquiry should be made as to the previous treatment which the patient has undergone, and he further states that had he been consulted about this special case treated by Finger he would not have advised the injection of salvarsan.

Salvarsan in the Treatment of Syphilitic Eye Affections.

(*a*) *Acute Conditions*

Although, when the drug was introduced, warning was given against its use in cases in which disease of the eye was present, treatment was after a time tried in such affections as iritis, iridocyclitis and neuroretinitis. The observations of Wechselmann, Gross, Schanz and Hirsch show that in these particular manifestations of syphilis, beneficial results have been obtained with salvarsan such as could never have been expected with any other form of treatment. Wechsel-

mann [4] records a case of extra-genital infection where, in spite of thorough inunction treatment, an iridocyclitis developed. While mercury and iodides were being administered a papillary growth appeared in the ciliary part of the iris. An injection of salvarsan was given and the tumour disappeared in a week. The iridocyclitis gradually disappeared, and the patient was dismissed from hospital a month after injection, the active syphilitic process having healed, although as a result of the structural damage sight was still somewhat impaired. Wechselmann refers to cases of choroiditis and affections of the lens in which marked improvement was observed. In some cases of interstitial keratitis in hereditary syphilitics the improvement was rapid and the result apparently permanent, while in other cases of this condition the response to treatment was slow and incomplete. The cases recorded by Schanz [2] demonstrate the efficacy of salvarsan in ordinary syphilitic eye affections. A case of recent syphilitic iritis with severe inflammatory symptoms received one injection of salvarsan in addition to atropin treatment. The patient was well in a week. The syphilis in this case had been acquired a year previously. Another patient with severe neuroretinitis and a history of syphilis had been treated with mergal without effect. The lesion was a progressive one, and in the interval between the admission to hospital and the administration of salvarsan haemorrhage into the papilla had taken place. On the day after injection an improvement could already be noted; the haziness of the papilla was less, its margins better defined, the haemorrhage itself was smaller and vision had improved. The patient continued to improve, but had to leave hospital a few days after treatment and before the process had healed completely. In another case with cerebral symptoms the papilla on each side was red and had indefinite margins. After an injection of salvarsan the papilla acquired a normal appearance and its margins became sharply defined. Another patient with double optic neuritis was treated. On the day on which the injection was given improvement was noticed. The swelling of the papilla receded. In a few days the cloudiness in the neighbouring retinal area cleared up and traces of a small haemorrhage at the border of the papilla disappeared entirely. The visual acuity which in one eye was $\frac{5}{18}$ before injection, was found to be $\frac{5}{6}$ fourteen days after injection. Schanz records his observations in five cases of interstitial keratitis in congenital syphilitics. In none of the

cases was there an obvious effect of treatment during the first weeks after injection. In three cases the process which at the time of treatment was active, continued to extend. In two cases, however, where the disease was still at an early stage the progress was arrested, and in one case the signs of disease absolutely disappeared. Schanz remarks that when it is considered how hopeless these cases usually are, and how they progress gradually in spite of treatment with mercury and iodides, the qualified success of salvarsan marks a great advance on anything that has been accomplished by previous treatment.

(*b*) *Chronic Conditions*

Hirsch [5] has recorded two cases of chronic syphilitic affection of the eyes in which salvarsan treatment had remarkable success. The first case was that of a man 36 years old, who fourteen years previously had acquired a syphilitic infection. Five years after infection he suffered from iritis in the right eye. Ten years after infection he had an oculomotor paresis in the left eye. At that time he was seen by Hirsch and the fundus and visual acuity were normal. In January, 1909, his eyesight suddenly became affected. In July of the same year he was seen by Hirsch and at that time he was unable to read. A large central scotoma was present on each side. The right pupil was wider than normal and reacted slowly to light; the left pupil was normal. The patellar reflexes were absent, Romberg's sign was present, and there was slight ataxy of the upper and lower extremities. Ophthalmoscopic examination showed pallor of one half of the right papilla, while the left papilla was quite pale. The vessels of the fundus appeared to be normal. In spite of the usual treatment with iodides, arsenic and strychnine the lesion progressed, and when examined at the end of December, 1909, the right papilla was found to be quite pale. The patient was examined immediately before receiving the salvarsan injection, and it was found that the finger-counting test showed visual capacity at $1\frac{1}{2}$ metres for the right side and $\frac{1}{4}$ metre for the left side. The field of vision on each side was narrowed concentrically, and in each eye there was a large central scotoma. On September 26, 1910, he got an intragluteal injection of 0·5 gramme salvarsan. On the day after injection he said that he felt that his eyesight had improved. The first objective examination, however, was made on October 1, and it

was found that the finger-counting distance on the right side was 1¾ metres and on the left ½ metre. The scotomata were decidedly smaller. The patient stated that while previously there was a sensation of grey or black in the region of the scotoma, there was now a sensation of red. On October 10, the scotomata had undergone further diminution, and the finger-counting test gave 2½ metres on the right side and 1 metre on the left. On November 5, the scotoma on the right side consisted of four small islets of different sizes. The scotoma on the left side had become greatly diminished in size; on the left side of the middle line it had completely disappeared, but there remained a small field on the right side of the middle line. Hirsch suggests that only a proportion of the fibres related to the region of the scotoma had become degenerate, while the others were merely functionally affected, and that this temporary interference with function was due to a syphilitic process, which was cleared up by the salvarsan. He regards the sensation of red in the region of the scotoma as an expression of the local reaction to the treatment. The rapid improvement in the condition, he concludes, is a proof of the superiority of salvarsan to all other forms of treatment. A second case is that of a man, 52 years of age, who was infected in 1906. At the time of the appearance of symptoms antisyphilitic treatment was begun and this was continued for about four years. The secondary symptoms, which cleared up, were followed in spite of treatment by periosteal lesions and gummata in the bones of the arms and in the orbital cavities of the frontal bone. In September, 1909, a severe optic neuritis of the right side developed; when this cleared up after two months there was atrophy of the papilla with extensive disease of the retinal vessels. The veins and arteries of the retina were thickened and showed a cloudy indefinite appearance from the presence of greyish streaks. Some of the smaller arterioles appeared to contain no blood, and had become transformed into a fine white branched network. A small aneurysm was present on one of the main arterial branches. This condition was observed from time to time during a whole year and did not change in spite of continued antisyphilitic treatment. He received an intragluteal injection of 0·5 gramme salvarsan and from the date of injection the improvement in his condition was continuous. It was possible to follow with the ophthalmoscope the change in the blood vessels, which proceeded *pari passu* with the gradual improve-

ment of sight. The vessel walls became gradually thinner and more transparent, and blood once more began to flow through the vessels which seemed to have been obliterated. The process of recovery did not appear to proceed either from the centre or from the periphery of the field, but to occur irregularly here and there. This case is of particular interest, demonstrating as it does the influence of treatment on syphilitic disease of the small blood-vessels. The walls of the blood vessels constitute the seat of change in many syphilitic conditions, especially in the nervous system, and this observation of Hirsch would suggest that these vascular changes are primary, and the result of active syphilitic processes, and also that they are not refractory to treatment.

We have reviewed only a few of the recorded observations which have a bearing on the relation of salvarsan to syphilitic diseases of the eye. We have shown that there is no question of the occurrence of amaurosis such as was experienced after the use of atoxyl or soamin. Neither experimentally nor clinically has evidence been forthcoming that salvarsan has a toxic effect on the fibres of the optic nerve. Atoxyl amaurosis was stated to occur in about one to two per cent. of the cases treated. Up to the end of 1910 the results of treatment with salvarsan in many more than 40,000 cases must have been recorded, and there is not a single instance in which blindness can be attributed exclusively to the use of the drug. Had atoxyl been given in those cases, instead of salvarsan, from 400 to 800 cases of blindness might have been expected. On the other hand, we have shown that in the ordinary ophthalmic complications of recently acquired syphilis salvarsan is no less effective than in the other manifestations of the disease. Cases of iritis, choroiditis, iridocyclitis, optic neuritis and neuroretinitis clear up almost invariably within a week or ten days after receiving a single injection. Even in the late manifestations of disease unexpected improvement has in some cases followed treatment. In the interstitial keratitis of congenital syphilis the results have not been so satisfactory, although even here a marked advance is recorded when compared with the results obtained with other anti-syphilitic remedies. It is not impossible that even in the congenital conditions, sustained treatment in the form of a combined therapy might yield more satisfactory results.

Salvarsan and Syphilitic Recrudescences affecting the Organs of Special Sense

Sight

While we have disposed of the suggestion that injection of salvarsan by itself exposes the patient to the risks of amaurosis, and while we have shown that salvarsan surpasses all other anti-syphilitic remedies in the treatment of syphilis of the organs of vision, there is yet another point of controversy which must be considered. This has reference to the occurrence of severe inflammatory affections of the eye following the administration of salvarsan. The following case came under our own observation. A man, 28 years of age, suffering from a recent attack of syphilis, with symptoms of sore throat, roseolar eruption and a small hard chancre on the dorsum of the glans penis, received an injection of 0·4 gramme salvarsan in the form of neutral suspension in the region of the vertebral border of the scapula. The chancre receded within a week, and within the same period the sore throat and rash had disappeared. Seven weeks afterwards he complained of severe pain in the right eye, and in the course of a day there developed a well marked iritis and choroiditis. Two days later there was dimness of vision in the left eye. Ophthalmoscopic examination showed the presence of optic neuritis in the left eye. There was a fluctuant mass about the size of a hen's egg at the site of injection. An intragluteal injection of 0·4 gramme salvarsan in the form of a neutral suspension was given. The choroiditis disappeared in five days. The optic neuritis receded more slowly, but in a month the papilla regained its normal colour and definition. The Wassermann reaction was very slightly positive when the choroiditis was present, but was negative a month after the second injection. This is a good example of a type of case which has been cited as evidence of the toxic effect of salvarsan. But it is obvious from the effect of the second injection that one is really dealing in such a case with a recrudescence of the syphilis and not with a toxic phenomenon. In this particular instance the clue to the real condition was given by the choroiditis. Had the optic neuritis occurred alone it is unlikely that at the time a second injection would have been risked. It is to be noted that the injection of the neutral suspension had not been properly absorbed. Reference has already been made to the case under our own

observation in which the subcutaneous injection of a neutral suspension of salvarsan was followed in two months by the development of an acute inflammatory syphilitic lesion of the uveal tract. This disappeared immediately after a second injection of a similar character, but developed again two months later. Neither of the injections was properly absorbed (*v.* p. 202).

Schanz [6] records two similar cases of optic neuritis following injection of salvarsan. The first case was that of a man with secondary syphilis, who was seen on August 26, 1910. He had a positive Wassermann reaction. Examination of the eyes revealed slight congestion of the papillae, but they were otherwise normal. On August 27 he received an intravenous injection of 0·5 gramme salvarsan and on August 30 he got an injection of 0·25 gramme in a neutral suspension into the muscles of the back. On September 3 all the symptoms had disappeared, and he was dismissed from hospital. At the beginning of November he suffered from left-sided headaches, and noticed that the sight of the left eye was impaired. When he was seen on December 2 he had an intense left-sided optic neuritis. On the same day he received an intravenous injection of 0·35 gramme salvarsan, and an equal amount in the scapular region. On December 4 his power of vision with the left eye had already considerably improved. On December 8 the swelling of the papilla had receded considerably and the vessels inside the papilla were more easily seen. A small haemorrhage which had been observed under the papilla on December 2 was now almost completely absorbed. On December 21 the improvement was being maintained. The colour vision which on December 5 was abnormal, was now quite normal, and the field of vision was also normal. On January 5, 1911, the condition showed still further improvement. The visual acuity on December 5 was $\frac{5}{7}_{2}$ and now it was $\frac{5}{5}$. The swelling of the papilla had completely disappeared. The patient was dismissed from hospital, having no complaint referable to his eyes.

The second case was that of a woman 32 years old. Her illness began in May, 1910, with stiffness and pain in the neck. She was treated for headache for a fortnight when a roseolar eruption was discovered. There was a generalised swelling of the lymph glands and papules on the genitalia. The Wassermann reaction was positive. Examination of the eyes at this time revealed nothing abnormal beyond chronic con-

junctivitis. On August 17, 1910, she received an intragluteal injection of 0·5 gramme salvarsan. In four days the specific symptoms, including the headache, had disappeared. On October 5 she reported herself, and a circumscribed papular eruption was found on her back. She refused to have a further injection. On December 19 she again reported herself, complaining of defective vision in the right eye of a fortnight's duration. With the right eye she was just able to detect movements of the hand at a distance of 2½ metres. The left eye had a visual acuity of $\frac{6}{6}$. There was double optic neuritis. On December 20 she received an intragluteal injection of 0·6 gramme salvarsan. On December 24 the condition had improved; the visual acuity on the left side was $\frac{6}{6}$. On this side the colour vision was normal. On the right side it was abnormal. On December 31 the left eye was normal; the right eye had a visual acuity of $\frac{6}{18}$ and the swelling of the papilla had receded considerably. On January 31, 1911, the colour vision was normal for both eyes. The visual acuity for the right was $\frac{6}{6}$ and for the left $\frac{6}{6}$. The patient was now quite well and had no symptoms referable to the eyes. Schanz states that in each of these cases the condition was a typical syphilitic optic neuritis.

Hearing.

Cases of the character described above have acquired an enhanced interest and importance in view of the fact that Finger observed among seventeen cases of recurrence three in which the organ of hearing was implicated. It was suggested that possibly the involvement of the auditory apparatus was a result of the toxic properties of the drug. Probability was given to this suggestion by the fact that certain organic preparations of arsenic were known to have a toxic influence on the auditory apparatus of animals. First of all, however, it is important to recognise the nature of the clinical course of these cases.

Case I.—This patient came under observation with a hard chancre and no secondaries. The blood reaction was negative. An injection of salvarsan was given in the form of a neutral suspension. Secondaries did not appear, but seven weeks after treatment he suddenly became giddy and noticed that his hearing was affected. On examination it was found that both the cochlear and vestibular regions of the labyrinth were involved. The Wassermann reaction was still negative.

