

## MODIFICATIONS OF WASSERMANN'S REACTION.

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THE value of the information which the Wassermann reaction affords is so great that the test should be available in every hospital.

The following cases, which have been seen at the Royal Military Infirmary, Dublin, between the end of the year 1910 and the beginning of 1912, show that without the guidance of the serum reaction, treatment would have been inappropriate, diagnosis would have been haphazard, prognosis mistaken, and the claims of the patient against the public would have been wrongly assessed.

A married non-commissioned officer alleged that his arm had been injured through a fall from his horse while on duty. There was a firm swelling which involved and partially paralysed the biceps and deltoid. The X-ray examination was negative. The delay which occurred in the subsidence of this tumour threw doubt on its being a hæmatoma. A blood test gave the clue. Captain A. T. Frost administered salvarsan. This patient's rapid recovery was reported in the *JOURNAL OF THE ROYAL ARMY MEDICAL CORPS* of April last.

An officer, who was suffering from a chronic pustular eruption of his scalp attributed to Indian service, was sent to the Royal Infirmary for vaccine treatment. A positive serum reaction indicated the suitable remedies.

An officer appeared before a Medical Board at the expiration of sick leave, which had been granted on account of slight mental abnormality. The Board was impressed with the gravity of his condition by reason of a positive Wassermann test. Signs of general paralysis of the insane, which were not then evident, are now present.

Syphilitic fever is uncommon, and easily escapes detection. Three cases remained under observation for enteric fever during considerable periods, until the true nature of the infection was discovered by means of a positive Wassermann reaction.

Medical Boards often experience difficulty in deciding whether a soldier's disability has been caused by military service, especially in those heart cases which are not of rheumatic origin. Two non-commissioned officers were discharged on account of aortic disease. In both the blood test indicated that syphilis was the cause.

We are frequently called upon to report on ex-soldiers who have

developed some disability which they assert has originated in military service. In three instances skin gummata were said to be the sequels of gunshot wounds. Hemiplegia has been found to be of specific origin, and not due to sunstroke. A plausible soldier was invalided from India in consequence of tabes; a kindly disposed officer listened to his tale, and altered the name of the disease to neuritis. A positive serum test, however, would seem to make it clear that the original diagnosis was correct.

On the other hand a negative response has often prevented injustice being done. A non-commissioned officer suddenly became unconscious, and remained in a state of coma for some days. He was pronounced to be suffering from cerebral syphilis. His blood, however, was negative when tested on three occasions. He was the father of healthy children, and he denied infection.

A young soldier gradually became hemiplegic without obvious cause. His blood did not react to the Wassermann test.

A non-commissioned officer consulted an ophthalmic surgeon while on furlough, who diagnosed disseminated choroiditis, and prescribed specific remedies. At the Royal Infirmary it was ascertained that he was suffering from chronic nephritis with albuminuric retinitis. He stated that he had never been infected, and that his wife and children were healthy. The reaction of his blood was negative.

The absence of a positive response in optic neuritis, tubercular laryngitis, ascites, glandular enlargements, suppuration of the antrum, ozoena, sycosis, seborrhœic dermatitis, psoriasis and scabies has prevented a mistaken diagnosis of syphilis, which was suspected.

The method devised by Wassermann entails the use of fresh guinea-pig's serum, sheep's red corpuscles from recently shed blood, and the serum of a rabbit which has received several injections of sheep's erythrocytes, by which an amboceptor, hæmolytic to them, is elaborated. If a guinea-pig is sacrificed on each occasion when the test is employed, the expenditure incurred becomes prohibitive. In order to obtain the cubic centimetre of blood, which may be all that is required, otherwise than by slaughtering the animal, vivisection licences are necessary for the laboratory and the experimenter. An inquiry, therefore, into the value of the modifications of the process which can be performed by unlicensed persons was demanded. For more than two years the writer has been occupied with this investigation. As the result of twelve hundred experiments, he has come to the conclusion that these modifications are trustworthy.

