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## THE COMPLEMENT-FIXATION TEST IN SYPHILIS.

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It is axiomatic that the clinical diagnosis of a disease can only be regarded as satisfactory in the statistical sense when certain conditions are strictly fulfilled as to the constancy of the signs and symptoms in the condition and their uniform absence in other diseases. In the case of the infections it is becoming more and more widely recognised that it is necessary always to be on the look-out for atypical manifestations—that is to say, one must not be satisfied with any given symptom-complex as a diagnostic feature. It is true that many infections produce remarkably characteristic pictures, and the skin-eruptions of syphilis, smallpox or measles at once suggest themselves as striking examples. The degree of diagnostic acumen which enabled typical cases of such diseases to be accurately differentiated was attained long ago by competent observers. It is certain, however, that the recognition of typical cases touches only the fringe of the question of infective disease, especially in connection with prevention. The essential problem which demands solution is—what relationship exists between the number of the typical cases and of those which cannot be recognised with certainty by ordinary clinical methods. Where the proportion of clinically dubious manifestations is considerable the result is failure to deal effectively with the condition by administrative methods, such as isolation. This is



well illustrated in the case of scarlet fever, the incidence of which can scarcely be said to have been influenced to a significant degree by the segregation of those recognisably affected with the disease. Considerations such as these apply with special force to syphilis; here practically every case passes through periods of clinical latency, while, in addition, the proportion of males in which the external manifestations are throughout dubious or suppressed is probably much greater than is generally credited. As regards women, the fact that the production of syphilitic foetuses may be the sole gross evidence of the infection is almost universally admitted. There is no doubt also that, especially in the earlier years after the disease has been acquired, absence of obvious signs does not denote lack of power to infect, hence in the interest of others, as well as with a view to preventing later lesions in the patients themselves, it is important to detect latent and atypical cases.

Any test which aids in the fulfilment of this object must prove of great value. The Wassermann reaction has steadily acquired a reputation as the most constant and reliable manifestation of syphilis. This conclusion rests on the generally concordant results of a large number of workers in different countries. Further, the confidence reposed in the test has survived that period of enthusiasm which is apt to follow immediately after the introduction of any novelty which merely adds to previous experience without seeking to subvert cherished beliefs. But both the method of the test and the significance of the results derived from it have been the subject of adverse criticism, which was sufficiently serious to warrant a further judicial examination of the subject. In view of the extreme importance of venereal diseases as undermining the health of the nation, and especially since conditions of war give great impetus to the dissemination of these affections, it is a matter of congratulation that the problem should have been reviewed anew by a responsible official body with a scientific status, such as the Medical Research Committee possesses. Both the method of the test and its diagnostic value have been dealt with.

#### THE METHOD OF THE TEST.

The general explanation that the reaction depends upon fixation of hæmolytic complement by a mixture of patient's serum and a watery



emulsion of alcoholic tissue extract is easily comprehended. The mass of detail which must be added to this statement, however, in order to describe adequately a reliable method of performing the test, is not likely to prove attractive to one who desires merely to exploit the results. It will be most unfortunate, however, if those who perform the test and those who utilise the data clinically are confined in watertight compartments. The significance of the essential minutiae cannot be appreciated fully except by repeatedly working through the details in the laboratory; thus, on the one hand, ignorance of the fallacies may lead to blind reliance on the results obtained by an incompetent worker; or, on the other hand, it is equally likely that an unreasoning prejudice may arise against the test owing to ignorance of the inherent limitations which beset it. The diagnostic value of the reaction, of course, rests upon the correspondence of the laboratory findings with the clinical circumstances in those instances where the latter are unequivocal. To effect this correlation demands the most intimate and intelligent collaboration on the part of the clinical and the laboratory worker. It is specially important that all apparent discrepancies between the findings on the two sides should be thoroughly investigated, for it may be said at once that the method of the Wassermann reaction is far from fool-proof; in fact, the test is beset with difficulties. As the Medical Research Committee's Report says, "the performance of the Wassermann test involves one of the most complicated methods that have been applied to diagnosis in medicine." But the means to avoid the most important sources of error by suitable controls are available and are well defined. The principal aim of those who are concerned with improving the test as a diagnostic method is to obtain a maximum of positive results in cases of undoubted syphilis, while at the same time recording only negative findings where syphilis can be definitely excluded. It is out of the question to expect 100 per cent. of results free from dubiety. This is clear when one considers what is known as to the nature of the change in the serum which causes it to react positively. In the first place, the difference between a negative and a positive serum appears to be quantitative and not qualitative. Thus, when a large number of syphilitic sera are examined by a method which shows the actual degree of complement-fixation, it is found that there is no sharp line of demarcation between these and non-syphilitic sera



