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A PROCEDURE FOR THE SEROLOGICAL DIAGNOSIS OF SYPHILIS BASED ON A COMPARATIVE STUDY OF FIVE MODERN SEROLOGICAL TESTS

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Numerous serological tests for syphilis have been devised; there is, however, little information available indicating which test or combination of tests gives the most reliable results in routine serological work. The present paper attempts to answer this question. Five modern serological tests, differing from each other as widely as possible, were applied to a series of 470 serums from syphilitic persons and 989 serums from non-syphilitic persons. Attention has been paid not only to the sensitivity and specificity of the results of the individual tests but also to the ease with which the tests could be performed. Finally a procedure has been evolved whereby all syphilitic serums can be identified and at the same time the occurrence of non-specific results almost entirely eliminated.

Tests used

The Wassermann test was performed by the Harrison-Wyler method (Wyler), the technique most widely used in Great Britain. The antigenic property of the antigen was carefully titrated and the optimum dose used in the test. Kahn’s standard test was carried out exactly as described by Kahn in 1928, great care being taken to use an antigen of standard sensitivity. Mazzini’s microscopic slide flocculation test (Mazzini) was selected because of its excellent record at the Washington Serology Conference (Parran, Hazen, Mahoney, Sanford, Senear, Simpson and Vonderlehr). The standardized reagents for the test were obtained directly from Dr. Mazzini.

The distinctive feature of the Victoria blue test (Berger1) is the utilization of the sensitizing and indicator action of certain dyes on the flocculation reaction for syphilis. The technique of the test is very simple and the interpretation of the results easy.

The agglutination test (Berger2), is based on the fact that antigen-coated collodion particles agglutinate in the presence of syphilitic antibody, the reaction being clearly observed with the naked eye. The test proved to be capable of greater sensitivity than the Wassermann or the Kahn tests and has been recommended as a screen test. This test also allows the accurate titration of the amount of syphilitic antibody present in serum.

Serums tested

In all, 1,476 successive specimens of serum were examined by the five tests. In the case of seventeen serums information with regard to the syphilitic status of the patients could not be obtained and accordingly these serums were excluded from the final tabulation. Of the remaining 1,459 serums, 470 were from syphilitic persons and 989 from non-syphilitic persons. Serums from syphilitic donors were classified in three groups in the manner adopted at the Washington Serology Conference.

Group A comprised seventy-eight serums from patients with early, primary or secondary syphilis, who had not received any specific treatment. Serums in group B were from patients suffering from syphilis for less than four years and who had received varying amounts of specific treatment. We examined 333 such serums. Serums from patients infected with syphilis more than four years ago, whether treated or untreated, were included in group C. Fifty-nine such serums were examined. Cases of congenital syphilis were classified according to these criteria.
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Serums from non-syphilitic persons were divided into two groups. Group D comprised 702 serums from hospital patients with diseases other than syphilis. These patients did not show any clinical evidence of syphilis and had not any history suggestive of syphilitic infection. They had various medical or surgical conditions, febrile illnesses, including tuberculosis in all stages, and malignant diseases. Serums from pregnant women who did not show clinical evidence of syphilis and did not give a history of syphilis were classified in group E. There were 287 such serums. It has been pointed out by Sachs that serums from pregnant women often give false positive results in tests of doubtful reliability. This view has been confirmed by our own experience.

Comparison of the five tests

Table 1 gives the total number of syphilitic and non-syphilitic serums which reacted in each of the five tests. The sensitivity and specificity of the tests were calculated by a method similar to that used at the Washington Serology Conference. Sensitivity, which applied only to reports from testing specimens from syphilitic donors, was computed by adding the percentage of positive reports to one-half the percentage of doubtful reports.

In the calculation of these percentages, the total number of syphilitic serums tested was arbitrarily fixed at 317. This figure was arrived at by the exclusion of 153 serums which did not give any reaction in any of the tests. Sixteen of these serums were from very early untreated cases (usually in the first week) and 137 from cases adequately treated early after infection. It seems reasonable to suppose that the blood from the persons concerned did not contain the syphilitic reacting substance. As the term, sensitivity, can apply only to serums containing the reacting substance, it was thought advisable to exclude from the calculation serums which presumably did not contain any syphilitic reacting substance. Specificity, which applied only to reports from testing specimens from non-syphilitic donors, was computed by adding the percentage of negative reports to one-half the percentage of doubtful reports.