No difficulty is experienced in determining the reliability of modifications of Wassermann's reaction. It is only necessary to experiment with a sufficient number of syphilitic and non-syphilitic sera. The value of the test is established if the former behave constantly in a manner different from the latter. As most of the writer's observations were made in the laboratory of the Military Hospital, Rochester Row, London, he has had the advantage of learning how the bloods reacted, when Major L. W. Harrison tested them by Wassermann's original method. He is greatly indebted to this officer for the information and facilities which he afforded him.

Three modifications have been investigated—the sheep's blood, the guinea-pig blood, and the human blood method.

*Sheep's Blood Modification.*—The first process was introduced by Hecht, and has been followed by Fleming, Gibbon, Babington, Skelton, Bassett-Smith and others. It depends on the presence in human serum of an amboceptor hæmolytic to sheep's red blood corpuscles. An emulsion of washed red corpuscles from freshly shed sheep's blood and an alcoholic extract of heart are required. The latter is prepared by macerating the heart muscle of the guinea-pig, rabbit, or man, which has been reduced to a pulp in a mortar, in five times its weight of absolute alcohol. About a third of a cubic centimetre of the patient's blood is collected in a glass capsule from a finger prick. It is preserved for four to twenty-four hours, to allow time for the elaboration of the complement. The serum, which will amount to rather less than half the quantity of blood drawn, is removed with a pipette. One part is mixed with four parts of 0.9 per cent salt solution in distilled water, free from lime salts, in a small (0.6 by 4 cm.) test tube. Into other tubes are placed similar quantities of serum, and of the dilutions of the heart extract in the physiological saline fluid, thus

Tube 1	contains	0.02 c.c.	serum	and	0.08 c.c.	salt solution.
„ 2	„	0.02	„	„	0.08	„ $\frac{1}{5}$ dilution heart extract.
„ 3	„	0.02	„	„	0.08	„ $\frac{1}{10}$ „
„ 4	„	0.02	„	„	0.08	„ $\frac{1}{20}$ „

The tubes are put into the incubator for half an hour, or into a water bath raised to the temperature of 38° C. for five or ten minutes. At the end of this period such an amount of a 5 or 10 per cent emulsion of sheep cells is added that the fluid in each tube contains 1 per cent red blood corpuscles. It is important to avoid the addition of an excess of sheep's blood; for

if the corpuscles are too numerous, lysis is delayed. Until the observer's eye is trained, he is advised to dilute one part of his own blood with 99 parts of saline solution, which he uses as a standard. The tubes are then examined every minute or two. Tube 1, which contains no extract, and serves as a control, will show complete hæmolysis in five or six minutes. If the blood has been taken from a person free from the disease, lysis will also take place in the other tubes; though there may be a delay of some minutes in the hæmolysis of the fluid in the tube in which the dose of extract is highest. Should the serum be that of a patient in the early stage of secondary syphilis, before salvarsan treatment has been begun, no lysis will occur in any of the extract tubes. Even after repeated shaking, the cells will subside to the bottom, leaving the fluid above as clear as water. If, however, the patient has undergone efficient treatment, tube 4 may become reddened in half an hour, and tube 3 may also exhibit a trace of lysis after that interval. It must be remembered that the heart extract when present in quantities above a certain limit prevents the lysis of the sheep cells by normal blood. Hence each supply of extract must be titrated with many non-luetic sera in order to fix this limit. It is necessary to observe caution in interpreting the results if hæmolysis has been slow in the control tube 1. This indicates either that the complement, or the hæmolytic amboceptor, is insufficient. If the serum is wanting in complement, less extract will be required to deviate it. Incomplete lysis may occur in tube 2, even though the blood be normal. In such an event it would be desirable to repeat the test with serum which has been kept for either a longer or shorter time than that used in the first experiment. For though complement is generally at its maximum between five and twenty-four hours after the blood has been shed, it sometimes happens that this point is reached earlier or later. If the amboceptor hæmolytic to sheep cells is absent, the test fails. Stern eliminates this contingency by rendering the sheep corpuscles sensitive to the action of the complement by treating them with rabbit-versus-sheep serum; that is, serum derived from the blood of an animal which has received several injections of sheep's erythrocytes.

Two hundred and sixty-one sera have been tested by Hecht's process. Readings were not recorded sixty-seven times in consequence of delay, or absence of lysis in the control tubes, that is in 26 per cent of the observations. The reactions of the blood of fifty-five persons who had not been infected were negative.