(it may be remarked that every observer should verify this important fact for himself). This result implies that in addition to frankly negative and frankly positive sera there will always be some which exhibit a "dubious," "suspicious," or "weak positive" behaviour. The number of such intermediate reactions is not likely ever to be negligible (in one of our hospital series, comprising 1067 cases, there were 5·3 per cent. of suspicious reactions), and the results of any worker who fails to obtain a proportion of equivocal reactions may be regarded with suspicion. It must be remembered also that the serum of every case of syphilis will at some period yield a transition reaction; this will be referred to again (p. 130). A practical point in this connection is that several essential constituents employed for the reaction are highly variable, especially the guinea-pig's serum (complement). When parallel tests are made with a given syphilitic serum, in which the only difference consists in the use of different specimens of complement, it is found that the degree of positiveness may vary considerably. Such differences in deviability shown by complement in the Wassermann test were originally pointed out by Browning and Mackenzie and are now generally accepted. Browning and Kennaway have again recently examined this point in detail; they employed the same positive serum and the same negative control in repeated examinations, and obtained results which indicate that it is practically impossible to attach any significance to minor variations in one day's tests as compared with those on another occasion. Therefore the worker who revels in many degrees of positiveness, *e.g.* ranging from *four plus downwards*, tends to impress on those ignorant of the actual details of the test a fictitious sense of gradation and reliability. The practical consequence of this variation is that a weak positive reaction obtained when a patient's serum is tested with a moderately deviable complement will be recorded as negative when a little-deviable complement is employed. Hence it will be to a considerable extent a matter of fortuity whether a border-line positive is returned as positive or negative in a single test.

A further factor of importance is the syphilitic "antigen." Since Browning, Cruickshank and Mackenzie showed that the addition of cholesterin to the alcoholic solution of the ether-soluble, acetone-insoluble fraction of alcoholic tissue extracts (impure "lecithin") greatly enhances the antigenic property, this method of "fortifying"



alcoholic extracts for use in the Wassermann reaction has been very widely adopted. Fildes and McIntosh have used an alcoholic extract of human heart-muscle along with cholesterin and this combination is now very popular, since in the case of the majority of syphilitic sera the lipoids of heart-muscle elicit a greater degree of complement-fixation than do those of other organs (see Browning, Cruickshank and Gilmour). But this is not an invariable rule. Browning and Kennaway recently compared the results when a number of sera (360) were tested in parallel with liver lipoid plus cholesterin and with heart lipoid plus cholesterin. It was found that in about 5 per cent. of cases the heart-extract gave a significantly greater degree of complement-fixation, while in other 5 per cent. the liver lipoid was the superior. On the whole, the heart-cholesterin mixture is the simplest to use where the test is to be performed with a single antigen, but our results supply a cogent reason for employing an antigen of liver lipoid plus cholesterin in addition. The use of two antigens is especially indicated where there is suspicion on clinical grounds that the case is syphilitic, but the Wassermann reaction carried out in the first place with one antigen has given a negative result. Several striking instances of this are seen among the cases of primary syphilis in the Medical Research Committee's Report; thus one serum (Case 7) examined by Browning and Kennaway with a frank positive result was reported by Harrison as "practically negative," while another case (No. 66) was found positive by both McIntosh and Harrison, but negative by Browning and Kennaway.

