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a small light eusol gauze patch in the wound and a Carrel tube. The original entrance wound was excised and a Carrel tube inserted into the tract. Two days later there was slight voluntary flexion of the toes of the right foot, followed twenty-four hours later by some movement in the arms, and the presence of a mild knee-jerk on the right side. Six days after the operation, the Carrel tubes were removed, and two days later the wound was closed by three S.W.G. stitches. Voluntary movement improved daily, and before the patient was evacuated to the Base he could raise both arms to his temples, and could flex the right knee to nearly a right angle. The relief from the excruciating pain referred to the left shoulder was rapid and gratifying. A suprapubic cystotomy was not performed. The metal catheter was left in situ for three days, to dilate the stricture, then six-hourly catheterization was carried out until some control over the bladder was established.

One is now convinced that it would have been wiser to drain the bladder suprapubically in order to avoid the danger of cystitis and to overcome the difficulties of catheterization in the presence of a stricture.

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A PRELIMINARY REPORT ON THE ICE-BOX METHOD OF PERFORMING THE WASSERMANN REACTION.

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In recent years accurate titrations of the reagents employed in the Wassermann reaction and the utilization of various non-specific lipoidal antigens have led to several undoubted improvements on the original Wassermann technique. The diversity of methods adopted has unfortunately produced an inability to correlate and compare the results of numerous competent immunologists. The special Committee, appointed by the Medical Research Committee, for the standardization of pathological methods [1] has helped considerably in overcoming this difficulty by advocating the adoption of one of three standard methods. As modern statistics, including those collected by the Research Committee [2], still show that a proportion of early primary, of late tertiary, and of latent syphilis yield negative Wassermann reactions, it would appear that in such cases still more sensitive methods are needed to detect the smaller quantities of immune body or lipotropic substance present in the peripheral blood.

In the present report an analysis is made of an investigation of 1,302 cases.

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1 Staff-Sergeant Sullivan died from the effects of malaria contracted in the Jordan Valley. He was a keen scientific worker and had acquired a very considerable knowledge of immunology.
A slight modification of Harrison's technique [3] has been adopted and a comparison is made between the results obtained by the ordinary and the ice-box methods of conducting the Wassermann reaction.

The Rationale of the Ice-Box Method.

The fundamental difference in the ice-box method is that in the first stage the system composed of antigen, complement and suspected syphilitic serum, is placed in the ice-box at 8° C. for a period of from three to twenty-four hours instead of the customary incubation of one hour at 37° C. Recorded observations to determine the “optimum temperature” for conducting the first stage of the reaction are comparatively few in number. Satta and Donati [4] state that complement is absorbed at 0° C. but perhaps more slowly than at 37° C. Jacobsthal and also Guggenheimer [5] found that in some sera more complement is absorbed at 0° C. than at 37° C. Thompsen and Boas [6] state that binding never occurs better at 0° C. than at room temperature, but that in the majority of cases the reaction took place better at room temperature (or at 0° C.) than at 37° C. Harrison's observations corroborate those of Boas and in practice both conduct the first stage (of one hour) for thirty minutes at 37° C. and thirty minutes room temperature.

In our opinion, however, the rationale of the method is dependent not so much on conducting the reaction at an optimum temperature as on increasing the time available for the reaction. The effect of low temperatures in increasing the thermostability of complement, without pari passu decreasing the power of syphilitic immune-body or lipotropic substance to unite with complement and antigen, enables this stage of the reaction to be conducted over a period of time that would be quite unsafe at 37° C.

Statistical observations on the ice-box method appear to have been exclusively made in America and have been reported on favourably by Archibald McNeil [7] in 1912, by Zinsser [8] in 1916, and by Wheeler Smith and W. J. MacNeal [9] in two papers appearing in 1916 and 1917 respectively.

Experimental Data Favouring the Method.

If the ice-box method is to be considered a technically sound procedure certain experimental conditions must be fulfilled. In a series of preliminary experiments we have investigated the effects of temperatures of 8° C. and 37° C. on certain serological reactions.

The conclusions reached are as follows:

(1) In complement stored at 8° C. no deterioration could be detected even after twenty-four hours or longer. On the contrary the complement of certain guinea-pigs when stored at 37° C. showed deterioration within three hours.

(2) The complement absorbing power of a mixture of pooled syphilitic serum and antigen was found to be much greater when the reaction was conducted for six hours at 8° C. than for one hour at 37° C. In a series of observations the excess of complement absorbed by the ice-box method over and above that

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1 The complement in all these experiments was obtained from guinea pigs and stood in contact with clot for six hours at 8° C. before being used.
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absorbed by the ordinary method was 2 M.H.D.'s for primary syphilis, 5 M.H.D.'s for secondary syphilis, 6 M.H.D.'s for tertiary syphilis, and 5 M.H.D.'s for treated relapsing syphilis.