	Total	Positive	Negative	Percentage of positive reactions
Primary syphilis .. ..	26	18	8	70
Early secondary syphilis .. ..	31	30	1	97
Late ,, ,, .. ..	19	17	2	89
Latent syphilis.. ..	62	43	19	70
Congenital ,, .. ..	1	1	—	—
Control, non-syphilitic .. ..	55	Nil.	55	—
Failures .. ..	67			
<b>Total .. ..</b>	<b>261</b>			

One hundred and seventy-two of these sera were submitted to the original Wassermann test by Major Harrison. The results agreed in 134 instances or in 78 per cent. On thirty-six occasions, or 21 per cent, the reading of the modification was positive, and of the original negative. These were cases of syphilis which had recovered clinically. Hence the modification is a more delicate test than the original.

The failures occurred for the most part before errors of technique had been overcome. A common fault was the addition of too large an amount of the emulsion of sheep corpuscles. Sometimes this emulsion had been preserved too long. Sheep's blood which has become dark red, and darkens again rapidly after shaking, is useless. In some early observations the dose of extract was too great, or the time when lysis took place in the control tube was not noted. The want of success, however, in 10 per cent of the sera was due to the absence of hæmolytic amboceptor. As was mentioned before, Stern remedies this defect by sensitizing the sheep cells. Major Harrison has experimented largely in this way. He finds that Stern's procedure is more delicate than the original Wassermann method. The writer can speak favourably of it.

*Guinea-pig Corpuscle Modification.*—This process is based on the presence in human blood of an amboceptor hæmolytic to guinea-pig's red corpuscles. This amboceptor is less frequently absent than the corresponding constituent which acts on sheep erythrocytes. The test is in every respect similar to the foregoing, except that guinea-pig cells are substituted for sheep's. Guinea-pig corpuscles are more easily laked than sheep's, hence the proportion of failures has been smaller, and the process has occupied less time.

Three hundred examinations have been made. Failures were noted thirty-one times, that is, in 10 per cent of the experiments.

	Total	Positive	Negative	Per cent positive
Primary syphilis .. ..	23	9	14	39
Early secondary syphilis ..	35	31	4	89
Late " " .. ..	47	30	17	64
Latent syphilis.. ..	118	63	55	53
Controls, non-syphilitic ..	46	<i>Nil.</i>	46	—
Failures .. ..	31			
Total .. ..	300			

Major Harrison tested 264 of these sera according to Wassermann's method. The readings agreed 230 times, that is, in 87 per cent. On 27 occasions the response by the modification was positive, and by Wassermann negative. Seven times only did the contrary happen. Hence latent infection can be detected more surely by this modification than by Wassermann's test. Half of the failures were caused by the absence of the hæmolytic amboceptor in the human serum.

*Human Corpuscle Modification.*—This method is that which is most suitable for general use, since the ingredients are stable, and can be preserved indefinitely. A serum diagnosis, therefore, may be made at any time. A description of the process was inserted in the *JOURNAL OF THE ROYAL ARMY MEDICAL CORPS* for October, 1910. The substances required are the heart extract which was before described, and a rabbit serum hæmolytic to the red blood corpuscles of man. This is prepared by injecting 10 to 40 c.c. of washed human erythrocytes into the peritoneal cavity of a rabbit at intervals of five days, on three or four occasions. The blood is aspirated from the heart nine days after the last dose. The patient's blood is allowed to remain in the glass capsule in which it is collected from a finger-prick, for four to twenty-four hours, as in the previous methods. The clear serum is then withdrawn. The pipette is introduced again into the bottom of the capsule, or into the blood-clot, and such a quantity of red corpuscles is taken up as to make a 5 per cent mixture when they are added to the serum. One volume of this mixture of serum and corpuscles is put into a tube with four volumes of saline solution, and similar quantities of serum and extract dilutions are placed in other tubes. The fluid in each tube, therefore, contains 1 per cent of red corpuscles derived from the blood under examination. Thus:—