Case II.—This patient also was treated during the primary stage of the disease, and secondaries did not appear. Nine weeks after treatment the patient had attacks of giddiness, and noticed that his

hearing was affected. The blood reaction, which was negative when he was treated, was still negative. Four weeks after the appearance of the auditory symptoms he developed a polyneuritis cerebralis menieriformis; in addition to the affection of the auditory nerve, the facial and trigeminal on the same side were involved. The Wassermann reaction was now found to be positive.

Case III.—This patient was treated in the secondary stage with 0·5 gramme salvarsan. The symptoms disappeared almost immediately. Five weeks after treatment he noticed that hearing on the right side had suddenly become affected. For some days he had severe attacks of giddiness, and found that on walking he tended to turn to the right. He vomited his food, and there was a tendency to vomit even when the stomach was empty. There was further a marked spontaneous nystagmus to the left side, in which horizontal and rotating components could be distinguished. With closed eyes and feet together he tended to fall to the right side. The Wassermann reaction was negative.

These cases bear a striking resemblance to those which we have just described in which the eye was the seat of affection. In these particular cases a reinjection of salvarsan was not resorted to. In other cases, however, as we shall see, antisyphilitic treatment produced an almost immediate disappearance of the symptoms. When the occurrence of these complications was reported to Ehrlich, he communicated with twenty-five different centres where salvarsan was being administered, and it was found that out of 7,000 cases which had been treated, disturbances of the auditory nerve had occurred in nine cases. In some of these the auditory nerve alone was affected and in others the affection was associated with implication of other nerves. A further investigation of these nine cases showed—

(1) that in every case the patient had been treated with one dose of salvarsan, and that in most cases it had been injected subcutaneously in the form of neutral suspension;

(2) that without exception the cases were of recent standing, the period of infection dating back to six or eight months;

(3) that in most of the cases the Wassermann reaction was negative at the time when the auditory complication occurred.

From these considerations Ehrlich came to the conclusion that in all probability one was dealing here with a recrudescence of the syphilis in the auditory nerve. In favour of this view was the fact that exactly similar conditions had been described after mercurial treatment, and also in cases where no treatment at all had been employed. It was also

significant that these complications had occurred in the patients of observers who had employed single doses and small amounts of salvarsan for treatment. The experience with atoxyl and arsacetin showed that the occurrence of toxic effects was determined largely by the amount of the dose. As against the idea of a toxic effect was the fact that the complications yielded readily to anti-syphilitic treatment. In some cases salvarsan was reinjected and the symptoms disappeared almost immediately. Had the complication been due to the toxic influence of salvarsan it is unlikely that a reinjection would have proved effectual in curing it.

Nevertheless the publication of Finger's [7] cases, which included four with involvement of the second and third cranial nerves and three with involvement of the eighth, raised considerable doubt as to whether after all the salvarsan, or salvarsan combined with syphilis, were not responsible for the complication. This doubt was shared by Finger himself, who emphasised the fact that other evidences of syphilis were absent in these cases at the time, and that in most of them the Wassermann reaction was negative. Added to this was the important statement by Finger that "symptoms of this kind are scarcely ever seen in recent cases of syphilis."

Affections of Hearing in Syphilitics who have undergone Mercurial Treatment

It was important, then, first of all, to settle definitely whether these affections of the organs of special sense in early syphilis were peculiar to cases treated by salvarsan. From the records which Ehrlich had of 7,000 cases treated by salvarsan, Benario [8] was able to find ten cases in which symptoms corresponding exactly with those described by Finger, had been observed before salvarsan was administered, and in which mercurial treatment had been employed before the occurrence of the complicating affection. We cite these cases as being of the utmost importance in the solution of this problem.

Case I.—A woman 23 years of age, infected in February, 1910. She had had two courses of salicylate of mercury consisting of fifteen and ten injections respectively. In the autumn of 1910 she was absolutely deaf in one ear and had difficulty of hearing in the other. On October 3, 1910, she got an injection of 0·4 gramme salvarsan in the form of a neutral suspension. On October 7 her hearing was perfectly normal.

Case II.—A man 27 years old, with syphilitic meningitis following

malignant syphilis acquired in April, 1910. He had injections of mercury in April. In July, 1910, he had pains in the head. These were followed by tinnitus and giddiness, with left side paresis of the 7th and 8th cranial nerves. He received an injection of 0·6 gramme salvarsan in neutral suspension between the shoulder blades. In three days the facial and auditory paresis had disappeared, and in a month there was no evidence of syphilis remaining.

Case III.—This patient was a woman who was infected in October, 1908. Immediately after the appearance of symptoms she had forty injections of mercury. In April, 1909, she had facial paresis, almost complete deafness, diplopia and ptosis. Energetic mercurial and electrical treatment cured the paresis, but the deafness remained. In June, 1909, she had giddiness and vomiting and could not stand straight. In July, 1909, the condition became worse. She was compelled to hold her head to one side ; she suffered from giddiness, vomiting and salivation, and a papular syphilide appeared on her skin. She could neither stand nor walk. A course of mercurial inunction produced slight amelioration of the symptoms, and after some time she was able to walk again. Early in 1910, she had another course of mercurial treatment. In October, 1910, before she received the salvarsan injection she could hear only when one shouted into her ear. She had pains in the neck and a papular eruption on the body. On October 18, 1910, she got an intravenous injection of 0·5 gramme salvarsan. On October 24 there was a distinct improvement in her hearing, and she was given a subcutaneous injection of 0·3 gramme salvarsan. On November 16 the hearing on the left side was still diminished, but on the right side it was normal. She could now follow conversation without difficulty, and the syphilitic eruption had completely disappeared.

Case IV.—A man 32 years of age had a primary sore in December, 1909. In the middle of January he had severe right-sided headache and a painful swelling on the right eyebrow. There was also a general roseola. In February and March he had forty mercurial injections, and the symptoms disappeared. Potassium iodide continued to be taken. In May of the same year there developed a right-sided facial paresis and deafness in the right ear. He got fifteen mercurial injections. In July the paresis and deafness were still present and forty injections of mercury were given. On September 24 he was seized with severe right-sided headache and pain in the right side of the neck. From October 10 the pain was extreme, shooting out from the ear and down the neck. So severe did it become that he threatened to throw himself out of the window. When he was seen on October 28 he was thin and wasted, and much depressed. He was absolutely deaf on the right side. He was given an intravenous injection of 0·5 gramme salvarsan. The headache and pains in the neck disappeared in three days. On November 13 he felt quite well. There had been an absence of headache and pain in the interval, but he was still deaf on the right side.

Case V.—This patient had a hard chancre in April, 1910. He had six injections of insoluble preparations of mercury. Since the middle of May he had become gradually more and more deaf. Disease of the labyrinth on both sides was diagnosed. On October 10, 1910,

he received an intragluteal injection of 0·6 gramme salvarsan. In addition to deafness there were evidences of syphilis in the mouth. On October 14 the hearing had improved considerably and the other signs of syphilis had completely disappeared. On November 7 his hearing was normal.

Case VI.—This patient was infected early in 1910. In July, August and September of the same year eighteen injections of salicylate of mercury were given. Almost immediately afterwards hearing became affected, and eight days after these symptoms were first noticed she could not carry on a conversation. Giddiness was present. A double-sided labyrinthine affection was diagnosed. On October 25 she was given an intragluteal injection of 0·5 gramme salvarsan in acid solution. Ten days later the patient was well, and was able to resume her vocation.

Case VII.—This patient was infected in August, 1910. On October 20, 1910, she was found to have an extensive papular eruption and buboes. She had, in addition, severe headache, and her hearing was affected. On October 22 she received an intragluteal injection of 0·5 gramme salvarsan in neutral suspension. The headache and rash disappeared in a few days. On November 15 there were no symptoms, and her hearing was normal.

Case VIII.—This patient was infected early in 1910, and received eight injections of mercury. At the end of July she developed symptoms of deafness, tinnitus and giddiness. Disease of the labyrinth was diagnosed. At the same time she developed an iridocyclitis. Under treatment the symptoms disappeared. The nature of the treatment is not recorded.

Case IX.—This patient was suffering from secondary symptoms of syphilis and had been treated with mercury without success. He was absolutely deaf. Three weeks after an injection of salvarsan he could hear words which were loudly spoken.

Case X.—This patient was treated in the early stage of the syphilitic infection with mercury, and the symptoms disappeared. A recrudescence followed which did not yield to mercurial treatment. He suffered from confusion, loss of memory, and deafness, accompanied by tinnitus. An injection of salvarsan was given and in a few weeks the symptoms gradually disappeared. The general bodily health improved, the mental condition became normal, and the hearing was fully restored.

Table I gives a review of the main features of these cases.

When it is remembered that these cases were chosen from a series which was not compiled with a view to estimating the relative number of such complications in syphilis, it is remarkable that so many as ten examples should be found in 7,000 instances. One may take it for granted that references to minor manifestations of this kind were omitted in a number of instances. These ten cases demonstrate beyond all doubt that the complications described by Finger and Rille as following the administration of salvarsan occur also after treatment

TABLE I

No. of Case.	Interval between Infection and the Onset of Auditory Symptoms.	Interval between Mercurial Treatment and Auditory Symptoms.	Duration of Symptoms under Mercurial Treatment.	Nature of After-Treatment.	Result.
1	8 months	Short time	6 months	0·4 gramme salvarsan as neutral suspension	Cured
2	3 months	3 months	3 months	0·6 gramme salvarsan as neutral suspension	Cured
3	6 months	4 months	18 months with repeated courses of mercury	0·5 gramme salvarsan intravenously	Left side improved, right side cured
4	5 months	2 months	5 months with repeated courses of mercury	0·5 gramme salvarsan intravenously	Deafness not improved; other symptoms disappeared
5	2 months	1 month	5 months	0·6 gramme salvarsan, alkaline solution intragluteal	Cured
6	3 months ?	Short time	1–1½ months	0·5 gramme salvarsan, acid solution intragluteal	Cured
7	2 months	Untreated	Untreated	0·5 gramme salvarsan, neutral suspension intragluteal	Cured
8	3 months ?	3 months ?	?	Not recorded	Cured
9	?	?	?	Salvarsan; dose ?	Improved
10	?	?	?	Salvarsan; dose ?	Cured

with mercury or without any treatment at all. Seven of the cases described presented evidence of implication of the auditory apparatus within a period varying from two to eight

months from the date of the primary symptoms of syphilitic infection. In seven cases salvarsan cured the ear disease, in five of which the condition had already proved refractory to mercury. In one case which had not been treated prior to the onset of symptoms salvarsan proved effective. In two cases improvement followed the administration of salvarsan, and in one case salvarsan had no effect on the deafness. The conclusion which must obviously be drawn from these results is that the affections of hearing which develop in the early months of a syphilitic infection are syphilitic in origin.

Analysis of Syphilitic Recrudescences in the Organs of Special Sense after Salvarsan Treatment

In a more recent communication Benario[9] has extended his observations on the occurrence of complications involving the organs of special sense in syphilis. He has collected his material from the communications and private reports (sent to Professor Ehrlich) bearing on the salvarsan treatment of 14,000 cases, his object being to determine in what proportion and to what extent, disease of the cranial nerves occurs after the administration of salvarsan. His analysis includes an inquiry into the period of incidence of these complications in the course of the disease, the nature of the infection in the cases involved as regards site of the primary sore and character of the rash, and also the character of associated symptoms which supervene, such as headache. His results are of the utmost importance in that they settle once and for all the controversy as to the toxic influence of salvarsan, and in addition they throw further light on the nature of some of the most important manifestations of syphilitic disease.

Out of a total of 14,000 cases there were 126 in which symptoms pointing to an implication of the cranial nerves developed after treatment with salvarsan. There were eight of these where the patients were in the tertiary or post-tertiary stages of the disease, and these cases are left out of account in the general analysis. Of the 118 remaining cases 5 (4·2 per cent.) were in the primary stage when first treated, 22 cases (18·6 per cent.) were in the primary and secondary stages, and 91 (77·2 per cent.) were in the secondary stage.

With regard to the five cases treated in the primary stage, two were the subjects of multiple infection, and in one of these a chancre was situated on the lip. The primary sores had been present for a considerable time before treatment, and the

Wassermann reaction was positive in both. In two cases the result of the blood test is not recorded. In the fifth case the blood reaction was negative at the time of treatment. This patient was treated with 0·5 gramme salvarsan in neutral suspension; two months after treatment the primary sore broke down again and a fluctuant mass was found at the site of injection. The patient was an alcoholic, and this, according to Benario, may have had something to do with the fact that the early treatment had proved ineffective. In one of the cases with a negative Wassermann reaction the injection of 0·6 gramme salvarsan in the form of neutral suspension was followed by extensive necrosis. This may have accounted for the recrudescence, since only a proportion of the injected drug is likely to have had an opportunity of exercising a therapeutic effect. The important fact to be recognised in these cases is that they constitute only a very small percentage of the total number of nerve recrudescences, and the importance of this is enhanced when it is recognised that a very large number of syphilitic cases come under treatment while they are still in the primary stage. *Thorough treatment in the primary stage minimises the probability of subsequent relapses.*

The following table (Table II) gives a summary of the nerves involved in all the 126 cases, showing the combinations where more than one nerve was affected.

TABLE II

	Total.	Affected alone.		Affected with others.	With I.	With II.	With III.	With IV.	With V.	With VI.	With VII.	With VIII.	With IX–XII.	With IV–VI.	With V–VII.	With VII–VIII	With III, IV & VII.	With V, VII & VIII.
I. Olfactory .	0	0		0														
II. Optic . .	41	31		10			1				1	4		1		1	1	1
III. Oculomotor.	8	8		0														
IV. Trochlear .	2	2		0														
V. Trigeminal .	0	0		0														
VI. Abducens .	3	3		0														
VII. Facial . .	10	9		1					1									
VIII. (*a*) Vestibular and Cochlear	62	17	51	11						1	8							
(*b*) Vestibular alone .		5																
(*c*) Cochlear alone .		29																
IX.–XII . . .	0	0		0														
Total . .	126	104		22														

The numbers opposite the individual nerves in the first three columns refer only to those cases in which the particular nerve was the *first* to be affected. (Private communication from Dr. Benario.)