(3) The complement absorbing power of the mixture of pooled negative serum and antigen was never more than \( \frac{1}{2} \) a M.H.D. greater when the reaction was conducted at 8°C for six hours than when it was conducted for one hour at 37°C.

These findings hold not only for the pooled sera of healthy individuals but also for the pooled sera of certain protozoal diseases, i.e., relapsing fever and malaria. Having determined the limits of the amounts of complement fixed at 8°C by pooled negative sera, and having demonstrated the thermostability of complement and the enhancement of the complement absorbing power of syphilitic serum and antigen at 8°C for six hours, one is enabled to evolve a satisfactory ice-box technique.

Technique Employed.

Owing to the rapid deterioration in titre of haemolytic serum and of antigen obtained from abroad and stored under Egyptian conditions it was found necessary to manufacture both in the laboratory. The titre of hemolytic serum utilized was never less than 1 in 2,000.

(1) The antigen advocated by Fildes and McIntosh [10] was used. The one per cent. alcoholic solution of cholesterin was stored at room temperature, the ten per cent. alcoholic extract of human heart at 8°C. Occasionally the antigen was tested for haemolytic properties and the anticomplementary dose estimated. Never more than half the anticomplementary dose was used.

(2) A fresh suspension of three per cent. of sheep corpuscles was always made. The blood was obtained from a sheep specially kept for the purpose and after citration (two per cent sodium citrate in saline) the corpuscles were repeatedly washed with saline and centrifugalized. The corpuscular suspension was sensitized by adding 4 M.H.D.'s of haemolytic serum and incubating at 37°C for half an hour. The mixture was then kept at 8°C till required.

(3) Blood was obtained from the patient within twenty-four hours of performing the test and stored in the ice-chest. Serum was diluted with four volumes of saline and heated to 55°C for twenty minutes. Complement and any thermolabile anti-complementary body present in the serum was thereby destroyed. Any serum exhibiting anti-complementary tendency in the serum control tubes or any serum in which microbic infection was suspected, was retested at a later date.

(4) Complement which was obtained from the serum of a healthy guinea-pig stood in contact with the clot in the ice-chest for six hours before use. The M.H.D. of complement was always accurately determined.

Arrangement of the System for the Final Test by the ordinary Method.—Small volumes of reagents were measured by Donald’s dropping pipettes. The total volume in the final stage of the reaction was five cubic centimetres, and each volume of the reagent equalled one cubic centimetre. Racks containing four rows of tubes were utilized and the system was put up as follows:

(1) The first row contained one volume each of antigen, serum and saline, and one volume of complement diluted so that it contained 3 M.H.D.'s of complement.
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(2) The second row contained similar volumes of the above reagents, except that the volume of complement used contained 5 M.H.D.'s.
(3) The third row contained similar volumes of reagents, but the volume of complement used contained 7 M.H.D.'s.
(4) The fourth row contained only serum, saline and complement (3 M.H.D.'s), and served to detect the presence of any anti-complementary tendency in the serum under examination.

Two antigen controls, one containing one volume, and the other two volumes of antigen, were included, and also a pooled negative, and a pooled positive serum.

In the ordinary method the above system was incubated for one hour at 37° C. and then one volume of sensitized sheep's corpuscles was added, the racks were repeatedly shaken and readings registered at intervals of fifteen minutes at 37° C. Final readings were generally made at the end of the hour.

In the ice-box method 4, 6, 8, and 4 M.H.D.'s of complement were used, an additional 1 M.H.D. of complement being added to compensate for the slightly increased tendency for fixation of complement by normal sera with this method.

The time employed for the first stage was always six hours at 8° C. Sensitized corpuscles were then added and readings registered quarter-hourly at 37° C., as with the ordinary method.

Recording of Results.—In both methods a P.+++ indicates an entire absence of hemolysis and is registered as a strong positive reaction. A P.+ means that there is an entire absence of hemolysis in tube of rows 1 and 2, and is a definitely positive reaction. A P.+ reaction indicates an absence of hemolysis in the tube of row 1 and is regarded as positive in the presence of suggestive clinical evidence of the disease or in a treated case. A P.± means a partial hemolysis in the tube of the first row, and sera giving such reactions are retested at a subsequent date.

An Analysis of Cases Investigated by the Two Methods.

The total number of cases investigated was 1,302. Of these 516 were regarded on clinical grounds as being either definite or probable cases of syphilis. In 216 of these cases no treatment had been instituted. In 300 treatment had been carried out.

The remaining 786 cases presented no clinical evidence of syphilis.

One hundred and eight of these cases were suffering from one of several protozoal diseases occurring in Egypt.

The remaining 678 cases constituted healthy soldiers or patients suffering from metazoal or bacterial infections.