When the sensitivity and specificity of the Wassermann and Kahn tests are compared, it may be observed that both values are almost identical; the small differences which do occur are not statistically of any significance. This confirms the results obtained by Berger and Sutherland who compared the two tests on a series of more than 15,000 serums. The sensitivity of the remaining three tests was higher than that of the Wassermann test or the Kahn test. The non-specificity (the reciprocal value of specificity) however, was increased proportionally with the increase of sensitivity of each test. The number of doubtful results with the individual tests had not any relation to the sensitivity and specificity of the test. The majority of serums giving doubtful results in various tests were from persons suffering from syphilis. These serums contained only small amounts of the syphilitic reacting substance when titrated by the agglutination test. With the agglutination test doubtful results were obtained very rarely.

Approximately the same proportion of serums from patients suffering from syphilis in different stages and classified into groups A, B and C reacted in each test. These results are summarized in Table 2 which shows clearly that none of the tests

<table>
<thead>
<tr>
<th>TEST</th>
<th>SYPHILITIC CASES</th>
<th>SENSITIVITY PER CENT</th>
<th>NON-SYPHILITIC CASES</th>
<th>NON-SPECIFICITY PER CENT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
<td>±</td>
<td>—</td>
<td>+</td>
</tr>
<tr>
<td>Wassermann</td>
<td>169</td>
<td>15</td>
<td>286</td>
<td>55-6</td>
</tr>
<tr>
<td>Kahn</td>
<td>172</td>
<td>24</td>
<td>274</td>
<td>58-0</td>
</tr>
<tr>
<td>Victoria blue</td>
<td>206</td>
<td>15</td>
<td>249</td>
<td>67-2</td>
</tr>
<tr>
<td>Mazzini</td>
<td>230</td>
<td>32</td>
<td>208</td>
<td>77-5</td>
</tr>
<tr>
<td>Agglutination</td>
<td>304</td>
<td>—</td>
<td>166</td>
<td>95-8</td>
</tr>
</tbody>
</table>

TABLE 1—SUMMARY OF RESULTS OBTAINED WITH 470 SYPHILITIC AND 989 NON-SYPHILITIC SERUMS EXAMINED BY FIVE DIFFERENT TESTS.
possesses a selective affinity for any particular group of syphilitic cases. The widely held view that a relatively greater number of serums from cases of early syphilis react only in the Wassermann test, and that certain serums from treated cases of long standing react only in precipitation tests, lacks any foundation in fact. It appears probable that the same reacting substance is present in the blood of syphilitics from whatever stage of the disease they may be suffering.

TABLE 2—ANALYSIS OF SERUMS REACTING IN FIVE DIFFERENT TESTS IN RELATION TO THE CLINICAL HISTORY OF PATIENTS

<table>
<thead>
<tr>
<th>GROUP TEST</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wassermann</td>
<td>37 ± 40 98 14 221</td>
<td>34 ± 25</td>
<td>1 ± 701</td>
<td>1 ± 286</td>
<td></td>
</tr>
<tr>
<td>Kahn</td>
<td>38 ± 38 101 19 213</td>
<td>33 ± 23</td>
<td>1 ± 701</td>
<td>2 ± 285</td>
<td></td>
</tr>
<tr>
<td>Victoria blue</td>
<td>42 ± 35 124 12 197</td>
<td>40 ± 17</td>
<td>2 ± 700</td>
<td>2 ± 284</td>
<td></td>
</tr>
<tr>
<td>Mazzini</td>
<td>42 ± 31 151 23 159</td>
<td>37 ± 18</td>
<td>3 ± 679</td>
<td>5 ± 279</td>
<td></td>
</tr>
<tr>
<td>Agglutination</td>
<td>60 ± 18 194 ± 139</td>
<td>50 ± 9</td>
<td>7 ± 695</td>
<td>8 ± 279</td>
<td></td>
</tr>
</tbody>
</table>

Discrepancies between the results of various tests

Discrepancies between the results are summarized in Table 3, which gives the number of serums reacting in the various tests and also shows the number of false negative results obtained with the test named in the first column on the left. Only persons suffering from syphilis were taken into consideration and doubtful results were tabulated as positive. Inspection of the table will show that a test of very high sensitivity may miss certain serums giving a positive reaction with a test of considerably lower sensitivity. Of the five tests under consideration the sensitivity of the Wassermann test was the lowest. Thus six serums which reacted in the Wassermann test did not give any reaction in the considerably more sensitive Mazzini test. Similarly some other syphilitic serums were positive only with some other test or group of tests. Only the agglutination test picked out all serums reacting in the two most widely used tests, the Wassermann and Kahn tests. The causes of the discrepancies between the results of different serological tests for syphilis are manifold and have been analysed in detail by Berger and Sutherland.