In these 126 cases it was found that the cranial nerves which were implicated numbered 158. A further analysis of the affected nerves is given in Table III.

TABLE III

	On Right Side.	On Left Side.	On Both Sides.	Not recorded.	Total.	Per cent.
II. Optic . .	11	11	15	4	41	26
III. Oculomotor	2	6	0	4	12	7·6
IV. Trochlear .	2	1	0	1	4	2·5
V. Trigeminal .	1	2	0	1	4	2·5
VI. Abducens .	1	3	0	0	4	2·5
VII. Facial . .	11	9	5	0	25	15·9
VIII. Auditory .	15	16	30	7	68	43
Total . .	43	48	50	17	158	

The next point of importance is the period of incidence of these complications. In three of the 118 cases of recent syphilis data were not supplied, but in the remaining 115 cases it was found that—

Thirty cases occurred in the first month after injection of salvarsan, that is, 26·1 per cent.

Forty-six cases occurred in the second month after injection, that is, 40 per cent.

Twenty-seven cases occurred in the third month after injection, that is, 23·5 per cent.

Eight cases occurred in the fourth month after injection, that is, 7 per cent.

A review of the total results of the analysis showed that in 88 per cent. of the cases affected the complication occurred within nine months from the time of infection, and it was exclusively the cranial nerves from the optic (II) to the auditory (VIII) which were involved. In 96·6 per cent. of the cases the nerve recrudescence occurred within four months from the time of receiving the injection of salvarsan. In the majority of cases only one nerve was affected, and when more than one nerve was affected, the onset was coincident in some cases and at different periods in others. There were in the majority of cases no other symptoms of syphilis in addition to the symptoms referable to the cranial nerves. In a few instances, however, there were secondary lesions affecting the skin and mucous membranes. In a large number of cases the Wassermann reaction at the time of the recrudescence was negative.

Comparison of Mercury and Salvarsan Treatment with Respect to Neuro-recurrences

We have already made extensive reference to Benario's communication on the occurrence of syphilitic affections of the cranial nerves in cases treated with mercury. In his more recent paper on the recrudescences after salvarsan he has been able to add other nineteen cases collected from the reports on salvarsan treatment, in which recrudescences affecting the organs of special sense followed the use of mercury. He illustrates further from the earlier literature on syphilis the relative frequency of such relapses in the early stages of syphilis. From Mauriac's work [10] he gives the following quotation: "Among all the internal complications of syphilis, the cerebral affections are not only the most frequent, but they are the first to make their appearance." Out of thirteen cases which Mauriac [11] himself observed, nine occurred within five months from the date of infection, and in 168 cases of cerebral syphilis which he collected from the literature, the nervous complication ensued in all during the first year of the disease. From a tabulated comparison of his own statistics with those of Mauriac, Benario gives further proof of his contention that there is no essential difference between the nerve complications following salvarsan treatment and those which occur apart from salvarsan treatment (Table IV).

TABLE IV

Mauriac: No. of Cases, 53.						Benario: No. of Cases, 118.					
Interval since Infection.	No. of Cases	Percentage.	Percentage in periods of Two Months.	Percentage in first Six Months.	Percentage in first Nine Months.	Interval since Infection.	No. of Cases	Percentage.	Percentage in periods of Two Months.	Percentage in first Six Months.	Percentage in first Nine Months.
Mths.			Per cent.	Per cent.	Per cent.	Mths.			Per cent.	Per cent.	Per cent.
1	3	5·5	12·8	54·5	62·7	1	5	4·2	12·6	86·1	93·7
2	4	7·3				2	10	8·4			
3	5	9·3	24·3			3	15	12·6	34·6		
4	7	15				4	26	22			
5	4	7·3	18·4			5	29	24·5	38·9		
6	6	11·1				6	17	14·4			
8	2	3·6	7·2			8	5	4·2	7·6		
9	2	3·6				9	4	3·4			
10–14	20	37·4	—	—	—	10–14	8	6·7	—	—	—

A comparison of these tables shows that while there is a difference in the percentage, that difference is accounted for by the fact that in Mauriac's series a larger number of cases fall within the period between ten and fourteen months. In both series the number of relapses is relatively small in the first two months. From the third to the sixth month inclusive, there is in each series a considerable increase, and again in the eighth and ninth months a definite fall.

What then are the general conclusions to be drawn from a correlation of these data in the light of our knowledge of syphilis ? It is obvious that the phenomena known as nerve relapses or neuro-recurrences are not peculiar to patients who have been treated with salvarsan. The affections are the expression of a syphilitic lesion, and are cured by specific treatment, most readily by salvarsan itself. With regard to the possibility that these nerve affections may be due to toxic effects of the drug itself, it must be noted that arsacetin is known to possess a toxic affinity for the organ of hearing, and Röthig has pointed out that arsacetin when it affects the organ of hearing singles out the vestibular nerve, and always on both sides. Now in Benario's series of cases treated with salvarsan the vestibular branch was affected by itself only in five cases, in three cases on one side, and in one case on both sides, while in the fifth case particulars are not given. In the single case in which the vestibular branch alone was affected on both sides, the patient was in the tertiary stage of the disease. It is thus clear that the action of arsacetin in this respect throws no light on the conditions under consideration. The evidence in favour of the view that the nerve affections in general are syphilitic is very definite. In the first place, Schanz points out that the ophthalmoscopic changes in cases of optic neuritis occurring after salvarsan treatment are not distinguishable from those which are admittedly characteristic of syphilitic optic neuritis, and that they are quite different from the changes characteristic of atoxyl amaurosis. In the second place, the phenomenon known as Herxheimer's Reaction (see p. 174) has been observed in at least nine cases in the cranial nerves, seven times in the auditory and twice in the facial. This means that a short time after the salvarsan injection, varying from a few hours to one or two days, transitory affections of hearing and sight occurred which resembled the complications we have described as nerve relapses. In one case described by Wechselmann, a facial paresis occurred

two hours after the injection. As has been pointed out, the occurrence of this phenomenon is in all likelihood due to a stimulation and reaction on the part of the spirochaetes, and in the case of the Herxheimer phenomenon in the cranial nerves the supposition is that the organisms are situated there. In the third place, it has been demonstrated by Hoffmann and Ehrmann [12] that spirochaetes do settle in the nerve sheaths and between the nerve bundles. Strassmann [13] has further demonstrated the presence of spirochaetes in the cranial nerves, and recently Verhoeff (cited by Benario) has found spirochaetes in the optic nerve of an eye removed from a patient suffering from secondary syphilis.

Factors Predisposing to Neuro-recurrences

The next point which calls for consideration is the nature of the conditions which predispose to syphilitic recurrences in the cranial nerves. We have already noted that very few cases treated in the primary stage of the disease are subject to these complications. It is for the most part in those cases in which an abundant general dispersion of the spirochaetes throughout the body has already taken place that the recrudescences occur. Speaking generally, these have been most frequently observed in patients who have received only one injection of salvarsan, and in many instances the salvarsan has been injected subcutaneously in the form of a neutral suspension. Recrudescences in the cranial nerves have, however, occurred in cases treated intravenously and in cases where two injections have been given. Still it is clear from the statistics that the less efficient forms of treatment in cases where the spirochaetes are widely disseminated constitute a predisposing factor, and it is a significant fact that Duhot, Gennerich and Neisser, who have given repeated injections of salvarsan, and who have combined salvarsan with mercurial treatment, have not had a single case of nerve recrudescence.

The anatomical relationships of the cranial nerves predispose, in Ehrlich's opinion, to the occurrence of relapses. Their passage through narrow canals and foramina expose them to pressure in the case of their becoming swollen. Hoffmann has shown the tendency of spirochaetes to traverse the lymph spaces in the connective tissue round nerves and blood-vessels. If the invasion of the cranial nerves were accompanied by any degree of swelling, the pressure exer-

cised by the narrow passages in which they lie would lead to an interference with their function. That the nerve affection is, in its early stages at least, a purely functional disturbance and not a degenerative lesion, is demonstrated by the rapid recovery which as a rule follows treatment. It will be seen that the nerves most liable to be influenced by these conditions are the II, VII, and VIII, and to a less extent the III, IV, and VI, while the I, V, X, XI, and XII are not likely to be interfered with at all. Thus, the optic nerve along with the ophthalmic artery passes through the optic foramen, and is enclosed for a considerable distance in a narrow dural sheath. The facial and auditory nerves pass together through the internal auditory meatus. They both lie in a narrow bony canal, and the facial nerve has a circuitous route through the temporal bone to its foramen of exit. On the other hand, the oculomotor, trochlear, and abducens, passing through the superior orbital fissure, have a relatively wide exit, although they are sheathed firmly in a fibrous covering. The glossopharyngeal, vagus, hypoglossal and spinal accessory are not likely to be exposed to pressure, as they pass through the wide jugular foramen. The significance of these anatomical conditions is recognised when it is remembered that the optic, facial and auditory nerves are most frequently affected. A further point of importance from the anatomical side is the proximity of the auditory and facial nerves to the throat. The throat is almost always affected in secondary syphilis, and not infrequently the middle ear becomes involved through the Eustachian tube. Where there is ulceration of the throat the spirochaetes undoubtedly penetrate the lymph spaces in the immediate neighbourhood. If the nerve sheaths are invaded and become oedematous the first effect of their becoming swollen in the bony canals will be to impair the local blood supply. When treatment is carried out in such cases, the spirillicidal substances which are carried by the blood will fail to reach the organisms in sufficient amount to produce sterilisation. Thus there will be left in the nerve sheaths isolated masses of spirochaetes which may exert their pathogenic action at a later period.

From this point of view it is easy to understand why a negative Wassermann reaction is found in a large number of these isolated nerve recurrences after salvarsan treatment. From experimental and clinical evidence there is every reason to believe that the injection of salvarsan is followed in most

cases by an extensive sterilising process so far as spirochaetes are concerned. It is natural that this process will be most active and most effective where the blood supply is most efficient. Thus the ordinary lesions on mucous membranes accompanied by hyperaemia heal rapidly, and the spirochaetes disappear from them almost immediately after treatment. With the disappearance of the lesions under thorough treatment the substances in the blood serum which produce a positive Wassermann reaction disappear also. There may remain, however, in tissues poorly supplied with blood, spirochaetes to which the spirillicidal substances do not gain access. Supposing that such a mass of inactive spirochaetes were lying in the lymph spaces round some portion of the auditory nerve, and that the rest of the body had become sterilised, then the production of those substances which give rise to a positive Wassermann reaction would probably cease, and the blood would become negative just as if complete sterilisation had taken place. We have pointed out, however, that immunity to protozoal diseases is of short duration, and before long, perhaps as a result of abuse of alcohol or tobacco, the organisms in the nerve may become active, and there will be set up what may be termed a reinfection. At the time of their occurrence the nerve recrudescences are in most cases the only evidence of disease. When the symptoms first make their appearance the blood will naturally give a negative reaction, but if the process be allowed to develop and extend, the blood will again become positive. This was seen to occur in the case described on p. 241, where the auditory nerve became affected while the blood gave a negative reaction. Soon afterwards the facial and trigeminal nerves became affected in addition and the blood was found to give a positive reaction. This interpretation is also in accordance with the well-known fact that a primary sore may be present for several weeks before the blood gives a positive reaction.

Attention has been called by some observers to the frequency with which the cranial nerve recurrences are associated with *extra-genital syphilitic infection*. Benario states that nine cases of chancre cephalique are included in his series. Rille [14] records two such cases; in one of them a primary sore on the tonsil was followed in three months by affection of hearing, optic neuritis and facial paresis; in the other a chancre on the lip was followed in three months by headache, giddiness, right-sided facial paresis and double optic neuritis.

Werther recently described two cases of a similar nature which were observed before the introduction of salvarsan. In one a primary infection of the right tonsil was followed in four months by disease of the labyrinth, which yielded to injections of salicylate of mercury. In the other case a primary infection of the tonsil was followed in six months by disease of the labyrinth on both sides and oculomotor paresis on one side. Injections of calomel did not prevent the patient from becoming absolutely deaf.

Cephalic infections of syphilis, just like rabies and anthrax, are recognised as often giving rise to a very malignant type of disease. The important factor, however, is most likely to be the proximity of the primary sore to the cranial nerves, and the extension of the spirochaetes in large numbers along the lymph tracks to the nerve bundles.

A proof of the malignant and refractory character of the infection in these cases of nerve recurrence is afforded by the fact that in a large number the original syphilitic exanthem was of the nature of a papular syphilide. This is universally recognised as being an evidence of the most refractory type of infection. In Benario's series a papular syphilide was present in thirty-six cases out of sixty-six in which particulars relating to the rash were given. A papular syphilide was present in five of the nine cases of extra-genital infection to which Benario refers ; in the remaining four the character of the rash is not referred to.

Of clinical importance is the fact that these recurrences are very often heralded by headache of extreme severity, sometimes confined to one side. Out of sixty cases quoted by Benario, in which particulars relating to this phenomenon were given, headache was present in thirty-six. When the headache becomes general, with its seat of maximum intensity at the back of the neck and with severe pains radiating down the back, the likelihood is that an invasion of the central nervous system has taken place.

The practical conclusions to be drawn from a study of these complications are—

1. Affections of the cranial nerves in recent syphilis, whether occurring after treatment with mercury or salvarsan or in untreated cases, are syphilitic in character, and should be treated as such without delay.

2. These complications may be avoided by early and energetic treatment of the syphilis, and in this respect special atten-

tion should be paid to cases of cephalic infection and to cases of a malignant or refractory character, such as those with papular eruptions.

3. Patients should be instructed to report without delay the occurrence of symptoms which would indicate the possible incidence of a recurrence. Such symptoms include severe headache, affections of hearing or sight, giddiness with sickness and vomiting, and signs of facial or oculomotor paresis.