It may be gathered from the above references that we are still far from possessing a standard method of performing the syphilis test, since the essence of standardisation, viz. constancy of results, cannot at present be obtained. The Medical Research Committee's report suggests four methods; three of these bear the names of their advocates, namely, (1) Harrison's, (2) Browning and Kennaway's modification of the method of Browning, Cruickshank and Mackenzie, and (3) Fildes and McIntosh's, while the fourth method is a composite one. The differences in these procedures are in part technical and in part involve differences in reagents. Provided, however, that suitable reagents are employed and accurate methods of quantitative work, it is probable that by any of these methods the majority of syphilitic sera will be detected and that all non-syphilitic sera



will be returned as negative (excluding of course certain diseases not common to this country). Suitable reagents comprise (1) an alcoholic extract of heart or liver or the ether-soluble, acetone-insoluble constituent of such an extract, preferably along with cholesterin, (2) guinea-pig's serum as a source of complement, (3) the employment of the serum to be tested after it has been heated to  $55^{\circ}\text{C}$ ., and (4) test corpuscles sensitised with an artificial immune body. The report does not recommend "short-cut" methods which employ unheated patient's serum and omit guinea-pig's complement. These may be of value in controlling the treatment of known cases of syphilis, but the tendency toward "over-sensitiveness" which they exhibit renders them unfitted for purposes of diagnosis. In regard to methods of measuring the reagents there is a difference of advocacy as between the use of droppers and of graduated pipettes. The accuracy of measurement by drops—a procedure which has been elaborated in its application to serological work by Donald—rests on the fact that, other things being equal, the size of a drop depends on the external diameter of the dropper, provided the drops do not form too quickly, and that the apparatus is not used at different angles. To measure the same amount repeatedly by means of a graduated pipette makes greater demands on the manipulative accuracy of the user; thus of the two procedures the drop method is perhaps the more "fool-proof." But, on the other hand, it has the objection that it tends to produce the idea that the reaction has reached a stage of mechanical precision, which is far from being the case.

As long as the test depends on the use of complement and of a hæmolytic system, so long will it involve constituents which are incapable of really accurate standardisation. These reagents are, however, merely the indicator which serves to render visible the difference between the state of a mixture of "antigen" with a positive serum on the one hand and a negative serum on the other. Many workers have endeavoured to detect by other means the difference, which is probably of a physical character. Recently Sachs and Georgi have stated that when using a suitable antigen composed of heart extract plus cholesterin, they observed the formation of a precipitate when the syphilitic serum was added, but not with a normal serum. The results in a large series of cases corresponded very closely with those obtained by the Wassermann test and this has been confirmed by others



using Sachs' and Georgi's antigens. But the preparation of the antigen appears either to have been imperfectly described or else its properties depend on conditions which can be reproduced only with difficulty, as we have been unable to obtain satisfactory results and we have been informed of similar failure by other workers.

#### THE DIAGNOSTIC VALUE OF THE WASSERMANN TEST.

The Medical Research Committee's report commences with a survey of the literature bearing on the diagnostic value of the Wassermann reaction. This is the most comprehensive treatment of the subject with which we are acquainted, and should be read by all who wish to be informed on the practical utility of the test.

The occurrence of discrepancies in the reports on specimens of serum, different portions of which have been submitted to several laboratories, has always been a vexatious question and has given rise to several controversies. Accordingly, it appeared desirable to the Committee to arrange a definite trial in which a series of specimens from patients whose clinical history was known should be examined by skilled observers of recognised experience, using identical specimens of serum in each case and employing the original Wassermann test with its full controls. Thus specimens of blood from a series of about 100 cases for the clinical notes of which Dr. Sequeira was responsible were submitted to Col. Harrison, Dr. James McIntosh and the present writers. The cases comprised syphilis at all stages, treated and untreated, and also a considerable proportion of non-syphilitic conditions. No series of cases hitherto submitted to the test has been published with such fulness of clinical detail. Thus the reader is as far as possible enabled to form his own conclusion regarding the conditions.