Tube 1	contains	0·02	c.c. serum and corpuscles	and	0·08	cc. saline.
" 2	"	0·02	" "	"	0·08	" $\frac{1}{50}$ dilution of extract.
" 3	"	0·02	" "	"	0·08	" $\frac{1}{100}$ " "
" 4	"	0·02	" "	"	0·08	" $\frac{1}{200}$ " "

The tubes are placed in a water bath at 38° C. for five minutes, or into the 37° C. chamber for half an hour. At the end of this time the red corpuscles are seen lying at the bottom of each tube; the fluid above them is untinted, as no lysis has occurred. To each tube are now added five hæmolytic doses of the rabbit-*versus*-man serum, namely, 0·01 c.c. The tubes are shaken and kept under observation. Lysis will be complete in tube 1 in from three to fifteen minutes. If the blood has been obtained from a non-luetic source, hæmolysis will take place in the extract tubes, though there may be a delay of a few minutes in tube 2, which contains the largest dose. If the blood be that of a patient in the early stage of secondary syphilis, no lysis will occur in any of the extract tubes. The cells fall to the bottom and the fluid remains colourless for twenty-four hours or more. In latent syphilis, or if intensive treatment has been adopted, lysis may appear in tube 4 in an hour, and a faint trace may be apparent in tube 3, but the fluid in tube 2 will remain free from colour: a quantitative estimate of the serum is thus made.

Serum placed in each tube is 0·02 c.c., a convenient amount. It may be reduced by one-half if the supply of blood is small. The test can be performed successfully with only 0·2 c.c. of blood, though twice or three times as much is desirable. In any case, blood collected from a prick of the finger is sufficient. Venesection is not necessary.

When practising the test, three series of four tubes are used. The first contains normal serum, the second the serum of a florid secondary case, and the third the suspected serum. Comparison of the last with the other two, enables the observer to say to which category it belongs.

Six hundred and twenty-five sera have been examined by this method.

	Total	Positive	Negative	Positive per cent
Primary syphilis .. ..	45	22	23	51
Early secondary syphilis ..	83	77	6	93
Late " " .. ..	103	88	15	85
Latent syphilis.. ..	223	127	96	58
Congenital " .. ..	5	3	2	—
Ague .. ..	6	4	2	—
Scarlet fever .. ..	7	4	3	—
Hodgkin's disease .. ..	1	1	—	—
Mania .. ..	1	1	—	—
Controls, non-luetic .. ..	132	<i>Nil.</i>	132	—
Failures .. ..	19			
Total .. ..	625			

Comparative tests by Wassermann's method were made by Major Harrison in 204 instances. The results agreed 177 times, which equals 87 per cent. In eighteen cases of well treated, or latent syphilis the blood gave a positive response to the modification, though negative to Wassermann. In nine the Wassermann was positive, and the modification was negative.

Eighty-five of the specimens of blood were tested by the sheep's corpuscle method. The readings were concordant in 76 per cent of the experiments. On fourteen occasions the sheep blood reading was positive when the human corpuscle method was negative. These sera were negative when tested by Wassermann's procedure.

The guinea-pig blood modification was applied to 123 of these sera; 89 per cent of the results agreed. Accordingly the methods confirm one another.

An advantage which the human erythrocyte modification possesses over the former two, lies in the fact that one of the variables is eliminated. In the first two, both the complement and hæmolytic amboceptor may fluctuate in amount. In the third process, we add a constant quantity of hæmolytic amboceptor. The complement alone varies. Now a minimum dose of complement cannot effect lysis except in the presence of multiple doses of amboceptor. It follows, therefore, that if the quantity of complement in a serum is small, its hæmolytic action can still be exerted through the agency of the five units of hæmolytic amboceptor added, an influence which is wanting in the first two methods, since the proportion of natural hæmolytic amboceptor in human blood is not so high.

The action of human complement on human blood corpuscles which have been rendered sensitive with rabbit-*versus*-man serum is nearly as powerful as that of guinea-pig's. If we take two tubes containing a 1 per cent emulsion of such sensitive human erythrocytes, and add to one  $\frac{1}{40}$  of its volume of six-hour-old human serum, and to the other a similar quantity of guinea-pig serum, it is often found that lysis is as rapid in the tube which contains the human complement as in the other.