4. It is of importance for the further study of this question to pay particular attention to other factors which may possibly predispose to recurrence. These may include (*a*) the habits of the patient in respect to the abuse of alcohol or tobacco, (*b*) the vocation of the patient, thus for example in the case of a blacksmith or iron-founder the ear is exposed to considerable shock or again, (*c*) the presence of intercurrent disease such as middle ear catarrh, influenza or diphtheria.

Alimentary and Urinary-Bladder Complications

Attention has been drawn already to the fact that sickness and vomiting occasionally follow injection. In the case of intravenous treatment these symptoms occur within two or three hours after the injection. We have observed three cases in which diarrhoea followed injection. Iversen [15] observed one case in which treatment was followed by transitory diarrhoea. Similar observations are recorded by Géronne and Huggenberg.[16] This complication seems to occur only rarely and possesses no serious significance.

Complications involving the urinary system and alimentary system have been described, and interpreted as affording evidence of the toxicity of salvarsan. The first report of this kind was published by Bohač and Sobotka.[17] In three of their cases they observed retention of urine ; in one instance the retention lasted a day and in another it lasted nine days. In all three cases the knee reflexes were absent, and in two there was severe tenesmus. Constipation was present in each case. The authors attribute these symptoms to a toxic influence of the drug on the spinal cord. Symptoms of a similar character have been observed by Eitner,[18] Malinowski,[19] Herxheimer,[20] Wechselmann,[21] Bering,[22] Schlesinger,[23] and others. From a review of the reported cases it is impossible to obtain evidence of a common cause for the appearance of the symptoms. Bohač and Sobotka in the preparation of the salvarsan for injection used methyl alcohol to bring it

into solution. It was thought that an excess of methyl alcohol or perhaps impurities in the methyl alcohol might have been responsible. In the cases reported by Schlesinger, Bering, and Eitner, however, methyl alcohol was not used. The complications in the cases reported by Eitner and Malinowski are to be attributed to the fact that the capsule containing the drug was not opened immediately before the preparation and injection. In Eitner's case the capsule was opened fourteen days previously and part of the drug withdrawn. The tube was then sealed and the contents used fourteen days later. In Malinowski's case the solution was prepared three days before injection. It has already been pointed out that salvarsan is preserved in sealed capsules containing inert gas, since it readily oxidises to poisonous compounds on exposure to the atmosphere ; on this account the capsule should be opened and the solution prepared immediately before injection. While there is nothing to show that the procedure adopted in the cases of Eitner and Malinowski was followed in any of the other cases described, it should be borne in mind that there is always a possibility that where subcutaneous or intramuscular injections were given, some of the material which was not absorbed may have become altered and may have given rise to the symptoms referred to. In any case it would appear that since the general adoption of the intravenous method of treatment, reports of these symptoms have ceased to appear in the literature. We have already suggested that the local effects of intragluteal injection on the nerves in the neighbourhood might account for some of the complications alluded to. In a case of general paralysis which we treated by the intragluteal method, there was considerable oedema and tenderness at the site of injection, and there was also retention of urine lasting two days. This case was treated about four months before the report of Bohač and Sobotka appeared, and we attributed the condition to the local irritation in the first instance, and to the fact that urinary disturbances of this character manifest themselves readily in patients suffering from general paralysis. In some of the cases described in the literature the relation of the symptoms to the injection of salvarsan is very indefinite. Herxheimer, for example, emphasises the fact that in his case the patient was neurasthenic. In Wechselmann's case the symptoms occurred a considerable time after the injection, and the appearance of the symptoms was coincident with the onset of a severe

febrile angina. In a further case, described by Deneke,[24] the patient had been subject to similar attacks prior to injection. It is obvious that these symptoms cannot be attributed in all cases to a common cause. Care should be taken, however, to examine every capsule thoroughly before opening, to make sure that it is quite intact, so as to obviate the dangers of using an oxidised specimen of the drug.

Nephritis

After the injection of salvarsan the arsenic is excreted to a large extent by the kidneys. Where intramuscular or subcutaneous injection has been adopted the excretion may extend over a period of weeks, or even months, although it is most abundant during the first week. Where the drug has been injected intravenously the excretion is probably complete in three or four days. Transient albuminuria has been observed during the period of excretion in some cases in which, prior to injection, the urine was free from albumin. On the other hand, several cases have been recorded in which albuminuria in a syphilitic subject has disappeared or become much diminished as a result of salvarsan treatment (Treupel and Levi [25]). We have ourselves observed a case of syphilis with albuminuria in which the albumin practically disappeared after treatment with salvarsan (*v.* p. 210). In a series of 350 cases, Sellei [26] records two instances in which a latent nephritis became active as the result of injection. In one case a man whose albuminuria registered 0·5 in Esbach's tube received a subcutaneous injection of 0·45 gramme of salvarsan. The albumin subsequently registered 4·5, but gradually subsided to the earlier amount. Two cases are recorded by Weiler,[27] in which acute nephritis followed the injection of salvarsan in patients who, prior to treatment, showed no evidence of renal disease. These two cases occurred in a series of 500. The first was that of a woman 25 years of age, who became infected with syphilis in January, 1909. In March of the same year she had a course of mercurial inunction lasting 25 days. A recurrence having appeared, the inunction courses were repeated in December, 1909, and in May, 1910. In November, 1910, there was still a papular eruption on the body, and she was given a subcutaneous injection of 0·45 gramme salvarsan on November 7. A week later the eruption had disappeared and she was dismissed from hospital. A week after leaving hospital she noticed that her legs were swollen, and six weeks

later she consulted a physician, who found her to be suffering from acute haemorrhagic nephritis. In addition to the nephritis there was albuminuric retinitis. The second case was that of a woman 26 years of age. She was in the early secondary stage of syphilitic infection. On February 3, 1911, she received an intravenous injection of 0·4 gramme salvarsan. Four days later there was oedema of the eyelids. She complained of headache and a feeling of lassitude, but could not sleep. The urine was found to contain albumin, red and white blood cells, hyaline, epithelial, granular and blood casts in considerable numbers. A week later the oedema had disappeared. In a month the albumin and blood had disappeared, and there were only a few cylindrical casts to be seen. She was dismissed from hospital apparently quite well. These two cases are exceptional in the general experience of those who have been administering salvarsan. For example, Wechselmann, who has recorded the results of the treatment of 1,200 cases, and Schreiber, who has treated over 1,000 cases, make no mention of any such complication. Weiler himself has expressed doubt as to whether his first case may not have been a coincidence. At any rate the instances of nephritis following salvarsan treatment are so extremely rare as to warrant doubt as to a causal relationship.

Jaundice

A few cases have been recorded in which jaundice appeared after treatment with salvarsan. Klausner [28] describes four cases which came under his own observation and refers to similar cases recorded by Rille and Pinkus and Waelsch. The first of Klausner's cases was a man, 41 years of age, who received an intramuscular injection of 0·6 gramme salvarsan in the form of neutral suspension on October 21, 1910. On the day after the injection there was a slight rise of temperature and a general feeling of weakness. On October 23, there was vomiting and diarrhoea, and a jaundiced discolouration of the skin appeared. The urine gave a marked urobilin reaction. The jaundice disappeared in the course of a few days, and there were no further symptoms. The second and third cases were treated on the same day with portions of the same suspension of salvarsan. One patient received 0·2 gramme and the other 0·4 gramme. In each case severe symptoms appeared a few hours after injection. The temperature rose to 103° F., and there were rigors and vomiting. The pulse was rapid and thready,

and the patients were in a collapsed condition. These symptoms disappeared rapidly, and on the following day a well marked jaundice made its appearance. In each case urobilin was present in the urine along with a considerable amount of albumin. The jaundice and albuminuria disappeared in a few days. In the fourth case the patient received an intramuscular injection of 0·6 gramme in the form of neutral suspension, and, fourteen days later, very marked jaundice developed, with urobilin and bilirubin in the urine. No further symptoms appeared, and the patient recovered. The case described by Waelsch corresponds exactly with the fourth case in Klausner's series. Here also the patient was treated intramuscularly and fourteen days later jaundice appeared. In neither case was the injection followed immediately by symptoms suggesting intoxication. In both cases the jaundice may have been due to the absorption of substances from the necrosed mass at the site of intramuscular injection. The occurrence of jaundice is, however, so extremely rare as compared with the number of cases treated, that it is difficult to assign a cause for its appearance. The second and third cases cited by Klausner, in which the symptoms appeared within a few hours after injection with the same suspension, suggest the possibility of a toxic action of the injected material. The fact that such a rare complication should appear in two cases treated with the same suspension at the same time, apart from some abnormality in the drug or its preparation, would be a peculiar coincidence. We have ourselves observed the occurrence of jaundice in a patient who manifested symptoms similar to those described by Klausner in these two patients. This was the case of a man who had been treated with mercury in the initial stages of the infection. Recurrence of the disease had taken place, and although the symptoms of relapse had disappeared under further mercurial treatment, the blood reaction at the end of two years was positive. He was given an intravenous injection of 0·5 gramme salvarsan. No disturbance of any kind followed the injection. Six weeks later the injection was repeated in the same amount and exactly in the same way. On this occasion the injection was followed in a few hours by very severe symptoms of rigor, headache and vomiting. The pulse was rapid and thready, and the patient was very weak and collapsed. He was restless and could not sleep. In six hours he began to recover, the sickness and vomiting ceased, the pulse became slow and steady

and he quickly regained strength. Twenty-four hours after the injection jaundice appeared. This lasted for several days, and then gradually disappeared. At the time two possible explanations suggested themselves. There was the possibility of a hypersensitiveness to the drug having been developed as a result of the first injection; or in the second place, the excretion of arsenic into the stomach might have intensified a catarrhal condition already present, thus giving rise to the sickness and vomiting, and subsequently to jaundice owing to a spread of the catarrh to the duodenum. There is no doubt that the patient was suffering from gastric catarrh immediately prior to the injection. This is the only instance of a complication of this kind which we have encountered, although as a matter of routine, intravenous injections are repeated at an interval of three weeks to a month in patients who show signs of syphilis or whose blood gives a positive reaction. In discussing his cases Klausner suggests that the jaundice may be due to destruction of red blood-corpuscles following the injection of salvarsan. He points out that after the injection the number of red blood-corpuscles may show great variations. In some cases the number is greatly reduced, in other cases it is greatly increased. During the first twelve hours or first few days after injection a considerable amount of urobilin may be present in the urine, a phenomenon which he interprets as indicating a large destruction of red blood-corpuscles. It is not improbable that jaundice following injection may result from a variety of factors. In any case its occurrence is so extremely rare as to possess in general, very little practical importance. As has already been indicated, care should be taken in every case to prepare the patient for injection, as is done prior to the adminstration of an anaesthetic. In the event of gastric catarrh being present, postponement of the injection is advisable.

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CHAPTER VI

FATAL CASES

DIFFICULTIES IN ESTIMATING THE PART PLAYED BY SALVARSAN IN CERTAIN FATALITIES—FATALITIES ASSOCIATED WITH FAULTY ADMINISTRATION AND WITH CONTRA-INDICATIONS—FATALITIES DUE TO THE TOXIC EFFECTS OF THE DRUG—DIABETES AS A CONTRA-INDICATION—FATALITIES IN CASES WITH EXTENSIVE NERVOUS AND CIRCULATORY LESIONS—FATALITIES ASSOCIATED WITH INTERCURRENT DISEASES—FATALITIES DUE TO FACTORS ASSOCIATED WITH THE ADMINISTRATION—CASES MORIBUND AT THE TIME OF TREATMENT—SEPSIS FOLLOWING INTRAMUSCULAR INJECTION—BENEFICIAL EFFECTS OF SALVARSAN IN ANGINA PECTORIS

IT is of the utmost practical importance in reviewing the fatal cases to consider (1) whether some of them might not have ended fatally without injection; (2) whether others were not treated by methods technically faulty, or whether they did not before treatment present some of those contra-indications to which attention has been repeatedly called; and (3) what proportion of the cases treated has succumbed to the toxic effects of the drug alone.

Difficulties in Estimating the Part played by Salvarsan in certain Fatalities

When it is remembered that the drug has probably been administered a million times (*v.* p. 278), and that the patients treated have been suffering from a disease whose complications involve in many cases organs and centres of vital importance, it would not be a matter of surprise should some of them have died a few days after treatment, from causes which had nothing whatever to do with the injection. The importance and significance of this aspect of the question is

enhanced by recognising how very serious some of the conditions have been which were selected for treatment. Ehrlich, who has had the best opportunity of reviewing the whole situation, cites from the reports which he has received, five cases already selected for treatment, in which a fatal issue supervened before the injection was carried out. Three of these cases suffered from general paralysis, one from malignant syphilis, and the fifth case, which on admission to hospital presented no evidence of serious disease, died before receiving treatment and the post-mortem examination showed no obvious cause of death. It was supposed that he had died from an attack of angina pectoris due to arteriosclerosis. In any case, had the patient been treated, his death would undoubtedly have been attributed to salvarsan, and there would have been little or no evidence on which to question the conclusion.

On the other hand, when the drug was first introduced, several cases were treated which were regarded as hopeless from the beginning. When these cases are cited as evidence of the serious accessory effects of salvarsan treatment, there is some reason for doubting the authority or reliability of the criticism. Mulzer [1] says, for example: "Wechselmann himself treated with salvarsan three infants with pemphigus neonatorum, and they died after the onset of fever and anaemia and in one case of opisthotonus." Wechselmann's [2] own account leaves a different impression. Referring to the responsibility attached to the administration of a new drug in comparatively large doses, and the avoidance of possible effects from hypersensitiveness induced by repeated injection, he says: "Since I could not overlook the danger of such a procedure, I determined in the first instance to make a trial on congenital syphilitics with pemphigus, because experience has shown that the internal organs of such cases are saturated with spirochaetes, and that they almost all terminate fatally with or without mercurial treatment. I found first of all that a weakly dying child, suffering from Little's disease, was able to stand an intramuscular injection of 0·03 gramme without harm. This child died fourteen days later, and the post-mortem examination showed neither macroscopic nor microscopic evidence of arsenical poisoning. Cases of pemphigus of the most severe character were then treated, and in all there occurred a remission of the symptoms. Some of them showed rise of temperature and marked anaemia, and in two cases there was a peculiar kind of opisthotonus. While a number

recovered, three died. In the fatal cases there was no post-mortem evidence of arsenical poisoning. On the other hand, it is possible that the destruction of enormous numbers of spirochaetes was followed by the liberation of endotoxines which the enfeebled constitution was unable to withstand. In support of this interpretation is the fact that later cases treated with 0·015 to 0·02 gramme, repeated in eight days, gave more satisfactory results."