In order to eliminate all bias from the tests the specimens reached the laboratories bearing no history, but only a number. We are strongly of opinion, however, that this practice, while fair under the circumstances in question, should not be the custom when specimens are submitted for routine diagnosis. The reason for this is clear: it does not imply that the laboratory worker lacks the courage of his convictions, but depends upon the fact that there exists no sharp line of demarcation between positive and negative sera; instead there is



an intermediate zone into which the serum of non-syphilitic cases may ascend owing to a variety of, so far, not well-defined circumstances (possibly during fever\*), and through which the serum of a syphilitic must pass in the initial stages of the infection or a relapse, as well as when latency supervenes spontaneously or through specific treatment. Thus there are two criteria of positive: firstly the absolute positive which is required for diagnosis in an unknown case, and to which one would swear in a court of law, and secondly the weak positive or suspicious reaction, which is as good as positive in a case of known treated syphilis or in one which had reacted positive prior to treatment; the latter reaction might be termed the *therapeutic* positive in contra-distinction to the former, the *diagnostic* positive.

The value of the diagnostic positive to the clinician depends upon its never being obtained where syphilis can be excluded with certainty. It is highly satisfactory, therefore, that of the 30 cases which Dr. Sequeira classed as *clinically non-syphilitic* an almost unanimous result was obtained in 29, of which 28 were negative† or merely suspicious and 1 positive (Case 99).‡ The latter case is described as one of carbuncles, ill one week, without sugar in the urine; the patient had received a dose of vaccine six hours before the specimen of blood was taken. As the Committee states, however, "on serological evidence it would appear therefore that this case was one of latent syphilis." It is indeed remarkable that more cases of latent syphilis with a positive reaction, but without obvious syphilitic history, were not encountered. In one other patient (No. 71), a case of

\* It should be noted that recent work has shown that malarial infection, even at the febrile stage, is seldom, if ever, responsible for a positive reaction (Fletcher, Thomson and Mills). The number of positive reactions recorded by various observers in the tropics is probably to be accounted for by the great prevalence of syphilis. The suggestion that trench fever and tick fever may be the cause of positive reactions is also without any definite basis. Leprosy, however, occupies a different position, and the careful observations of Thomsen and Bjarnhjedinson can scarcely have any other interpretation than that leprosy is a frequent cause of positive reactions unless syphilis in Iceland is clinically very much modified.

† Case 2 is included among the unanimous negatives, since the positive result attributed to us in Table XI was a *clerical* error (see footnote, p. 48).

‡ Dr. McIntosh reported this case as negative in the first instance, but a portion of the same specimen was positive in the hands of Dr. Donald, and a later sample from the same case was found positive by Dr. McIntosh also.



scleroderma (morphœa band type left arm and forearm) in a girl, aged 18 years, with no history or evidence of syphilis, Harrison obtained a positive reaction, but the other observers recorded negatives. It would appear therefore as if Harrison's results had a tendency toward the positive side, since he also records two doubtful reactions, whereas the other observers report only one each, but these do not really disturb the diagnosis.