TABLE 3—FALSE NEGATIVE REACTIONS OCCURRING WITH DIFFERENT TESTS

<table>
<thead>
<tr>
<th>TEST</th>
<th>Total number of reacting serums</th>
<th>Total number of negative serums</th>
<th>Number of syphilitic serums detected with other tests</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>W.R. Kahn Victoria blue Mazzini Agglutination</td>
</tr>
<tr>
<td>Wassermann</td>
<td>184</td>
<td>133</td>
<td>20</td>
</tr>
<tr>
<td>Kahn</td>
<td>196</td>
<td>121</td>
<td>8</td>
</tr>
<tr>
<td>Victoria blue</td>
<td>221</td>
<td>96</td>
<td>7</td>
</tr>
<tr>
<td>Mazzini</td>
<td>262</td>
<td>55</td>
<td>6</td>
</tr>
<tr>
<td>Agglutination</td>
<td>304</td>
<td>13</td>
<td>0</td>
</tr>
</tbody>
</table>

Recommended procedure for testing serums

At present in routine serological work sensitivity is sacrificed in favour of specificity. This practice is justified to a considerable extent since a false negative result may be considered to be of less significance to the individual than a false positive one would be. To the community at large, however, false negative results are of equal if not of greater importance than false positive results. A perfect method of testing should give neither false positive nor false negative results. Such a test cannot be devised so long as the antigenic substance, the syphilitic hapten, has not been isolated in the pure state.

It has been pointed out by Kahn (1942) that by the use of several different serological tests on each serum perfect results cannot be obtained, since any increase of
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sensitivity may be counterbalanced by a corresponding decrease of specificity. There is however a way out which, with our present-day knowledge, permits identification of all syphilitic sera containing the reacting substance and yet does not give a greater number of non-specific results than would be obtained by a single test of very high specificity.

The following procedure is recommended. In the first instance all sera are submitted to the agglutination test. Serums giving a negative result are reported and all the remaining sera re-tested by the Wassermann and Kahn test. Serums giving a positive result in both these tests are reported. The remaining sera giving a negative, doubtful or discordant result with these tests are then re-tested by the quantitative agglutination test and by Kahn's verification test.

Rationale of the procedure

The procedure outlined has been devised on the basis of the following three observations. First, a negative result with the agglutination test excludes the presence of any presumed syphilitic reacting substance. Secondly, false positive results are, as a rule, obtained only in low dilutions of serum and, conversely, a strong positive reaction with a test of high specificity is good evidence of syphilitic infection. Thirdly, patients whose sera give weak positive or doubtful reactions should be re-investigated not only serologically but also clinically.

The use of the agglutination test as a screen test requires some comment. Precipitation tests cannot be utilized as screen tests because of their inherently low sensitivity. The inherent sensitivity is defined by the smallest amount of syphilitic antibody which a test is capable of detecting. The sensitivity of a precipitation test can be increased only within narrow limits without an undue decrease of specificity. When a precipitation test is adjusted by various technical modifications to give a positive reaction with all syphilitic sera, it will also give positive results with 40 or 50 per cent of normal sera (Kahn, 1942). The agglutination test of 100 per cent sensitivity gives, however, only 1 to 2 per cent non-specific results. The Wassermann test possesses the same high inherent sensitivity as the agglutination test. The sensitivity of the Wassermann test varies, however, in an unpredictable way with the fixability of the complement and the presence of "complementoid" substances in serum. The great advantage of the agglutination test is the fact that its sensitivity is independent of the factors causing variations in the sensitivity of the Wassermann reaction. The agglutination test can easily be adjusted to a certain sensitivity and kept constantly at this level. Therefore it is easy to reproduce the results of the agglutination test at will, whereas with the Wassermann reaction the results will vary from day to day, especially in the case of weak sera.

The results of the agglutination test were compared with those of the Wassermann and Kahn tests in a series of more than 8,000 sera. All syphilitic sera which gave a positive or doubtful reaction in either the Wassermann test or Kahn test or in both gave a positive result, without exception, with the agglutination test. Moreover, many syphilitic sera giving a negative result in both Wassermann and Kahn tests, gave a positive result with the agglutination test. The agglutination test therefore fulfils all the requirements of a screen test and we do not know of any other test giving equally good results when used for this purpose.

The re-testing of all specimens giving a positive reaction with the agglutination test by the Wassermann and Kahn tests is necessary for two reasons; first to eliminate laboratory errors and secondly to decrease the incidence of non-specific positive reactions. Serums giving strong positive reactions in both tests will undoubtedly be syphilitic sera and can safely be reported as such without further examination. The relatively few remaining problem sera giving a positive result with the agglutination test and discordant or doubtful or weak reactions with the Wassermann and Kahn tests should be examined more thoroughly. These comprise two different kinds of sera, namely, non-syphilitic sera giving false positive reactions and those syphilitic sera containing only small amounts of the syphilitic reagin. The most helpful tests in differentiating between these two kinds
of serums were the quantitative agglutination test and Kahn's verification test (Kahn, McDermott and Marcus). Non-specific positive reactions with the agglutination test occur, as a rule, only when undiluted serum or low dilutions of serum are tested; most of these false positive reactions do not appear with a 1 in 8 dilution of serum. Most of the syphilitic serums, however, still reacted when diluted with eight or more volumes of saline. Some indication as to the syphilitic or non-syphilitic nature of a problem serum can therefore be obtained by determination of the titre of the reacting substance by means of the quantitative agglutination test. Kahn's verification test, which should also be carried out with these serums, often proved very helpful. We have examined several syphilitic serums which reacted negatively in Kahn's standard test while giving a marked syphilitic reaction in the verification test.