The following cases comprise all the fatalities which have been reported among the first forty thousand cases the results of whose treatment have been recorded in the literature. Cases of congenital syphilis are not included; we have, however, included cases of advanced tabes and general paralysis, as well as cases of disease of the circulatory system, in order to emphasise the importance of regarding the presence of such conditions as contra-indications for salvarsan treatment.

Fatalities Associated with Faulty Administration and with Contra-indications

In the first place, those fatal cases must be considered which before treatment presented evidence of the contra-indications of which warning had been given, or which were treated by methods which are now recognised as inadvisable. When the remarkable therapeutic properties of salvarsan became generally known, it was only natural that the victims of syphilis who had heard of the drug, should desire to give it a trial. It is not difficult to understand how patients in the last stage of the disease, or the relatives of patients in the last stages of general paralysis, should appeal to the new remedy as a last resource. Such cases have admittedly been treated, but where accidents have occurred and these are cited as evidence of the toxic effects of salvarsan, it should be remembered in extenuation that when Ehrlich distributed the drug for trial, it was given on the following condition: "Patients are not to be treated who have irritable hearts, valvular disease, arterial degeneration, or aneurysms. Old people, and those with a history of cerebral haemorrhage, are not to be treated." Attention was also called to the danger involved in treating cases of advanced disease of the nervous system. Cases of severe nephritis, diabetes, and gastric ulcer were also excluded. In view of such a definite warning the responsibility for accidents where these contra-indications are present, does not lie with the drug.

Case I.—The first case of death as a probable result of injection is recorded by Spiethoff.[3] This was a young woman, 28 years of age, very anaemic and badly nourished, suffering from tertiary syphilis of the throat. Three years previously she had had a course of atoxyl treatment. She was given an intramuscular injection of 0·5 gramme salvarsan in the form of the monohydrochloride (acid solution). She died on the evening after injection. The post-mortem examination revealed the presence of extensive ulcerative and cicatricial processes in the throat and larynx, cicatrices in the liver, and hypoplasia of the heart and aorta. The internal organs showed no evidence of arsenical poisoning. The patient in this case is supposed by Ehrlich to have died from shock. The mono-acid solution of salvarsan is extremely irritating, and this taken in conjunction with the fact that she was anaemic, poorly nourished, had respiratory disability, and circulatory inefficiency, is probably sufficient to account for the fatal issue.

Case II.—This man was under the care of Professor Anton [4] in Halle. The infection was of five years' standing, and the disease at the time of treatment manifested itself in the form of a moderately large encapsulated area of softening in the left parietal region of the brain. Three and a half hours after the intravenous injection of a strongly concentrated solution (15 c.cm.) of 0·4 gramme of salvarsan in the acid form, he died. There were symptoms of arsenical poisoning in the form of sickness, vomiting and abdominal pains, with a soft rapid pulse. Both Ehrlich and Anton are of opinion that the method of administration in this case probably determined the fatal issue. The drug in the acid form was believed by Ehrlich, Hata, and Alt to be more toxic than in the alkaline form, and this view has been recently confirmed by Hering [5] of Prague (*v.* also Joseph [24]). The post-mortem examination revealed the following condition : " Extensive cortical softening of the left temporal and a part of the left parietal lobes ; internal hydrocephalus ; anaemia and oedema of the brain ; chronic leptomeningitis of the convexity ; hyperaemia and oedema of the lungs ; a flabby atrophic heart with fatty infiltration of the right side ; hypoplasia of the left kidney, enlarged spleen, and hyperaemia of the liver." The wall of the right ventricle of the heart was only 2 mm. thick and was covered and infiltrated with fat. The cardiac muscle was flabby in consistence, greyish-red in appearance, and the cut surface was speckled. Ehrlich draws particular attention to the state of

the cardiac muscle in this case, and suggests that had the patient died subsequent to administration of chloroform, the cardiac changes would have been held responsible.

Case III. (Ehrlich[6]).—A man, 39 years of age, who for about six months prior to treatment presented symptoms of heart disease. Clinical examination showed the area of cardiac dulness to extend abnormally towards the left. There was a diastolic murmur over the aorta. There was no evidence of cardiac failure. The pupils were unequal and did not react. He was given an intravenous injection of 0·5 gramme salvarsan in an alkaline solution. He died five hours after the injection. Before the injection he received a hypodermic injection of scopolamine-morphia. Post-mortem examination revealed the following condition: "Great hypertrophy of both ventricles of the heart, with marked aortic incompetency; syphilitic aortitis, sclerosis of the coronary vessels with narrowing of the lumina at their origin from the aorta; diffuse myocarditis with thinning of the heart wall, and aneurysmal dilatation at the apex." Ehrlich is of opinion that the organic changes in the heart were undoubtedly responsible for the fatal issue in this case.

Case IV. (Ehrlich[7]).—A woman, 26 years of age, who apart from a history of two miscarriages, and a positive blood reaction, showed no evidence of syphilis. She complained of pain in the region of the heart. She was desirous of having healthy children, and was given an intravenous injection of 0·4 gramme salvarsan in an alkaline solution. She died three days after treatment, with evidence of increasing respiratory difficulty. She had, contrary to orders, engaged in heavy garden work immediately after the injection. Post-mortem examination in this case showed the presence of a large thrombus in the vein at the site of injection, and an embolism of the pulmonary artery. There was in addition syphilitic aortitis, sclerosis of the coronary arteries, and degeneration of the cardiac muscle. In this case an unfortunate association of circumstances had combined to produce the fatal result. There was first of all the technical fault in the administration, resulting in the formation of a venous thrombus. Further, had the patient been kept under strict medical supervision, and not been allowed to engage immediately after treatment in heavy manual labour, it is unlikely that pulmonary embolism would have occurred. In addition, the cardiac abnormality possibly contributed to the fatal result.

Case V. (Morata [8]).—A young man suffering from an infection of two years' standing, who had syphilitic placques on the tongue, lips and pharynx, and a diffuse papular exanthem. He had previously been treated with injections of biniodide of mercury and subsequently with injections of 40 per cent. grey oil. He weighed 43 kilos. There was no albuminuria and the heart and lungs appeared to be healthy. For injection 40 centigrammes of salvarsan were first dissolved in serum and then neutralised with 15 per cent. caustic soda, and this was diluted with 200 cubic centimetres of normal saline solution and injected intravenously. Three hours after injection, vomiting, diarrhœa and sweating supervened. The vomited matter was greenish in colour and tinged with blood, the diarrhoea was frequent, the perspiration copious, and there was severe palpitation. He died in a state of coma fifty-one hours after the injection, and during the last forty-six hours he passed no urine and the bladder was empty. Post-mortem details are not given, although it is suggested that there was congestion and swelling of the renal epithelium. No reasons are given why the drug was prepared for injection in the extraordinary manner described.

It is of great practical importance to recognise that in the first four of these cases the post-mortem examination showed an abnormal condition of the circulatory organs. In Case I there was hypoplasia of the heart and aorta, and in addition there must have been embarrassment of the circulatory system due to respiratory inefficiency following the cicatricial narrowing of the larynx. In Case II the heart was atrophied and there was thinning of the right ventricular wall with marked fatty infiltration. In Case III there was great hypertrophy of the heart, with incompetence of the aortic valves. Syphilitic aortitis was present, with sclerosis of the coronary vessels and narrowing of their lumina at the point of origin from the aorta. There was also diffuse myocarditis with thinning of the ventricular wall and aneurysmal bulging at one part. In Case IV there was syphilitic aortitis, sclerosis of the coronary arteries and degeneration of the cardiac muscle.

It is further to be noted that the method of treatment in Cases I, II and V, did not conform with the instructions originally given for administration. In Case I the drug was given in the form of the mono-acid compound; this particular form is extremely irritating and, as has been pointed out already, it is possible that shock contributed to the fatal issue. In Case

II the drug was injected in the acid form in a highly concentrated solution. The acid form is of itself considered to be more toxic than the alkaline form, and when injected intravenously in a concentration of 0·4 gramme in 15 c.cm. water, it is not surprising that in a patient otherwise debilitated a serious result should have followed. In Case V the fatal issue cannot be dissociated from the method of preparation of the salvarsan for injection. In Case IV the sequel was determined primarily by faulty technique, or an extraordinary tendency to thrombus formation in this particular patient. The lack of proper after-treatment was, however, an important and most easily avoidable factor in the process. The general conclusion to be drawn from a review of these fatalities is that they occurred in patients, three of whom presented clinically, and four of whom presented post-mortem, evidence of conditions which have from the commencement been regarded as contra-indicating the injection of salvarsan. In three cases the method of preparation and injection employed is now regarded as inadvisable, and so far as we know, was never recommended by Ehrlich. In the fourth case the actual technique resulted in an accident which may happen with any one, although there was a neglect of the proper after-treatment, which should be carried out rigidly in every case. Thus, there is very little evidence that the drug itself was directly the cause of death in these cases.

Fatalities due to the Toxic Effects of the Drug

In the second place, there remain to be considered those fatal cases in which it would appear that death was due to the toxic effects of the drug alone. We have already seen that many of the synthetic preparations of arsenic possess extremely toxic properties, and it is reasonable to suppose that a drug which is strongly parasiticidal may on some occasions and under certain conditions give rise to toxic effects in the host. Idiosyncrasy is observed in the reaction of certain individuals to almost every drug of therapeutic importance, and it would be strange if salvarsan were an exception. Cases are known where small doses of phenacetin, opium, cocaine, iodoform, or mercury have produced most alarming symptoms. Ehrlich emphasises the fact that the toxicity of the drug is a property related to the constitution and health of the patients under treatment. It might be possible to give chloroform to 50,000 soldiers without causing a death; on the other hand, the mortality rate from

chloroform alone in the ordinary course of events is 1 in 2,050 to 1 in 2,080; in cases of heart disease or lung disease the mortality from chloroform administration may be 1 or 2 per cent., or even higher. These facts should be borne in mind when it is observed that out of forty thousand cases treated with salvarsan, which have been recorded in the literature, we have been able to find only three in which the toxic properties of the drug as such would seem to have conduced to a fatal issue.

Case VI.—This was a case of relapsing fever treated by Iversen.[9] The patient was an alcoholic, was hysterical and had been treated fifteen years previously with mercury. She received 0·3 gramme in a clear alkaline solution into the gluteal muscles. Fourteen hours after the injection, the enormous numbers of spirilla which were previously present in the blood had entirely disappeared. On the fourth day after the injection the temperature rose and she appeared to be suffering from a double broncho-pneumonia. A scarlatiniform rash appeared, which afterwards became haemorrhagic. Acute nephritis and general oedema developed, and she died on the eighth day after injection. Post-mortem examination revealed the presence of advanced arteriosclerosis, myocarditis, and gross macroscopic changes in the internal organs. Iversen is inclined to attribute the fatal complication to an idiosyncrasy to the drug, although he recognises the probably important part which must have been played by a feeble and debilitated constitution.

Case VII. (Ehrlich[10]).—A strong young man, 23 years of age, was injected in September, 1910, and died in November, 1910. He died on the way to hospital, with evidence of intense jaundice and profuse bleeding from the nose. Post-mortem examination showed the presence of a severe interstitial nephritis with fatty degeneration of the liver. No further details of this case have been published so far, and in the absence of these it is futile to speculate on the probable cause of death. The case resembles that described by Iversen, in that acute nephritis played a part. The patient was in all likelihood treated by an intramuscular method, since he was injected at a time when the intravenous method had not yet been widely adopted. Ehrlich suggests the possibility of death having occurred from the absorption of the toxic decomposition products of salvarsan from the injection-depôt.

Case VIII. (Ehlers [11]).—This patient was 40 years of age, and had contracted syphilis eleven years previously. He had not

been efficiently treated. In 1906 he had girdle sensations and pains in the chest and legs. Improvement followed a course of mercurial inunction. In April, 1908, he had an apoplectiform seizure. In August, 1908, he developed other symptoms of general paralysis, and was removed to an asylum. After six months' treatment a remission occurred, and he was allowed to go home. In July, 1910, he had another seizure and from that date the symptoms again progressed. In August, 1910, he received an injection of 0·5 gramme salvarsan in the form of a neutral suspension in the scapular region. He died five days later, with symptoms of intoxication of the central nervous system, tremors, sweatings and gradual loss of strength. Post-mortem examination showed acute parenchymatous degeneration of the internal organs.

Unfortunately, the published records of these three cases (VI, VII, and VIII) are very short. From such details as have been given the diagnosis of arsenical intoxication would seem to be justified, especially in case VI where Iversen himself seems to have had no doubt that he was dealing with a case of idiosyncrasy. Case VIII where the patient was a general paralytic of two years' standing, is perhaps doubtful. Although the course, the acute termination and the post-mortem appearances suggested arsenical poisoning, it must be borne in mind that in general paralysis the general metabolism is far from normal, and these cases in their advanced stages are not suitable subjects for salvarsan treatment. The published details of Case VII are still more scanty. If death were due to arsenical poisoning it could only have been from a decomposition product of salvarsan, and not from the salvarsan itself, seeing that the acute illness supervened a month after treatment. In this case, as in Iversen's, there were acute degenerative changes in the kidneys and liver. The occurrence of jaundice has been observed in a few cases without any serious consequence. In some of these the jaundice appeared a day or two after treatment, in others it did not appear for several weeks (*v.* p. 259).

Diabetes as a Contra-indication

Case IX. (Willige [12]).—A man, 44 years of age, suffering from syphilis and severe diabetes. He received an injection of 0·35 gramme salvarsan in the form of neutral suspension under the scapula. This was followed by symptoms of intoxication, the appearance of acetone in the urine, and a gradually deepening diabetic coma, ending in death.