Turning now to the *untreated secondary stage of syphilis*, in which positives are the rule, McIntosh and Harrison each obtained 100 per cent. of positives in 20 cases; Browning and Kennaway out of 19 cases obtained 15 diagnostic positives, 2 weak positives and 2 negatives (it was subsequently found by the latter observers that the negatives and one of the weak positives were all obtained in one day's tests, in which an under-deviable complement had been employed, since these sera when re-tested all reacted frankly positive). Seven cases of *untreated late (tertiary) syphilis* (6 cases of cutaneous gumma and 1 of ulceration of the fauces with much loss of tissue) were unanimously returned as positive. In *primary untreated cases* Harrison and Browning and Kennaway each obtained in all 11 positives out of 15 cases and McIntosh 11 out of 14. But discrepancies in individual cases were more in evidence in this series. Thus Browning and Kennaway obtained a positive (No. 7) where the other observer recorded negative; in another case (No. 66) the findings were reversed. Also, Browning and Kennaway recorded suspicious and very suspicious reactions in 2 cases in which the others found negative reactions. In the 4 *primary treated cases* the results of all the observers were negative. In the series of *treated secondary and tertiary cases*, in which, of course, any trace of reaction short of a definite negative possesses clinical value from the point of view of treatment, Browning and Kennaway and Harrison obtained respectively 9 and 6 negatives out of 18 cases and McIntosh 8 out of 17. As one would expect, discrepancies between the individual results in particular cases are more numerous in this than in the other groups; thus, in 2 cases Browning and Kennaway returned negatives where the other workers found positive results, while in 1 case McIntosh alone returned a positive.

In comparing the results of the different observers it should be noted first of all that Harrison and McIntosh employed practically



identical methods, and any difference between their records is probably of the same order as those which would be obtained by a single observer using either of these procedures on repeated occasions. Donald, who tested a portion of the samples submitted to McIntosh, employed a method which differs from that of Fildes and McIntosh only in the employment of smaller amounts of the reagents. Browning and Kennaway, on the other hand, have used an antigen consisting of liver lipoid plus cholesterin instead of heart extract plus cholesterin, and have fixed their criteria of a positively-reacting serum according to its behaviour in comparison with a known negative serum tested at the same time and with the same reagents. This undoubtedly is a far more rational criterion than the one commonly employed, which is based on the fixation of an arbitrary amount of complement, since the complementing property of serum is so variable and complex in its characters. The use of a negative control serum, and preferably one obtained always from the same person, provides automatically a control upon the variable deviability of complement and upon differences in the behaviour of the antigen. The result given by such a serum should therefore be the chief basis of diagnosis in the Wassermann test.

The general conclusion will be reached by every reader of the report that in experienced hands remarkably concordant results are obtained in the majority of cases provided that there has been no active anti-syphilitic treatment. This is a highly satisfactory issue, which vindicates anew the reliability of the test. The method of the authors, as is seen especially in the series of treated cases, gives a somewhat smaller yield of positives than that of the other workers; this is probably due to the antigen. Such a result is more satisfactory from the point of view of diagnosis than if there were the suspicion of too many positives being recorded. Thus the investigations carried out at Glasgow on the incidence of syphilis in the hospital population (see Browning) obtain a strong measure of support as being rather an under-statement than an exaggeration of the wide prevalence of this disease as revealed by the complement-fixation test. On the other hand, where the reaction is employed for the control of treatment it is in the interest of the patient even to err on the side of positiveness. Accordingly, the therapeutic Wassermann reaction should always be performed with the heart-cholesterin



antigen in addition to any other which may be employed, and it is advisable to use both antigens, as Browning and Kennaway have recently recommended. Many observers have noted that liver lipoids were less potent syphilitic antigens than heart lipoids, and Browning, Cruickshank and Gilmour demonstrated the fact clearly. A number of American workers have expressed the view that the heart-cholesterin antigen was over-sensitive in the sense that it yielded positive reactions in non-syphilitic cases, and it was on account of a similar observation in their own work that Browning, Cruickshank and Gilmour originally discarded heart-antigen in the form in which they employed it. But the conditions under which an antigen is used determine its reliability, and, as recommended by Fildes and McIntosh, it is extremely valuable and highly specific.