There is not any single test available at present by which the syphilitic or non-syphilitic nature of every serum can be established. It is therefore necessary to examine these problem serums by both tests and to evaluate the findings in conjunction with the clinical history of the patients. It will then be possible to arrive at a correct diagnosis even in the majority of these problem serums.

The procedure outlined is neither laborious nor time-consuming. Two workers can easily deal with 400 to 600 serums every day. The day's work is planned in the following way. All serums are inactivated first thing in the morning, and while inactivation is taking place the suspension of collodion particles is sensitized. The results of the agglutination test may be read and the negative results reported about one and a half hours later. The relatively few reacting serums may then be examined on the same day by the Wassermann and Kahn tests and those giving a positive reaction with both tests reported immediately. The re-examination of the problem serums is carried out on the following day.

Alternative methods of testing
The procedure described has been devised to meet the requirements of large laboratories dealing with hundreds of specimens per week. Harrison and Osmond and other experts believe that the best results are obtained in large laboratories. It is difficult to devise an entirely satisfactory procedure which would fulfil the requirements of smaller laboratories. Mazzini's microscopic slide flocculation test however deserves to be more widely used in Great Britain. The excellent results obtained with this test are apparent from Table 1 and do not need any further comment. The Victoria blue test can be carried out so easily and quickly, even without laboratory facilities, that it may be recommended for use in small isolated hospitals or even in the surgery. An antigen of suitable sensitivity which should be obtained preferably from a central laboratory should be used. The results obtained with this test are almost equal to those obtained with the Kahn standard test.

Summary
A series of 470 serums from syphilitic patients and 989 serums from patients suffering from other diseases was examined by five different serological tests for syphilis.

Harrison-Wyler's Wassermann test and Kahn's standard test gave non-specific results very exceptionally but picked out only about 60 per cent of serums from syphilitic donors. The agglutination test, on the other hand, gave a positive reaction with almost all syphilitic serums but recorded 1-5 per cent of non-specific results.

A procedure is described which allows identification of almost all syphilitic serums and at the same time almost entirely eliminates the occurrence of non-specific results. In the first instance all serums are tested by the agglutination test and those giving a negative reaction are reported without further examination. The remaining serums are re-tested by the Harrison-Wyler, Wassermann and Kahn's standard tests, and those giving positive results in both tests are reported. The remaining problem serums are re-tested by the quantitative agglutination test and by the verification test.

The rationale of the procedure is given and the interpretation of the results is discussed.
CLINICAL RECORDS

We wish to thank Prof. P. L. Sutherland for his interest and valuable advice while we were carrying out this work and for his assistance in the preparation of this paper. We are also indebted to Dr. Mazzini for a supply of reagents for his test.

REFERENCES

CLINICAL RECORDS

ULCERATING GRANULOMA OF PUDENDA—TREATMENT BY ZINC PEROXIDE PASTE

This ulcer is for the most part a rare lesion occurring in the Tropics and Subtropics. The infection appears to be acquired in coitus. The stock treatment is by various antimony preparations given parenterally, usually in repeated, prolonged courses, combined with local antiseptic irrigations and dressings.

Historically this antimony therapy is insecurely based on a once supposed kinship of certain intracellular and extracellular bodies demonstrated in the ulcer tissue and Leishmania parasites; and hardly more securely on the clinical experience that under the combined treatment mentioned above a few of these ulcers heal in a few weeks and many more in a few months.

Against this however is the not rare experience of locally applied antiseptics and courses of injections of various antimony preparations given for one, two and three years before the case ends by complete healing or death after the ulceration has opened up rectum or bladder or both.

The following brief reports on two cases may perhaps reinforce doubt about the specificity of the antimony therapy if not yet the prime agency of the intracellular and extracellular parasite-like bodies.

Case reports

Case 1.*—A naval rating noticed a small abrasion on dorsum of shaft of penis in November 1935, seven days after coitus in Hong Kong. The abrasion became painful ulcer; later right inguinal bubo developed, was incised, and ulceration spread from this wound also. On admission to hospital in Hong Kong "the grossly undermining ulceration" had flayed penis and extended all over right lower abdomen and right perineum. Seven months later the patient was sent home to the

Fig. 1. The ulceration in May 1938.
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