Post-mortem examination showed the presence of a large flabby heart with parenchymatous degeneration of the muscle fibres. The kidneys were large and flabby, and showed signs of cloudy swelling. The liver also showed signs of parenchymatous degeneration. There was oedema of the brain. In estimating the influence of the injection in determining the issue in this case it must be remembered that a condition of coma might have supervened under any circumstances. On the other hand, the metabolic processes in the body in diabetes are already in an abnormal state, and the introduction of a potent drug like salvarsan might quite well determine the onset of coma. This is the only case of the kind recorded in the literature, and naturally does not in itself afford evidence on which to base a general conclusion. It would be well, however, under the circumstances, to regard severe diabetes as a contra-indication for salvarsan treatment.

Fatalities in Cases with Extensive Nervous and Circulatory Lesions

The following fatal results (Cases X–XIII) occurred in patients where there was heart disease, along with disease of the central nervous system, which was in most instances extensive.

Case X. (Martius [13]).—A man, 48 years old, whose heart appeared to be normal on clinical examination, and who on account of the symptoms of locomotor ataxy was given an intragluteal injection of 0·6 gramme salvarsan in 8 c.cm. olive oil. The injection caused no disturbance, and the girdle sensations from which he suffered completely disappeared. On the sixth day after treatment he died suddenly. Post-mortem examination showed syphilitic aortitis with sclerosis of the aorta and peripheral arteries. There was very advanced sclerosis of the coronary arteries, with dilatation and hypertrophy of the left ventricle. There were islets of cicatricial tissue and of recent myocarditis in the cardiac musculature. Internal hydrocephalus was also present. Extensive necrosis was found at the site of injection.

Case XI. (Spiethoff [14]).—This patient suffered from an indefinite form of gastric crisis. There was aortic incompetency, but no subjective evidence of cardiac disease. The Wassermann reaction was positive. A subcutaneous injection of 0·3 gramme salvarsan was given, and there were no immediate disturbing effects. After a few days, however, symptoms of heart weak-

ness appeared, and these increased, death occurring on the eleventh day after injection. Post-mortem examination revealed the presence of a syphilitic aortitis, with advanced disease of the coronary vessels and degeneration of the cardiac muscle.

Case XII. (Martius [15]).—A man 47 years of age, suffering from locomotor ataxy and syphilis of the aorta, and with a positive Wassermann reaction, received 0·6 gramme salvarsan in the form of an intragluteal injection. After the injection he became gradually weaker. On the fifteenth day after treatment a painful haemorrhagic pemphigoid eruption appeared over the arms and legs; on the twenty-first day he died. Post-mortem examination showed dilatation and hypertrophy of the left ventricle, with moderate dilatation of the aortic arch. There was extensive syphilitic degeneration of the aorta, with evidence of old and recent changes, fatty degeneration of the liver and parenchymatous degeneration of the kidneys. The muscle tissue at the site of injection was necrotic.

Case XIII. (Werther [16]).—A man 60 years of age, who for years had been treated for cerebral syphilis. He was pale and weakly and there was a paresis of one side of the body. Clinically his circulatory system appeared to be normal. He was given an intramuscular injection of 0·4 gramme salvarsan, and he died four days afterwards. Post-mortem examination showed the presence of a large area of softening in the brain, and gummatous endarteritis of the cerebral arteries. There was also a condition of syphilitic aortitis with diffuse degenerative changes in the cardiac musculature.

Fatalities Associated with Intercurrent Diseases

In the following fatal cases (XIV–XVII), which have been recorded, it is difficult to say whether the patients may not have died as the result of an intercurrent malady.

Case XIV. (Martius [17]).—A woman, 56 years of age, who suffered from angina pectoris. Clinically she was supposed to have syphilitic aortitis with diffuse dilatation of the aorta and aortic incompetency. The anginous attacks were severe and occurred very frequently, and as the treatment which had been employed had proved of no avail, she was given an interscapular injection of 0·5 gramme salvarsan in the form of neutral suspension. For a fortnight after the injection the attacks were absent. On the fifteenth day there was a slight attack. On the follow-

ing day she left the hospital, and on the eighteenth day after receiving the injection she died suddenly, apparently during a severe attack of angina. The post-mortem examination showed hypertrophy of the left ventricle, vascularisation of the mitral cusps, extensive syphilitic aortitis, with aortic incompetency and narrowing of the lumina of the coronary arteries at their origin. There was also oedema of the lungs, hydrothorax and hydropericardium.

Case XV. (Martius [18]).—This patient was 41 years of age, and had contracted syphilis thirteen years previously. For some months prior to injection he showed signs of rapidly advancing general paralysis, and at the express wish of his friends received an injection of salvarsan. For the first few days there was an apparent improvement in his condition. Then he developed lobar pneumonia and died. Post-mortem there was found lobar pneumonia. There was also hypertrophy of the heart, and atrophy of the convolutions of the brain.

Case XVI. (Jadassohn [19]).—A man with an aortic aneurysm causing severe symptoms. There was no history of syphilis, but his blood gave a positive Wassermann reaction. His general condition was poor. He was given a subcutaneous injection of 0·4 gramme salvarsan in the form of neutral suspension. During the week following injection there were occasional rises of temperature, and he died on the ninth day after treatment. Post-mortem examination revealed the presence of an aortic aneurysm, infarctions of the spleen and kidneys, and pneumonia of the lower lobe of the left lung.

Case XVII. (Martius [20]).—This man was 44 years of age, and had shown symptoms of general paralysis for four years. He received an injection of 0·5 gramme salvarsan. A few days after injection he suffered from retention of urine and tenesmus. An acute inflammatory condition of bed sores, which had been present before injection, set in, and he died five weeks after receiving the drug. The post-mortem examination showed, in addition to the brain changes, syphilitic aortitis and signs of septic infection in the internal organs.

Fatalities due to Factors Associated with the Administration

The fatal issue in the following two cases cannot be ascribed to salvarsan itself. Death was really due to factors associated with the administration.

Case XVIII. (Martius [21]).—A man, the subject of syphilitic

aortitis, died while the injection was being given. Post-mortem there was found syphilitic aortitis, with an aneurysm of the aorta immediately above the diaphragm. The aneurysm, which had not been diagnosed clinically, had ruptured.

Case XIX. (Martius[22]).—A man, 35 years of age, strong and well-developed, with obvious signs of general paralysis. An injection of salvarsan was given intravenously. Half an hour after the injection he showed signs of cardiac weakness. Ether and caffeine were administered and the symptoms passed off. From the doctor's consulting-room, where he had been treated, he walked to his hotel. Four hours after the injection he died, after increasing signs and symptoms of heart failure. The post-mortem examination showed hypertrophy of the heart, nephritis, cirrhosis of the liver, haemorrhage in the brain and arteriosclerosis.

Cases Moribund at the Time of Treatment

The following three cases are cited in the literature as deaths following the administration of salvarsan. It is quite obvious, however, that when the condition of the patients at the time of treatment is taken into consideration, there is little ground for concluding that death was due to salvarsan. The injection in each case was given as a last resort.

Case XX. (Martius[23]).—A man with very advanced cerebral syphilis. He received an intragluteal injection of 0·6 gramme salvarsan in clear alkaline solution. He died three days later, with signs of cardiac failure. The doctor under whose care he was, points out that he was already in the last stages of the disease when he was treated. He was quite demented, and he was in poor general condition.

Case XXI. (Jadassohn[19]).—A man 40 years old, with severe cerebral syphilis and myocarditis. He was in a very advanced stage of disease, the least exertion producing evidence of cardiac weakness. His condition was obviously hopeless, but his relatives were anxious that salvarsan treatment should be tried. He was given 0·1 gramme. He died on the following day.

Case XXII. (Bloch[25]).—This patient was suffering from very advanced cirrhosis of the liver and degeneration of the cardiac muscle. As a last resort an injection of salvarsan was given, and he died soon afterwards. It is stated that the patient was moribund when the injection was given.

Sepsis following Intramuscular Injection

Among the fatalities there remains to be recorded the case to which reference has already been made on p. 230, the fatal issue in which was undoubtedly due to the method of administration employed.

Case XXIII. (Martius[26]).—A man 47 years old, with commencing general paralysis. He received an intragluteal injection of 0·5 gramme salvarsan in neutral suspension. He died a fortnight after the injection. Post-mortem examination revealed the presence of a large abscess at the site of injection, with septic venous thrombosis and pulmonary embolism.

Summary and Conclusions

We have included in this general review the deaths which have been recorded in the literature, as well as those which were reported privately to Ehrlich, and which have been summarised in a recent contribution from the Frankfort Pathological Institute by Martius.[27] It must be clear that the treatment was responsible only to a very limited extent for the fatal issue in these cases. Even if it be admitted that the injection was the determining factor in some instances, it is still evident that almost every fatal case presented the contra-indications which have been repeatedly emphasised. In some cases the time which elapsed between the administration and the occurrence of death is so long as to give rise to reasonable doubt whether the drug played any part at all in causing death.

In two cases, II and V, the method of preparation and injection might be held responsible for the result. In other two cases, IV and XIX, the after-treatment of the patient undoubtedly contributed to the fatal complication. The proportion of cases among the fatalities which were in a hopeless condition when treatment was undertaken, is also very considerable. We have cited certain of the cases, because they appear in the literature as fatalities following salvarsan, although the authors who record them make it quite clear that the chances of recovery were absent from the beginning. The most striking feature of the whole series is the number of cases in which there was a combination of extensive disease both of the nervous and circulatory systems. The warning which has been given in regard to the treatment of these cases is well founded. Syphilitic aortitis with narrowing of the coronary vessels and myocarditis, would seem to constitute the strongest contra-indication; this would suggest that death in cases of this kind may be due

to the local reaction caused by the drug in the syphilitic vessels. But even of those cases only a small proportion have proved fatal, and these were almost exclusively combined with nervous disease.

Beneficial Effects of Salvarsan in Angina Pectoris

On the other hand, numerous cases of angina pectoris have been recorded in which the administration of salvarsan has been attended with most successful results. It is suggested that where there is no evidence of disease of the coronary arteries and cardiac muscle, the danger is slight, and that in those cases of angina which have been successfully treated, the heart itself was probably sound. Martius [27] cites the following instances, as proving the efficacy of salvarsan in cases of angina pectoris.

Case 1.—A man who had suffered continuously for ten months from attacks of angina, supposed to be due to syphilitic aortitis (Wassermann reaction positive) received an intramuscular injection of 0·5 gramme salvarsan. The attacks, which had been of a most threatening character, ceased.

Case 2.—A man, with syphilitic infection of twenty-five years' standing, had suffered severely from attacks of asthma for three years. He was given an injection of 0·7 gramme salvarsan. The attacks immediately ceased to occur.

Case 3.—A man 50 years old, who had contracted syphilis twenty-five years previously, and had a positive Wassermann reaction, received an injection of 0·6 gramme salvarsan on account of attacks of angina pectoris which had been recurring constantly for a year. The attacks ceased immediately after the injection, and during the following six weeks did not return (case reported at the end of six weeks).

These are only a few instances from the large number of cases of angina which have benefited by salvarsan. While, as we have already pointed out, the combination of syphilitic aortitis, disease of the coronary arteries and myocarditis is to be regarded as an absolute contra-indication, it must be admitted that the exclusion of any one or more of these conditions by clinical examination in a given case is extremely difficult. Although cases of aortic syphilis, where the heart was apparently sound, have benefited from the treatment, the injection of salvarsan must always be regarded as accompanied by a certain amount of danger in such conditions.

We have appended in tabular form the fatalities, and have

included in addition to all the fully recorded cases, those cited by Martius, which were privately reported to Ehrlich. Other reported fatalities may have escaped our notice, and some cases may not have been reported; but when every allowance is made for these possibilities, it appears that the mortality of cases treated with salvarsan is not merely "under 0·2 per cent." (Manuel and Bayly [28]), but it is evident that the deaths constitute only a small fraction of this figure. As these authors say, "a considerable number of fatalities reported on the continent have been described as cases of death 'following the administration of salvarsan,' which would have been more justly recorded as cases of death 'occurring in spite of the administration of salvarsan.'" But even including all the deaths, there is no statistical basis for a mortality at all approaching 0·2 per cent. The manufacturers of the drug, Messrs. Meister, Lucius and Brüning, have estimated that the drug has been administered almost a million times. On the basis of this estimate, the deaths which have followed the administration of salvarsan either as a result or in spite of treatment would require to number somewhere about 2,000, to give a mortality of 0·2 per cent.; whereas a search of the literature has revealed less than fifty deaths! The results of Willige [12] of the Asylum at Halle, who has reported five deaths in thirty-five cases, have been seriously misinterpreted, as the following analysis of his cases, made by himself, shows. To make quite clear the significance of the statement that five deaths occurred in thirty-five cases, Willige writes: "Out of the thirty-five cases which we treated with '606' five have died. One of these died seven weeks subsequent to injection, after a series of epileptiform convulsions" (this was a case of general paralysis). "The second case died from suffocation, as a result of aspirating a large amount of food, which became lodged in the trachea." "The third case was a woman who died from general paralysis." Willige adds: "There was certainly no causal relationship between the treatment and the death in these three cases." The fourth case is the well-known one at Halle, which has been referred to by Anton and Ehrlich [4] and reported by Fraenkel and Grouven [29] and also by Willige [12]; this is the case which we have described as No. II in our review, and in which death was undoubtedly associated with faulty administration of the drug. The fifth case is that of syphilis with severe diabetes in which coma and death ensued a few hours after treatment (No. IX in our series).