With a fixed number of corpuscles, and a fixed dose of hæmolytic amboceptor, the time of lysis is a measure of the complement. Hence arises the necessity of examining the tubes every few minutes after the addition of the hæmolytic amboceptor. If lysis is complete at the same time in the control tubes which contain the normal, syphilitic and suspected blood respectively,



then the quantity of complement in each is similar, and the readings of the corresponding extract tubes are strictly comparable. The titration of complement, however, can be easily carried out. A ten-fold dilution of the serum in saline fluid is made; 20-, 40- and 80-fold dilutions are prepared from this. One volume of each of these is added to one volume of a 2 per cent emulsion of human red corpuscles, which have been rendered sensitive by means of five doses of rabbit-*versus*-man serum. The measure of the complement in each tube is therefore  $\frac{1}{20}$ ,  $\frac{1}{40}$ ,  $\frac{1}{80}$ , and  $\frac{1}{160}$  of the volume of fluid, which now contains 1 per cent of red corpuscles. The period which elapses before hæmolysis occurs, is noted. Hæmolysis is complete in the  $\frac{1}{20}$  and  $\frac{1}{40}$  tubes within fifteen minutes, if the complement is present in normal quantity, or in other words, the titre of normal human serum is  $\frac{1}{40}$ . Since the proportion of serum employed in the modifications of Wassermann's reaction is eight times as great—for  $\frac{1}{5}$  of the volume of the fluid in each tube is serum—the number of units of complement employed in these processes is eight.

If the blood be examined four to twenty-four hours after shedding, the amount of complement is not subject to great variations. In less than 2 per cent of the tests by the human blood method has the lysis in the control tube been delayed longer than twenty minutes. Though sufficient complement will have been elaborated at the end of four hours after taking the blood, it will be more convenient generally to perform the test on the following day. There is often still enough complement present at the end of forty-eight hours. It is not unusual to find it existing in small amount in three or four-day-old blood. As an exceptional experience, it was once discovered in human serum which had been preserved for nine days. It is in those cases in which we have not had an opportunity of examining the blood within twenty-four hours of its abstraction, that we scrutinize the rapidity of hæmolysis in the control tube. If there is retardation we estimate the deficiency of complement by titration and reduce the doses of extract in like proportion. Consequently the human blood modification admits the same precision as Wassermann's original method.

In both scarlet fever and ague the serum reaction was positive during the attack. It became negative in the convalescent stage.

Caan (*Münch. med. Woch.*, May 10, 1910, p. 1002) reported a positive Wassermann reaction in four cases of Hodgkin's disease; and Trembar (*Cent. f. Bakt.*, Ref. April 23, 1912, p. 523) stated

that he obtained a very strong Wassermann reaction in a fatal case of lymphosarcoma. In the patient examined by the writer there were neither signs nor history of a specific infection.

Syphilis could not be excluded with certainty in the soldier suffering from mania, whose blood gave a positive response.

Nineteen failures occurred in the 625 tests, which is equal to 3 per cent. They were caused for the most part by a delay in the examination of the blood beyond twenty-four hours.

Captain A. T. Frost, Specialist in Dermatology, Royal Infirmary, Dublin, to whom the writer is indebted for many samples of blood, and clinical reports, has himself employed the human erythrocyte modification for the examination of nearly 300 sera.

#### CONCLUSIONS.

(1) The modifications of Wassermann's reaction are trustworthy.

(2) The human corpuscle method is that which is most generally applicable. The components required are the complement in the serum, and a small portion of the erythrocytes of the blood under examination, heart extract, and rabbit-*versus*-man serum. The latter are stable.

(3) The human corpuscle method can be rendered as precise as Wassermann's original test by titrating the complement in the serum.

(4)<sup>1</sup> With a supply of Gunter and Wagner's "Pelican" Indian ink, heart extract, and rabbit-*versus*-man serum the detection of the *Treponema pallidum* can be secured, and the diagnosis of syphilis accomplished in every hospital where a microscope, pipette and tubes are available.

Heart extract and rabbit-*versus*-man serum may be obtained on application to the writer, at the Royal Army Medical College.

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<sup>1</sup> JOURNAL OF THE ROYAL ARMY MEDICAL CORPS, August, 1912, p. 203.