Although the Committee states its view "that any intelligent reader will be able to form his own opinion of the clinical value of the Wassermann reaction," it is perhaps to be regretted that the onus of a detailed critical consideration of the findings of the different observers fell upon the clinical side alone. This appears especially where those cases are dealt with in which the results of the blood-test differed; thus Dr. Sequeira in his summary and conclusions seems to have missed the opportunity of showing the full value of the composite report. *Inter alia*, he remarks on a case (No. 66) of primary untreated syphilis in which our return of negative differs from the clinical diagnosis (the other observers found positive), and he states that this is "important clinically"; he fails, however, to advert to the significance of the equally striking instance in a similar case (No. 7) in which we obtained a definite positive, while the other report on the serum (Harrison) was "practically negative." Again, in contrasting Harrison's and McIntosh's results it is stated, "in no syphilitic cases were the differences serious," and Dr. Sequeira classes as a "minor variation" a "practically negative" ( $\pm$ ) return by Harrison where McIntosh gave a positive report (Case 8—tertiary syphilis: treated), whereas a weak positive returned by Browning and Kennaway in a case of treated secondary syphilis (No. 14) as compared with full positives by Harrison (+ +) and by McIntosh is described as "important clinically." The critical comments, therefore, appear to lack judicial consistency.



An important source of mistake to which, *à propos* of one of our results, attention is drawn by the Committee, is clerical error as contributing to the apparent uncertainties of the test. Dr. Sequeira's report itself furnishes other examples in point, since it contains three errors which have been rectified in a list of corrigenda.\*

It cannot be too strongly urged that the Wassermann reaction of the serum should be tested in the case of every patient who comes under observation, quite irrespective of the condition for which he seeks treatment. This may appear to be a drastic proposal, but it is at least as essential as the recording of the patient's age, sex and social state, and the determination of the reaction of urine to litmus paper. It must be remembered that an untreated syphilitic affection is a most undesirable possession, and a procedure which indicates the presence of this infection is of the utmost importance. The value of the Wassermann test in revealing syphilis or in confirming what otherwise would merely remain a vague suspicion is continually receiving fresh proofs. Thus Boas has recently reported a case which is interesting in this connection and also from the rarity of the manifestation described: A boy, aged  $3\frac{1}{2}$  years, came to hospital on account of failure to thrive; beyond a continued state of lassitude there was no other history. In hospital he was found to show the very uncommon tertiary ring erythema. No stigmata of congenital syphilis were detected, but the Wassermann reaction was positive. The father had had gonorrhœa nine years before, but he gave no history of syphilitic manifestations; Wassermann reaction positive. The mother had had no abortions or stillbirths or other syphilitic symptoms; Wassermann reaction positive. The other child of the family, aged 2 years, had had no illnesses and showed no stigmata; Wassermann reaction negative. In confirmation of the diagnosis of syphilis is the fact that during treatment the boy developed double hydrops of the knees. One of the most striking of therapeutic calamities is the failure to recognise the nature of a syphilitic bone lesion in childhood. Where there is necrosis and sinus formations such cases seem commonly to be regarded as tuberculous, and in consequence there result years of suffering and permanent deformity, all of which could have been obviated. One of the earliest cases treated

\* An additional error in this section is the inclusion of Case 10 among treated cases on p. 47, line 6 (*cf.* Table I, p. 33, and Table XIV, p. 50).



with salvarsan in this country was of this type (Browning and Edington), and it seems almost incredible that a patient should have come under hospital treatment repeatedly for years without syphilis being suspected, especially when there was choroiditis in addition. The routine application of the syphilitic blood-test would, at least, minimise such misfortunes. Of course, it must never be forgotten that a positive reaction with the blood does not prove the syphilitic nature of every lesion which the patient presents. There is always need for the application of all methods to the problem of diagnosis, and when the laboratory has proved beyond doubt that the patient is syphilitic an invaluable contribution has been made to the data of the case, and one the therapeutic bearing of which is quite unequivocal.

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