TABLE OF FATAL CASES

No. of Case.	Disease.	Treatment.	Period between Treatment and Death.	Post-mortem Examination.
I	Tertiary syphilitic lesion of throat and narrowing of larynx	0·5 gramme salvarsan in mono-acid solution, intramuscularly	A few hours	Tertiary syphilitic ulcers in throat; cicatrices in larynx and liver; narrowing of larynx. Hypoplasia of heart and aorta.
II	Extensive cerebral softening	0·4 gramme salvarsan dissolved in 15 c.cm. water and injected intravenously (strongly acid solution)	3½ hours	Extensive cerebral softening. Atrophy of heart; wall of right ventricle only 2 mm. thick, and infiltrated with fat; fatty layer on the wall of the right ventricle 5 mm. thick.
III	Cardiac disease. Fixed and unequal pupils	0·5 gramme salvarsan injected intravenously in dilute alkaline solution	5 hours	Great hypertrophy of heart; incompetency of aortic valves; syphilitic aortitis; sclerosis of coronary arteries with narrowing of lumina at aorta; diffuse myocarditis; aneurysmal dilatation of heart wall.
IV	History of miscarriages. Positive Wassermann reaction. Pains in cardiac region	0·4 gramme salvarsan intravenously in dilute alkaline solution. (Hard manual labour immediately afterwards.)	3 days	Thrombus attached to wall of vein at site of injection. Embolism of pulmonary artery. Syphilitic aortitis; sclerosis of coronary arteries; degeneration of cardiac muscle.
V	Syphilitic infection of two years' standing	0·4 gramme salvarsan dissolved in serum, neutralised with NaOH and diluted with 200 c.c. normal saline solution, injected intravenously	2 days	No details given. It is, however, suggested that there was congestion and swelling of the renal epithelium.

TABLE OF FATAL CASES (*continued*)

No. of Case.	Disease.	Treatment.	Period between Treatment and Death.	Post-mortem Examination.
VI	Relapsing fever in an hysterical alcoholic	0·3 gramme salvarsan injected intramuscularly in alkaline solution	8 days	Haemorrhagic exanthem. Acute nephritis. Arteriosclerosis and myocarditis.
VII	Syphilis	No details. Salvarsan probably injected subcutaneously or intramuscularly	1 month	Acute nephritis. Intense jaundice. Fatty degeneration of liver.
VIII	General paralysis	0·5 gramme salvarsan in a neutral suspension, injected subcutaneously	5 days	Acute parenchymatous degeneration of the internal organs.
IX	Syphilis and diabetes	0·35 gramme salvarsan in a neutral suspension, subcutaneously	A few hours	Large flabby heart with parenchymatous degeneration of muscle fibres. Cloudy swelling of kidneys. Oedema of brain. (Died in a state of diabetic coma.)
X	Locomotor ataxy. No clinical signs of heart disease	0·6 gramme salvarsan in 8 c.cm. olive oil, intramuscularly	6 days	Syphilitic aortitis; sclerosis of coronary arteries; hypertrophy of left ventricle; old and recent myocarditis. Necrosis at site of injection.
XI	Gastric crises. Aortic incompetency. Positive Wassermann reaction	0·3 gramme salvarsan, subcutaneously	11 days	Syphilitic aortitis; disease of the coronary arteries; degeneration of the cardiac muscle.
XII	Locomotor ataxy. Aortitis. Positive Wassermann reaction	0·6 gramme salvarsan into the gluteal muscles	21 days	Dilatation and hypertrophy of the left ventricle; extensive syphilitic degeneration of the aorta. Fatty degeneration of the liver and parenchymatous degeneration of the kidneys.

TABLE OF FATAL CASES (*continued*)

No. of Case.	Disease.	Treatment.	Period between Treatment and Death.	Post-mortem Examination.
XIII	Cerebral syphilis of long standing	0·4 gramme salvarsan intramuscularly	4 days	Large area of cerebral softening: extensive gummatous endarteritis of cerebral vessels. Syphilitic aortitis and diffuse degenerative changes in the heart muscle.
XIV	Syphilitic aortitis with attacks of angina pectoris	0·5 gramme salvarsan in a neutral suspension, injected subcutaneously	18 days	Hypertrophy of the left ventricle; syphilitic aortitis with aortic incompetency and narrowing of the lumina of the coronary arteries at their origin. Oedema of lungs.
XV	General paralysis	Salvarsan (no details)	A few days	Lobar pneumonia. Hypertrophy of heart. Atrophy of brain convolutions.
XVI	Aortic aneurysm. Positive Wassermann reaction	0·4 gramme salvarsan in a neutral suspension, subcutaneously	9 days	Aortic aneurysm. Infarctions of the spleen and kidneys. Lobar pneumonia.
XVII	General paralysis. Bed sores	0·5 gramme salvarsan (no details)	5 weeks	Usual brain changes of general paralysis. Syphilitic aortitis. Evidence of septic infection in the parenchymatous organs (an acute inflammation had set in on the site of the bed sores).
XVIII	Syphilitic aortitis. Aortic aneurysm (not diagnosed clinically)	Salvarsan (no details)	Died during injection	Syphilitic aortitis; ruptured aneurysm of aorta immediately above the diaphragm.
XIX	General paralysis	Salvarsan intravenously. (Half an hour after injection patient felt faint; recovered and walked to hotel; died in 4 hours)	4 hours	Hypertrophied heart. Nephritis. Cirrhosis of liver. Haemorrhage in brain. Arteriosclerosis.

TABLE OF FATAL CASES (*continued*)

No. of Case.	Disease.	Treatment.	Period between Treatment and Death.	Post-mortem Examination.
XX	Advanced cerebral syphilis	0·6 gramme salvarsan in clear alkaline solution (intragluteally)	3 days	No details. (Treatment given as last resort.)
XXI	Advanced cerebral syphilis. Myocarditis	0·1 gramme salvarsan	1 day	No details. (Treatment given as last resort.)
XXII	Advanced cirrhosis of the liver. Myocarditis	Salvarsan (no details)	A few hours	No details. (Patient in moribund condition when treated.)
XXIII	General paralysis	0·5 gramme salvarsan in a neutral suspension, injected into the gluteal muscles	14 days	Extensive necrosis at site of injection. Septic infection of necrotic mass and thrombosis of iliac veins. Pulmonary embolism.

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ADDENDUM

Acute Haemorrhagic Encephalitis following Salvarsan Treatment

While the preceding chapter was in the press three fatalities following the administration of salvarsan have been recorded *Münch. med. Woch.*, No. 34, 1911). All three were cases of syphilis within a year after infection and in each the acute symptoms which ushered in the fatal issue began on the third day after an intravenous injection of the usual dose. In two of the cases (Fischer, Kannengiesser) the illness followed the second intravenous injection administered at an interval of six weeks and two weeks respectively after the first injection ; in the third (Almkvist) the illness followed the first injection (cases 2 and 3 had been treated with mercury also). In every instance the period immediately after injection was free from untoward symptoms of any kind. The acute illness began with cerebral symptoms, including convulsions and loss of consciousness. The first two cases died on the second day and the third on the third day of illness. In all three the post-mortem examination showed the presence of *acute haemorrhagic encephalitis*. There was no evidence of any bacterial infection and the microscopic appearances did not suggest a syphilitic lesion. The parenchymatous organs showed protoplasmic degeneration, but the appearances did not resemble those of acute arsenical poisoning. The course of these cases makes it probable that the condition was not due directly to the toxic action of salvarsan, but that the encephalitis resulted from the effect of the drug on a brain which had been rendered specially susceptible by the syphilitic infection. This explanation is supported by the fact that a similar condition has been observed by Plötzl and Schüller (*Zeitschr. f. d. ges. Neurol. u. Psychiat.*, Bd. III., 1910, p. 139) after mercurial treatment. These authors ascribed the haemorrhagic encephalitis in their case to hypersensitiveness to mercury. The occurrence of similar lesions following both salvarsan and mercury suggests that the syphilitic infection is an essential factor in the production of this rare condition.

CHAPTER VII

GENERAL CONCLUSIONS ON THE TREATMENT OF SYPHILIS

THE EFFICIENCY OF SALVARSAN AS AN ANTISYPHILITIC REMEDY, COMPARISON WITH ARSENOPHENYLGLYCIN—REQUIREMENTS FOR EFFICIENT TREATMENT AS INDICATED BY THE NATURE OF SYPHILITIC INFECTION—PROCEDURE IN THE TREATMENT OF SYPHILIS, COMBINED THERAPY CONTROLLED BY THE SERUM REACTION

The Efficiency of Salvarsan as an Antisyphilitic Remedy

THERE can be no doubt as to the powerful effect of salvarsan in dissipating the signs and symptoms of syphilis in its various manifestations. In this respect it is greatly superior to all other anti-syphilitic remedies. Mercury and its compounds, also atoxyl, arsenophenylglycin and other organic preparations of arsenic, possess strong antisyphilitic properties and in a proportion of cases apparently produce cure. In isolated instances even a comparatively short course of mercury has been known to effect complete disappearance of symptoms and apparently permanent cure. Thus Wechselmann [1] records two cases which received each a single course of mercurial inunction; these patients were examined after eighteen and twenty-four years respectively and in each case the blood gave a negative reaction; there had been no evidence of syphilitic disease in the intervening period. Such cases are, however, comparatively rare, and prolonged treatment with mercury, even when early begun, frequently fails to prevent the onset of secondary symptoms. With salvarsan, however, the majority of cases treated in the primary stage with one or two intravenous injections, have shown no further signs of the disease. Thus in twenty-two cases which we treated in the primary stage of the disease, there has

not been any appearance of fresh symptoms since the injections were given. Ten months have elapsed since five of these were treated, and six months have elapsed since twelve of them were treated. As regards the immediate action of salvarsan, the various acute manifestations disappear with astonishing rapidity; severe sore throat, ulcerations of other mucous membranes, eye affections, condylomata and exanthemata yield to salvarsan treatment in a week or ten days. Acute tertiary phenomena, disease of the bones and gummata are treated with equal success. These conditions, of course, respond to other antisyphilitic remedies, but the opinion as to the great superiority of salvarsan is practically unanimous. As a matter of fact there are no symptoms which yield to mercury which are not removed more rapidly and more effectively by salvarsan. It is also a matter of general experience that numerous cases refractory to mercury administered for months and even years, have been treated with salvarsan with conspicuous success.

The dangers incident to salvarsan treatment are not greater than those attending the administration of mercury, antipyrin or cocaine, and not nearly so great as those associated with the administration of general anaesthetics.

We have purposely omitted reference to the adverse criticisms of those who cite the fatalities as evidence of the dangers of the drug. The fatalities have already been discussed in detail, and it is evident that if attention is paid to the contra-indications and reasonable care is exercised in the preparation and injection of the drug, the treatment involves very little element of danger.

By some critics great emphasis is laid on a distinction which they draw between "healing the symptoms" and "curing the disease." It is implied that whereas the symptoms may be made to disappear by the injection of salvarsan, a different process, culminating in cure, is produced by the administration of mercury. It has never been claimed either by Ehrlich or his collaborators that a single dose of salvarsan, no matter what the manner of administration may be, can cure any case of syphilis by complete extermination of the spirochaetes. In the preparation of drugs for the treatment of spirillosis and trypanosomiasis, Ehrlich has aimed at the production of substances which, when administered in experimental infections, should lead to a complete sterilisation of the host. This has been

accomplished. It was naturally hoped that in the treatment of syphilis complete sterilisation could be effected in a large proportion of cases. There is good reason for believing that this hope has been justified, but to maintain that properties have been attributed to salvarsan which could not have been tested, is merely to impute claims and assertions in order to refute them. Obviously years must elapse before a definite judgment can be pronounced on the ultimate effect of salvarsan treatment. The extensive experience, however, which has been gained during the past two years admits of tentative conclusions of the highest importance. Thus it is clear that salvarsan acts as a spirillicidal agent, just as does mercury, atoxyl or arsenophenylglycin. The spirillicidal action of mercury has been conclusively demonstrated in syphilitic infections in apes and rabbits. The spirillicidal action of salvarsan has been proved in similar conditions. In experimental spirillosis of fowls and in infections with the spirilla of relapsing fever in animals, rapid and complete sterilisation is produced by an injection of salvarsan. Iversen has shown that complete sterilisation is probably effected by salvarsan in relapsing fever in the human subject.

That *arsenophenylglycin* is apparently capable of producing complete sterilisation in syphilis is shown by its effect in three cases cited by Ehrlich.[2] These were treated with arsenophenylglycin by Herxheimer in 1907. The first case was that of a child with hereditary syphilis which received 0·06, 0·07 and 0·05 gramme of the drug at intervals between August 13 and October 17, 1907. During the following two and a half years there were no symptoms, and the Wassermann reaction at the end of that period was found to be negative. The second case was that of a man with secondary symptoms of a malignant type; there was a papular eruption of the skin, and severe ulceration in the mouth and nose. Between September 6 and 26, 1907, he received at intervals 0·3, 0·4, 0·5, and 0·6 gramme of arsenophenylglycin. There was no further treatment. On October 12, 1909, he was found to be in good health, and no symptoms of syphilis had been present in the interval. His blood gave a negative reaction. The third case was that of a man who also was suffering from malignant secondary symptoms. Between September 17 and October 19, 1907, he received at intervals 0·3, 0·4, and 0·5 gramme of arsenophenylglycin. On October 12, 1909, he was in good health, and his blood gave a negative reaction;

since the treatment there had been no evidence of disease. Although a period of two years is perhaps too short to admit of definite conclusions as to the ultimate success of the treatment in these cases, still, in the absence of recrudescences and with negatively reacting blood sera, the presumption is that a cure has been effected in all three. The potency and permanent result of treatment with arsenophenylglycin are further illustrated by the researches of Alt,[3] who found that in a proportion of cases of general paralysis a positive blood reaction could be converted into a negative one as a result of treatment, and that the blood remained negative in some of these over a period of one and a half years.

When it is remembered that arsenophenylglycin stands in close chemical relationship to salvarsan, and that experimentally salvarsan has been proved to be less toxic for the host and more lethal to the parasite, one would expect that salvarsan should exhibit clinically the curative properties of arsenophenylglycin, but in a higher degree. As a matter of fact, it has been found that the injection of salvarsan does convert a positively reacting serum into a negatively reacting one, and that in a large proportion of cases the blood reaction remains negative. The blood from some of our cases was examined nine months after injection and was found to be negative.

