in which we were unable to detect any other clinical evidence suspicious of congenital syphilis beyond, perhaps, the doming of the cusps of a solitary molar tooth. The physique and mentality were excellent. The Wassermann was persistently a strong positive. In some cases the unaffected eye became subsequently involved and went through the phases of a typical attack.

One might describe here the case of a surgeon, aged 29, who, whilst operating upon a non-syphilitic patient, received a splash of pus into one eye which was immediately washed out with some too strong perchloride of mercury lotion (1 : 1000). The resulting chemical conjunctivitis resolved, but was subsequently followed by an attack of interstitial keratitis in that eye. He was then examined for evidence of congenital syphilis—a possibility entirely unsuspected hitherto. His Wassermann reaction was strongly positive with other corroborative evidence.

Now if any of these cases had contracted malaria prior to the interstitial keratitis we are positive that syphilis would not have been suspected, and malaria would have been held responsible for the production of a strongly positive Wassermann reaction. It is well to point out that of the eight cases in our series giving a positive Wassermann reaction three were found on examination to be congenital syphilitics, a fact hitherto unnoticed in their military career.

Undetected Cases of Acquired Infection.

Numbers of acquired cases occur where the patient is entirely ignorant of his having been infected with syphilis; these come under the observation of the medical man if the severity of the secondary manifestations become such as to necessitate the patient seeking advice. It frequently happens that the patient only presents himself for examination after certain proprietary "blood mixtures" have failed to disperse the lesions. (There are many in whom the secondary lesions are trivial, and these resolve spontaneously undetected.) The type of case, by no means as rare as the literature might lead one to suppose, is that in which inoculation occurred on the tonsil, tongue, or fossa navicularis of the urethra. As an illustration it was found at Rochester Row Military Hospital, over one period of 18 months, that the incidence of a chancre in the fossa navicularis was 1 in 14 of the total admissions for primary and secondary syphilis; and the primary sore was by no means infrequent on the lip, tonsil, tongue, and finger in the order given in a period of four years.

The number of cases that have attained the age of, say, 40 and onwards before syphilis is diagnosed for the first time upon the development of an active gummatous process convinces one that many must pass entirely undetected. A large number might pass unsuspected should they develop, say malaria, at an earlier date while presenting no gross lesions. It is only reasonable to suppose that the number of syphilitics, especially females, who carry their infection to the grave undetected, must be considerable.

We are convinced that the further this investigation proceeds, in the hands of careful clinical observers, upon the behaviour of the Wassermann reaction in the various diseases other than syphilis and yaws, the more light will be thrown upon the number of undetected syphilitics, and it will not be assumed so lightly as hitherto that these other diseases are responsible for the reaction.

A positive Wassermann reaction in malaria demands that syphilis be excluded by a most careful and searching inquiry.

Authors' Conclusions.

1. The Wassermann reaction conducted according to a recognised standard method does not give a positive reaction in malaria at any stage of the disease.

2. If a positive Wassermann reaction is obtained in a case of malaria it is either due to undiagnosed syphilis or to faulty technique.

We wish to tender our grateful thanks to all the staff at Rochester Row Military Hospital for their kindness. Colonel Harrison, D.S.O., officer in command, Rochester Row; Captain D. Thomson, pathologist; and Mr. Nöel Clarke gave us every facility and help, and it is wholly due to them that these observations were made possible. Captain T. Gardner, R.A.M.C., and Dr. Jamieson on the malaria research staff gave invaluable assistance.

Literature.—1. Boehm (1909): Arch. f. Schiffs- u. Trop.-Hyg., 1909, xiii., Beiheft 6. 2. Schoo (1910): Nederl. Tydschr. voor Geneesk, 1910, No. 5. 3. Meier and Bonfiglio (1910): Results not published.

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WASSERMANN TESTS:

THE OCCURRENCE OF VARIATIONS IN THE RESULTS OF SUCCESSIVE TESTS; THE CLINICAL APPLICATION OF THE DATA.*

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THE clinical value of the positive Wassermann reaction as a "specific" indication of syphilis is now generally accepted. This statement, of course, applies to the performance of the test under adequately controlled conditions.[1] The subject, however, assumes another aspect when an attempt is made to draw conclusions for the control of treatment, &c., from quantitative differences in the reaction.

Quantitative Differences in the Reaction.

Owing, probably, to the fact that few observers test the reaction with a sufficient series of varying quantities of complement, it has not been widely recognised how variable the results are when the same specimen of serum is examined repeatedly. An indication of this fact is afforded by scattered observations on "paradoxical" reactions—i.e., the obtaining of a positive result on one occasion and a negative on another with certain sera. But the importance of such variations has not been recognised as affecting the value of the results of those who grade the positiveness of the reaction, e.g., by a series of plus signs, &c. Also it has not been sufficiently realised that conclusions regarding the state of a patient can scarcely be drawn from the result of repeated tests unless a definite positive reaction is replaced by a definite and repeatedly obtained negative. On the contrary, a number of observers (see Boas [2]) base conclusions on minor quantitative differences in consecutive examinations. Thus there is the danger of imbuing the reaction with an illusory attribute of precision in the quantitative sense.