(A) An Analysis of the Reactions in Untreated Syphilitics.

<table>
<thead>
<tr>
<th>Stage of the Disease</th>
<th>Total Examined</th>
<th>Positive by Ordinary Method</th>
<th>Positive by Ice-box Method</th>
<th>Additional cases detected by Ice-box Method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Per cent.</td>
<td>Number</td>
<td>Per cent.</td>
</tr>
<tr>
<td>Primary</td>
<td>70</td>
<td>44</td>
<td>62</td>
<td>74.3</td>
</tr>
<tr>
<td>Secondary</td>
<td>60</td>
<td>60</td>
<td>62</td>
<td>100.0</td>
</tr>
<tr>
<td>Tertiary</td>
<td>80</td>
<td>67</td>
<td>76</td>
<td>95.0</td>
</tr>
<tr>
<td>Latent</td>
<td>4</td>
<td>3</td>
<td>4</td>
<td>100.0</td>
</tr>
<tr>
<td>Totals</td>
<td>216</td>
<td>174</td>
<td>194</td>
<td>89.3</td>
</tr>
</tbody>
</table>

TABLE I.—"Untreated Syphilitics."
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An analysis of Table I demonstrates the superiority of the ice-box method in all stages of the untreated disease.

In early primary syphilis the ice-box method is much more sensitive than the ordinary method, but even here one must emphasize that the mode of diagnosis *par excellence* lies in the examination of the serum from the primary chancre by the method of dark-ground illumination. Frequently in early primary syphilis we have demonstrated *Spirocoma pallidum* where the Wassermann reaction was negative to both methods.

The diagnosis of secondary syphilis is satisfactory by both methods but in tertiary syphilis the ice-box method yields more sensitive results. This we have noted not only in interstitial and parenchymatous syphilis of the nervous system but also in such lesions as aortitis, syphilitic osteitis, etc.

(B) An Analysis of the Reaction in Treated Syphilitics.

<table>
<thead>
<tr>
<th>Stage of the Disease</th>
<th>Total Number</th>
<th>Positive by Ordinary Method</th>
<th>Positive by Ice-box Method</th>
<th>Additional Positive by Ice-box Method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Number</td>
<td>Number</td>
<td>Number</td>
</tr>
<tr>
<td>Primary</td>
<td>109</td>
<td>28</td>
<td>25.7</td>
<td>32</td>
</tr>
<tr>
<td>Secondary</td>
<td>139</td>
<td>44</td>
<td>34.1</td>
<td>60</td>
</tr>
<tr>
<td>Tertiary</td>
<td>62</td>
<td>41</td>
<td>66.1</td>
<td>61</td>
</tr>
<tr>
<td>Totals</td>
<td>300</td>
<td>113</td>
<td>37.7</td>
<td>143</td>
</tr>
</tbody>
</table>

In a majority of the above cases treatment was carried out by Captain R. T. Silverton, A.A.M.C. It consisted in the administration intravenously of 1.2 to 2.4 grammes of arsen-benzol (four injections) and of 3.6 cubic centimetres of gray oil (six injections) given intramuscularly over a period of six weeks. Potassium iodide was given in tertiary syphilis.

An analysis of this table illustrates certain important features:

1. The earlier in the disease antisyphilitic treatment is established the better is the prognosis. Thus after two months intensive treatment 70.6 per cent of cases of primary syphilis presented neither serological nor clinical evidence of the disease, 54.5 per cent of secondary syphilics yielded similar results, while only 17.7 per cent of tertiary cases yielded these results.

2. The comparative estimation of cure as yielded by the ice-box and ordinary methods is most important. And it is seen from Table II that ten per cent of all treated syphilics (i.e., thirty cases out of 300) which yielded a negative reaction with the ordinary technique were positive with the ice-box method.

Immunologists still agree that the Wassermann reaction affords the most reliable known index to the presence of living spirochetes and the decision of cure must ultimately be made not alone on the absence of clinical evidence of syphilis but in conjunction with at least four negative Wassermann reactions at intervals of six months.

In the following table an analysis is made of the Wassermann reaction in certain protozoal diseases, the two methods being utilized throughout:

1. It will be noted that during the pyrexial period it is not uncommon in both benign and malignant tertian malaria for 3 M.H.D.'s of complement to be fixed. In one case 7 and 8 M.H.D.'s of complement were fixed by the two methods respectively, but within three weeks of efficient quinine treatment this blood yielded negative results.
(C) INVESTIGATION OF THE BLOOD IN CERTAIN PROTOZOAL DISEASES BY THESE TWO METHODS.

TABLE III.—"PROTOZOAL DISEASES."