Further evidence of the influence of salvarsan is to be found in the fact that soon after treatment spirochaetes can no longer be found in the syphilitic lesions. Reference has been made to Sieskind's observations on this subject. In one case in which spirochaetes were present in abundance, they could not be found twenty-four hours after injection. Dr. Haswell Wilson recently examined the serum from the base of a chancre in a man; numerous spirochaetes were present prior to treatment, but twenty-four hours after intravenous injection of 0·5 gramme salvarsan none was found.

Probably the most convincing evidence in support of the view that salvarsan produces complete sterilisation is the occurrence of cases of *reinfection with syphilis*. Several cases of reinfection subsequent to treatment with salvarsan have been recorded, whereas prior to the introduction of the drug this was looked upon as one of the rarities of medicine. Three instances of such reinfection have been reported from a single clinique (Schreiber[4]). The significance of reinfection is important; reference has already been made to the fact that the supposed immunity to

reinfection possessed by syphilitics is really to be attributed to the presence of a latent infection. Real immunity to protozoal diseases is short-lived, and when infection with syphilis is contracted in the usual fashion after treatment with salvarsan, there is a very strong presumption that the salvarsan had in the first instance effected complete sterilisation of the patient's tissues.

Requirements for Efficient Treatment as indicated by the Nature of Syphilitic Infection

Relapsing fever is essentially a septicaemia, and the parasites are present in enormous numbers in the blood during the height of a paroxysm. The organisms of syphilis, on the other hand, cannot as a rule be found in the blood on microscopic examination. Their presence has been noted in very few instances, and only after considerable search ; the majority of observers have not been able to find them in the blood at any stage of the disease, although, of course, small numbers are present, as shown by positive results of inoculation experiments. Histological examination has proved that the spirochaete pallida has a predilection for firm connective tissue structures. In the early stage of infection it is to be found in the dense base of the chancre, and in the connective tissue of the thickened vessels in the neighbourhood. The infection spreads from the initial site of inoculation mainly by the lymphatic system along the lymph spaces surrounding muscles, nerves and blood vessels (Ehrmann[5]). Examination of the tissues in congenital syphilis by the silver impregnation method of Levaditi shows the great tendency for the organisms to settle in the connective tissues and elastic coatings of vessels and in nerve sheaths. Further, the spirochaetes are disposed in isolated groups in many instances. This is best seen in infants several months old, in whose organs spirochaetes are not so abundant as in aborted foetuses, which show a very wide and general distribution of the organisms. Thus, six portions of the liver of a syphilitic child which had lived three months, were examined in sections without any spirochaetes having been seen. In sections from a seventh block of this liver large numbers of spirochaetes were present, but they were in isolated groups. It is obvious that the parasites which have settled in dense connective tissue will be protected from the action of spirillicidal substances in the blood.

These facts are of importance in interpreting the phenomena which characterise the course of syphilitic infection in the

secondary stage when modified by treatment insufficient to produce complete sterilisation. In the primary stage of the disease, as we have seen, the organisms are at first confined to the site of inoculation and the neighbouring lymph spaces and small vessels which have become thrombosed. The onset of fever and sore throat with the appearance of a generalised rash is evidence of the previous dissemination of the parasites throughout the body. The administration of anti-syphilitic agents, if not sufficient to produce complete sterilisation of the host, will leave isolated groups of organisms in tissues where the blood supply is deficient. Thus, spirochaetes will tend to persist in dense connective tissue and in areas where there is thrombosis. Although there is temporary disappearance of all symptoms, these isolated organisms may produce pathogenic effects at a later period. We have seen evidence of this in the occurrence of recrudescences affecting the cranial nerves after salvarsan treatment, more especially in those cases treated with one injection of a neutral suspension of the drug. It is a well known fact also, that after apparently successful treatment the tissues at the site of the healed chancre may again become indurated and break down, and where multiple chancres were present, they may reappear at the former sites. Further, Wechselmann, Katzenstein and Guszmann[6] have shown that spirochaetes may be demonstrated at the site of healed lesions of the skin, tonsils and genitals, months and even years after the disappearance of all symptoms of syphilis. Hoffmann (cited by Wechselmann) has shown by inoculation experiments that the organisms, from a latent infection, are still capable of exhibiting virulence by causing typical lesions when introduced into monkeys. The vascularisation of small thrombosed vessels and, where a recrudescence occurs, the increased blood and lymph supply occasioned by the inflammatory reaction at the site of the lesions, probably render the spirochaetes in the related areas more accessible to the specific drug. Complete cure of the host may thus ultimately be produced by fractional sterilisation as the result of prolonged treatment, since it is a matter of common experience that recrudescences occurring during the course of mercurial treatment tend to appear in more and more isolated situations as time goes on.

Successful treatment is prejudiced not only by the peculiar anatomical distribution of the spirochaetes, but also by the length of time which elapses between inoculation and the

administration of the specific remedy. These two adverse factors, however, cannot be considered separately, because delayed treatment permits the development of prejudicial anatomical conditions. Over and above this it is important to remember that in certain circumstances, and chief among these is chronicity of infection, the host and the parasite become mutually adapted, so that the administration of spirillicidal agents produces only a slight effect as compared with that which follows treatment in the early stage of acquired infection. This consideration applies more particularly to cases of congenital syphilis and of so-called parasyphilitic disease. In most of these conditions mercury is of little or no value, and the effect of salvarsan is only slight when compared with the striking results obtained in the earlier stages of acquired syphilis. For example, the interstitial keratitis of congenital syphilis is refractory to mercurial treatment ; with salvarsan, good results have been obtained in some cases, but in others the lesions have extended in spite of treatment ; on the other hand, the inflammatory diseases of the eye in acquired syphilis yield readily to salvarsan, and in the case of experimental syphilitic keratitis in the rabbit, the response to salvarsan injection is immediate and complete. In the same way, general paralysis, while undoubtedly combated and arrested, temporarily at least, in a number of cases, cannot be said to respond to salvarsan in the striking fashion which has been observed in cases of early cerebral endarteritis and meningitis of syphilitic origin.

The significance of these facts from the point of view of the efficient treatment of syphilis may be summed up thus : (1) Treatment must be as energetic as possible. The disappearance of gross lesions is generally effected when the majority of the spirochaetes have been killed off, and therefore such clinical " cure " must not be taken as the index of efficient treatment. Spirochaetes tend to settle and persist in dense connective tissue structures, where they are comparatively inaccessible to spirillicidal agencies. It is on the extermination of these residual parasites that the ultimate success of treatment depends. The destruction of these residual spirochaetes is most likely to be effected when the treatment is energetic. Since it is impossible, however, to determine whether complete destruction has occurred until proof is afforded by continued absence of all phenomena of the disease, such destruction must be ensured, as far as possible, by the employment of energetic treatment at the beginning.

(2) The importance of commencing treatment as soon as possible after infection and of effecting rapid sterilisation is further enhanced by the fact that the persistence of living spirochaetes in the tissues renders the conditions for effecting complete sterilisation more and more unfavourable, and perhaps impossible in many cases, in the late stages of the disease.

Procedure in the Treatment of Syphilis—Combined Therapy controlled by the Serum Reaction

The observations detailed and discussed in the preceding chapters have been made with a view to determining the efficacy of salvarsan as an antisyphilitic remedy. As a result of this work, important facts regarding the nature of syphilitic infection have been brought to light. Thus the predilection of the spirochaetes to affect certain sites, as well as the chronicity and the latency of syphilitic infections, have been strikingly demonstrated. It is probable that in the natural course of events, if the syphilitic infection were uninfluenced by any form of treatment, a chronic type of the disease would result in every case, with, perhaps, prolonged latent periods intervening between the various manifestations. The phenomena of the disease even in the absence of treatment may be very variable. As an example of the unexpected and anomalous course which an untreated infection may follow, we cite the case of acute syphilitic iritis in a man who, six months previously, had a chancre; the primary sore disappeared without treatment, and there were no subsequent symptoms until the appearance of the iritis. The course of a syphilitic infection is, however, obviously influenced to a marked degree by treatment, but it is impossible to say prospectively, whether in any particular case the abolition of symptoms denotes complete sterilisation and hence cure, or merely the reduction of the infection to a latent state, with the possibility of subsequent relapse. In the majority of cases as ordinarily treated with mercury, it is highly probable that the infection is rendered merely latent, or at most a very gradual "fractional" sterilisation is brought about in some cases after years of treatment. Rapid and complete cure must be the aim of any form of treatment which is to be of permanent value, and this is much more difficult to attain than the production of a latent state. It has been shown that salvarsan possesses great advantages over mercury: (1) salvarsan acts with great rapidity and a single dose is at least as efficient as a prolonged course of mercury, and (2) salvarsan is extremely

active in cases which resist mercury, or which are susceptible to the toxic effects of mercury. At the same time, years will have to elapse before it can be demonstrated in what proportion those cases which have received one or two injections of salvarsan and no other treatment, have been completely cured. This proof will be afforded only when tertiary and parasyphilitic manifestations fail to occur, and when the serum reaction remains permanently negative.

It will be obvious from the foregoing considerations, that treatment should be begun immediately the condition is diagnosed. It is all the more satisfactory if the disease is recognised before the blood gives a positive reaction. In a suspicious case films of the serum expressed from the base of the sore should be examined for spirochaetes. Even if it is impossible to find spirochaetes, and the blood reaction is negative, the presence of a sore suggesting primary syphilis where there is the history of exposure to infection, should be taken as an indication for immediate treatment.

Excision should be resorted to, especially when the chancre is extensive and has a large indurated base. It is, of course, known that this does not prevent the spread of the disease, but it removes tissue in which the organisms have a particular tendency to persist in a latent condition after symptoms have disappeared.

The ease with which cure can be effected varies in different instances. There are probably certain cases in which the individuality of the patient or the advanced stage of the infection makes it impossible by any means to produce sterilisation. Where the treatment is begun in the primary stage, or early in the secondary stage, two intravenous injections of salvarsan should be given at an interval of three weeks, with a course of mercurial inunction following each injection.* In more chronic cases, and especially where the disease has been refractory, three or even four injections may be advisable, with a corresponding number of courses of mercurial inunction. The serum test should be carried out three or four months after treatment has ceased, and should be repeated at intervals of not more than six months. It is not

* The principle of *combined therapy* has been established by Ehrlich (*Berlin klin. Woch.*, 1907, Nos. 9–12), who showed in the case of trypanosome infections, that therapeutic agents belonging to different chemical groups attack the parasites at different points. In this way one drug reinforces the action of another of a different type.

impossible that further experience may lead to the adoption of even more energetic methods than those which we have suggested, but it is certain that these methods represent a very great advance on all preceding forms of treatment.

It is important to impress on the patient the importance of leading a regular hygienic life, especially during the period when treatment is being carried out. Alcohol and tobacco should be forbidden.

In no case can absolute assurance be given that a cure has been effected. If the patient is treated during the primary stage and before the blood gives a positive reaction, then the continued absence of further symptoms and a persistently negative serum reaction point almost conclusively to a complete cure. It must not be supposed, however, that when once the blood becomes negative in its reaction a cure has necessarily taken place. As has been shown already, the reaction must remain negative. Even where it may be assumed that treatment has been successful, if marriage is contemplated the period of a year should be allowed to elapse from the time of ceasing treatment, and at the end of that time the blood should be examined again. If then the reaction is still negative and if in the meantime there have been no symptoms of syphilis, the presumption is that the treatment has been successful. In any case, the patient can receive a more confident assurance than would be justifiable after two years of mercurial treatment.

As the serum reaction is of such vital importance in the diagnosis, treatment and prognosis of syphilis, it cannot be too strongly urged that the so-called simplifications of the original test are inadvisable. The greatest accuracy can only be attained by adopting the more delicate methods and in our experience there is no procedure which is so reliable as that in which lecithin and lecithin-cholesterin emulsions are employed.

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[2] Ehrlich, *Münch. med. Wochenschr.*, 1911, p. 1.
[3] Alt, *Münch. med. Wochenschr.*, 1909, No. 29.
[4] Schreiber, *Münch. med. Wochensch.*, 1911, p. 893.
[5] Ehrmann, *Archiv. für Dermatol. und Syphilis*, vols. 68 and 81.
[6] Wechselmann, *Behandlung der Syphilis*, Berlin, 1911, p. 130.

APPENDIX TO PART II

The Composition of Salvarsan

The suggestion of Lesser [1] that "salvarsan" as placed on the market differs in constitution from the specimens of the drug previously supplied for the purpose of trial (labelled "Ideal" and "Hyperideal," the latter being slightly less toxic for mice) is denied absolutely by Ehrlich [2] (*v.* also Benario [3]). Lesser's conclusion was based partly on the results of his own observations on the solubility and the therapeutic action of the specimens and partly on the results of blood-pressure experiments in rabbits injected with acid solutions (Hoke and Rihl [4]). As Ehrlich points out, Lesser's method of administering the drug (intragluteal injections of 0·1 gram suspended in almond oil, repeated weekly) is unsatisfactory. With regard to experimental results, Joseph [5] has shewn that the drug is much more toxic in acid than in alkaline solution, and when concentrated than when more dilute. Ehrlich points out further that differences in toxicity may be due to the use of solutions which have been allowed to stand for some time after being made up—oxydation leading to increased toxicity. Salvarsan as obtained commercially has a toxicity for mice corresponding with "Hyperideal" (tolerated dose = 0·25 gram per kilogram). The observations of Schreiber,[6] as well as our own clinical results lend no support to Lesser's contention that commercial salvarsan is at all inferior in therapeutic action to the original specimens of the drug.

REFERENCES.

[1] Lesser, *Berlin. klin. Woch.*, 1911, p. 1025.
[2] Ehrlich, *Berlin. klin. Woch.*, 1911, p. 1090.
[3] Benario, *Verh. d.* 28 *Kongr. f. inn. Med.*, Wiesbaden, 1911.
[4] Hoke and Rihl, *ibid.*
[5] Joseph, *Journ. of Exper. Med.*, Vol. XIV, 1911, p. 83.
[6] Schreiber, *Verh. d.* 28 *Kongr. f. inn. Med.*, Wiesbaden, 1911.

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