A consideration of the fact that, other constituents apart, different guinea-pigs' sera are used as a source of complement, as well as different specimens of red corpuscles in the hæmolytic system on successive occasions, should entail a cautious attitude; but we shall indicate later that the sources of discrepancy are probably even more extensive. In the report furnished to the Medical Research Committee [3] the conclusions which we had reached in this matter were stated as follows:—

"It has been found as the result of many years' routine tests with longer series of tubes than those above noted (i.e., more than three amounts of complement for each specimen of serum) that no single amount of complement, measured either by volume or by hæmolytic doses, will serve as a criterion for positive, owing to slight or no lysis occurring with it or for negative, owing to complete lysis occurring with it. This is due to the following facts:—(*a*) The absence of any fixed relationship between hæmolytic power and deviability on the part of different specimens of complement; (*b*) the difference between the amount of complement necessary to cause complete lysis and the amount which causes only a trace of lysis, varies greatly with different complements when the same antigen and the

* A report to the Medical Research Committee.

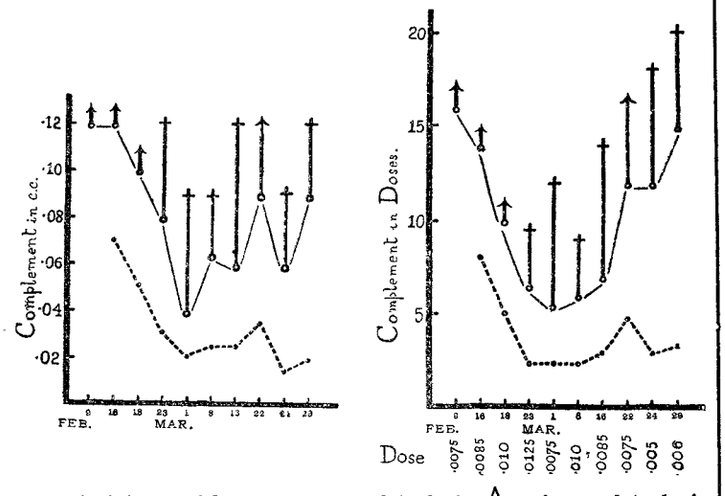
(*Continued from previous column.*)

4. Wassermann (1910): Annual Meeting of the British Medical Association, 1910, THE LANCET, 1910, ii., 475. 5. Ferrari and Gioseffi (1911): Biochimica e. Terap. Sperim., 1911. 6. Zschucke (1913): Berlin. klin. Wochenschr., 1913, 1. (No. 37), 1716-1719. 7. De Haan, J. (1913): Arch. f. Schiffs- u. Trop.-Hyg., xvii. 8. Sutherland and Mitra (1914): Indian Jour. of Med. Research, No. 2, 1914. 9. Thompson (1916): Syphilis, Diagnosis and Treatment of Syphilis, Philiadelphia, 27, 1916. 10. Hirsch (1917): Ztschr. f. Hyg. u. Infektionskr., 1917, lxxxiv. (No. 2), 323-324. 11. De Jong and Martin (1917): Presse Méd., 1917, xxv. (No. 60), 617-618. 12. Meyerstein (1917): Münch. med. Woch., 1917, lxiv., (No. 2), 366-368. 13. Prins (1918): Zur Klinik der Malaria, Deut. med. Woch., 1918, xliv. (No. 5), 132-133. 14. Craig (1918): The Wassermann Test, 1918, London, 149. 15. Cziknawerow (1909): Russky Vratsch, 1909, 876. 16. Manu Muscel and Vasiliu (1910): C. R. Soc. Biol., 1910, lxviii., (No. 4). 17. Bates (1912): Archives of Internal Med., 1912, x, 470. 18. Fletcher (1914): THE LANCET, 1914, i., 1677. 19. Harrison (1918): Special Report on Wassermann Test, Series 14, published by Medical Research Committee. 20. Mathis and Heymann (1915): Bull. Soc. Path. East., May, 1915, viii. (No. 5), 256-259. 21. Kimura (1917): Jl. Formosa Med. Soc. 1917, No. 175, 324-325.

same patient's serum are employed. Hence, it is impossible to judge accurately from the degree of lysis, with one fixed amount of complement, what degree of lysis would be obtained with a given smaller or larger amount. The same holds good when different positive sera are tested along with the same antigen and the same complement."

Illustrative Series of Tests.

The following series of tests illustrates the points clearly. The figure represents the amounts of complement necessary to produce different degrees of lysis (from a trace to complete) on different occasions when the same positive serum was tested along with syphilitic antigen and complement. The results of a parallel series of tests with the same negative serum are also given; but in this case, owing to the small amount of complement fixation, only the point of complete lysis is shown. In the left-hand figure the complement is expressed as c.cm. of guinea-pig's serum; the right-hand figure shows the same series of experiments, but the complement is reckoned in hæmolytic doses.



o = first trace of lysis, + = complete lysis, ↑ = incomplete lysis with the highest amount of complement used, with the positive serum. •----• = complete lysis with the negative serum.