<table>
<thead>
<tr>
<th>Disease</th>
<th>Total Cases Investigated</th>
<th>Number cases Examined</th>
<th>Ice-box</th>
<th>Ordinary</th>
<th>Total Pyrexial Period</th>
<th>Number cases Examined</th>
<th>Ice-box</th>
<th>Ordinary</th>
<th>Pyrexial Period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign Tertian Malaria</td>
<td>19</td>
<td>12</td>
<td>P+ =</td>
<td>P+ =</td>
<td>3 cases</td>
<td>7</td>
<td>0</td>
<td>P+ =</td>
<td>P+ =</td>
</tr>
<tr>
<td>Malignant Tertian Malaria</td>
<td>44</td>
<td>34</td>
<td>P+ =</td>
<td>P+ =</td>
<td>8 cases</td>
<td>10</td>
<td>0</td>
<td>P+ =</td>
<td>P+ =</td>
</tr>
<tr>
<td>Relapsing Fever</td>
<td>32</td>
<td>10</td>
<td>P+ =</td>
<td>P+ =</td>
<td>1 case</td>
<td>22</td>
<td>3 cases</td>
<td>P+ =</td>
<td>P+ =</td>
</tr>
<tr>
<td>Oriental Sore, Cutaneous Leishmanisis</td>
<td>5</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>1 case</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Amoebic Dysentery</td>
<td>8</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

In relapsing fever, both during the pyrexial stage and between the early relapses, pseudo-positive reactions were observed.

(2) Using the technique described above there was no greater tendency to pseudo-reaction observed with the ice-box than with the ordinary method.

(D) REACTIONS IN NON-SYPHILITIC SERA.

In four cases out of 678 in which there was no clinical history of syphilis P+ reactions were registered. In the remaining cases negative results were yielded by both methods.

CONCLUSIONS.

The substitution for the first stage of the Wassermann reaction, of the ice-box method (six hours at 8° C.) and the use of the cholesterinized alcoholic heart extract of Fildes and McIntosh affords a superior technique to that usually employed.

(1) It is a more reliable method in the diagnosis of syphilitic infection, especially in early primary and late tertiary syphilis.

(2) It affords a more accurate index to the cure of the disease.

(3) Using the ice-box technique as above described there is no increased tendency to pseudo-positive reactions either in normal sera, or in the various protozoal diseases investigated.

(4) It converts a number of border-line cases or partial positive reactions as yielded by the ordinary method into strongly positive reactions.

ACKNOWLEDGMENTS.

For facilities in this investigation and for permission to publish these notes we are indebted to Colonel W. E. Summons, A.A.M.C., commanding officer, 1st Australian General Hospital.

Captain R. T. Silverton, A.A.M.C. of No. 2 Australian Stationary Hospital, Moascar, has supplied a great deal of the clinical data on which this paper is based.

It is deeply regretted that just prior to the completion of this article Staff-Serjt. Sullivan, B.Sc., succumbed to malignant malaria. By his untimely death the scientific world has lost a most promising and capable bacteriologist.
REFERENCES.

[1] "Report of the Special Committee upon the Standardization of Pathological Methods."
   No. 1.—The Wassermann Test.
[2] Ibid. No. 4.—The diagnostic value of the Wassermann Test.
[3] HARRISON. Ibid., ref. [1].

NOTE: ON AN ANOMALOUS CASE OF MENINGITIS IN WHICH PNEUMOCOCCI WERE FOUND IN CLEAR CEREBROSPINAL FLUID.

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AND

CAPTAIN A. T. NANKIVELL.
Royal Army Medical Corps.

PRIVATE J. P., aged 22, with twenty months' service in France, was in good health one morning in the trenches. That evening when marching back to rest-billets he was seized with severe pains at the back of the neck and in the legs. He fell out but was able to continue his march with assistance, taking his own time. Next morning he "went sick" and was excused duty for three days. No improvement taking place he was evacuated to the Base through the usual channels. The first record of his temperature was on the sixth day—104°F. His temperature was at the same level when he came under our observation on the tenth day; in the meantime it had never been below 102°F. When we first saw him his condition was suggestive of an infection by a member of the enteric group; he was very ill, very drowsy, had a dry, furrowed tongue, sores on the lips, tenderness in the splenic region, constipation, a temperature of 104°F. and a feeble pulse of 88. He complained of intense headache. Feeble tendon-reflexes could be obtained with difficulty, abdominal reflex was absent. There was a raised reddish blotchy rash chiefly on the limbs and especially noticeable on pressure points about the knees, elbows and ankles, but extending also to the soles of the feet. A blood-culture was negative and agglutination reactions gave no evidence that the case was one of enteric or paratyphoid. There was a leucocytosis of 20,800 per cubic millimetre.

Meningitis was suggested by the history of nuchal pains at onset, by slight stiffness of neck and back, by persistent intense headache, a "very doubtful"