Amounts of Complement giving just Complete Lysis in the Antigen Control.—Feb. 9th, 2– doses; 16th, 7– doses; 18th, 2– doses; 23rd, 2– doses; March 1st, 2– doses; 8th, 2– doses; 16th, 2– doses; 22nd, 4–doses; 24th, 3– doses; 29th, 2·5– doses.

The tests were made by the method already described (Browning and McKenzie [4]). The antigen employed was an alcoholic solution of ox-liver "lecithin" with addition of 1 per cent. cholesterol, 1 volume of which was slowly mixed with 7 volumes of normal saline. A series of tubes was taken, each containing 0·3 c.cm. of the emulsion of antigen and 0·025 c.cm. of patient's serum previously heated for half an hour at 56° C.; increasing amounts of complement (the pooled serum of two or more guinea-pigs, bled about 18 hours previously) were added. On each date a different specimen of ox blood was used to furnish the hæmolytic system; the red corpuscles were sensitised with at least five doses of immune body from the rabbit.

The positive serum consisted of a mixture of sera of two cases; small amounts were placed in a series of tubes which were preserved frozen at −15 to −22°C. throughout; a specimen was withdrawn from the refrigerator and thawed before use. The negative serum was derived from one person and was that habitually used; it was treated in the same manner as the positive specimen; but two batches of the negative serum were employed, the first being used for five tests, and the second for the last four. The controls with serum and saline only, but no antigen, gave in all cases complete lysis with two doses of complement.

Value of Use of Negative Control Serum.

The results vary irregularly from week to week, hence there is evidence that a *progressive* alteration in the frozen sera is *not* responsible for the variations. The results given in the figure illustrate the fact that it is not possible to fix upon any single amount of complement the behaviour of which, along with "antigen" and patient's serum, will determine whether that serum is positive or negative. In the original technique of Wassermann 0·05 c.cm. of complement was employed, under specified conditions, in all cases; sera showing no lysis with this standard amount were regarded as positive, and those showing complete lysis as negative. But the results with the positive serum represented in the figure above show that on one day (March 1st) 0·04 c.cm. complement sufficed to produce commencing lysis, whereas on other occasions (Feb. 9th and 16th) no less than three times this amount was required.

It is clear, then, that the diagnosis must be based, not upon the behaviour of the unknown serum with any fixed amount of complement, but upon *comparison with the behaviour of a known negative serum.* In spite of the great fluctuations in lytic power for the test corpuscles which were exhibited also by the mixture of the negative serum along with antigen and complement, as shown in the figure, there is an obvious parallelism between the series of positive and negative results. This fact strongly suggests the use of a negative control serum as a standard in diagnosis. The value of such a procedure lies in its being independent of the variations of complement, except, of course, in so far as deficient deviability of the complement in a given test will cause failure to detect weak positives. The employment of a criterion of this nature will be considered in greater detail in a forthcoming paper.

Variability of Results.

With regard to the variability of results in the Wassermann test the figures show that (1) the same positive serum may require from 0·04 to 0·12 c.cm. complement, or from 5 to 16 doses to produce commencing lysis; (2) the same negative serum may require from 0·017 to 0·07 c.cm. complement, or from 2·5 to 7 doses, to produce complete lysis; (3) further, when the amount of complement which produces commencing lysis is ascertained, one cannot formulate any rule which will determine the further amount necessary to produce just complete lysis. This is shown by the varying length of the vertical lines in the figure. Thus the same serum may give the observer very different impressions of positiveness according to the particular sample of complement employed. Hence results recorded by varying numbers of plus signs, while purporting to give valuable information as to the state of the patient, are to a large extent merely expressions of the variability of the reagents employed in the test. Observations on the use of heart-cholesterol antigen indicate that the variations above noted are not pecular to a liver-antigen.

The complement is an important source of variation, since, as was pointed out by Browning and McKenzie,[4] there is no constant relationship between its hæmolytic power and its capacity for being bound by the mixture of "antigen" and antibody. Attempts to preserve a constant stock of complement by freezing the pooled serum of a number of guinea-pigs have not, however, solved the difficulty and irregularity in the degree of complement fixation remains a marked feature in spite of this precaution. It would appear, therefore, that minute physical differences in the emulsion of the "antigen," as well as variations in the test red corpuscles, may be responsible.

It is not asserted that variations which have been attributed to alteration in the reacting power of the same patient's serum when examined at short intervals are entirely explicable on the above basis—e.g., those quoted by Craig.[5] But we would point out that this, so far, apparently uncontrollable irregularity which is met with in the biological syphilis test, has not been sufficiently taken into account by many workers.

Summary.

1. When the same specimen of syphilitic serum is repeatedly examined for complement fixation in the Wassermann test the actual amounts of complement fixed vary greatly on the different occasions. These variations are quite irregular, and depend on factors which cannot, so far, be rendered constant.

2. This result prevents the attaching of clinical significance to minor variations in positiveness obtained on repeated tests of the same patient's serum—e.g., under treatment.

3. The evidence shows that the criterion of positiveness or negativeness of a serum should not be determined by the absolute amount of complement fixed, but by the amount of fixation relative to that produced by a known negative serum.

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