NOTES ON A MODIFICATION OF THE WASSERMANN REACTION.*

WITH A PRELIMINARY EXAMINATION OF 102 CASES AT THE NORTHCOTE POLICE HOSPITAL, BOMBAY.

BY

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AFTER a preliminary account of the early experiments on immunity, including the biological test for human blood worked out by Tristchowitz, Nuttall, and others, Dr. Bana described complement deviation phenomenon in syphilis as follows:

Complement Fixation.

In 1901-2 Bordet and Gengou, working at the Pasteur Institute, Paris, made known an advance in practical immunity, now known as the "Bordet-Gengou phenomenon," which forms the basis of many immunity observations at present. They showed that if typhoid bacilli were injected into a rabbit at suitable intervals and in suitable doses, the animal developed in its blood serum a body capable *in vitro* of breaking down *Bacillus typhosus*. The antibody so produced is called "bacteriolysin," and is specific. It consists of two substances—a thermostable substance resisting heat at 56° C. for half an hour, called the amboceptor or immune body, and a thermolabile substance destroyed by heat at 56° C. for half an hour, and called the complement. They showed that to bring about bacteriolysis of *B. typhosus*, antityphoid body, and complement, all three must be present in the mixture, and that the fresh antiserum contained the complement, which can be destroyed by heat or prolonged keeping, but can be restored by addition of some fresh serum of normal guinea-pigs. They applied this same phenomenon to organized cells, that is, red blood cells of different animals, such as the rabbit, sheep, etc., and found that on injection of red blood corpuscles of the sheep into a rabbit, a specific haemolysin developed which in presence of complement can produce haemolysis of the cells *in vitro*. They showed further that during this reaction the complement gets used up or disappears, and can no longer be demonstrated on adding more blood cells and complement-free amboceptor. This, then, was a proof of the action of body, antibody, and complement. The complement being no longer free was said to be fixed, and the reaction a "complement fixation." This fixation is a specific phenomenon, and strictly speaking should be applied to known body, antibody combinations such as *B. typhosus* and antityphoid serum, *B. tuberculosis* and tuberculous antiserum, etc.

Complement Deviation.

This can be merely a deviation in case of syphilis, in which Noguchi¹ has proved that fixation does not occur with syphilitic serums in presence of pure extracts of spirochaetes, but that the so-called "fixation" occurs with suitable aqueous, alcoholic, or acetone-insoluble lipoidal extracts of normal organs. The antibodies occurring in syphilitic persons are not, at any rate partially, of immune nature, that is, body antibody, and so long as the reaction is positive, unlike immunity produced during an attack of typhoid fever or pneumonia and after recovering, the patient is still in a state of infectivity. The Wassermann reaction is only a side issue, showing the degree of action and interaction of the syphilitic virus and tissues of the body, the degradation or the tissue-reaction products which can be shown by the complement being deviated by suitable organ extracts.²

In the light of modern research it is possible to show that spirochaete extracts can be made to "fix" complement in cases of syphilis possessing enough immune antibodies, thus showing that the patient has really recovered. But the sense in which the present Wassermann reaction is carried out is meant to show and to detect the evidence and presence of active infection of the patient's system.

In a patient clinically syphilitic, the antibody contents may be so small and the reaction so feeble as to be almost negative (especially if a feeble antigen and a strong

haemolytic system are used), showing that the complement, subject to the action of the two antibodies—(a) the syphilitic antibody ("reagin" of McDonagh³) and (b) the amboceptor for the red cells—will be drawn to the one or the other, according as the one or the other is the stronger. In these circumstances the Wassermann reaction, in its present form, cannot be called a fixation, but merely a deviation phenomenon.

The Original Wassermann Reaction.

In 1906 Wassermann applied the Bordet-Gengou phenomenon to syphilis. Using as antigen an extract of syphilitic fetal liver, he demonstrated that on addition of fresh guinea-pig's serum to the mixture of such an extract and a known syphilitic patient's serum, the subsequent addition of a complement-free haemolytic system (sheep's red blood corpuscles and antishoop amboceptor) did not produce haemolysis. Neisser, Bruck, and Detre, working independently, arrived at the same conclusions.

Since then the reaction has been modified at various times by various authorities. As regards the antigen, aqueous extracts of syphilitic liver used by Wassermann, Neisser, Bruck, Bauer, and Stern, were replaced by alcoholic ones at first of syphilitic, then of normal organs. For a long time the composition and the nature of the action of antigen was the subject of hot debate, and further research showed that, provided a proper "autolysis"⁴ of the organs to be extracted has been allowed to take place, a good reliable antigen can be obtained after treatment with alcohol, ether, and acetone. That such an extract as used in my reactions is truly antigenic, not haemolytic nor anticomplementary, can be proved in the following way:

The Antigenic Titre.

Seventeen tubes are taken. To these add the following, making the total at the end up to 1 c.cm. (using sheep's red cells; 1½ unit of amboceptor, and 1½ unit of complement, for the determination of which see later).†

Antigenic Titre.

Tube.	1 in 10 Antigen.	Reagents.	Result.
1	500 c.mm.	+ 100 c.mm. of red cells	I.
2	400 "	+ 50 c.mm. complement; mix well; incubate at 37° C. for 1 hour (water bath); then add 100 c.mm. red cells and 50 c.mm. amboceptor; mix; incubate at 37° C. for half an hour	C. H.
3	300 "		C. H.
4	250 "		C. H.
5	100 "		C. H.
6	100 "		C. I.
7	80 "	+ 50 c.mm. complement and 100 c.mm. of 50 per cent. known syphilitic inactivated serum; incubate at 37° C. for 1 hour, then add 50 c.mm. amboceptor and 100 c.mm. red cells; incubate at 37° C. for half an hour (water bath)	C. I.
8	60 "		C. I.
9	40 "		C. I.
10	20 "		C. I.
11	100 c mm.		C. I.
12	80 "		C. I.
13	60 "		C. I.
14	40 "		C. I.
15	20 "		S. H.
16	10 "		D. H.
17	Nil	+ 50 c.mm. complement, red cells and amboceptor as above	C. H.

I.=Inhibition; C. H.=complete haemolysis; C. I.=complete inhibition; S. H.=slight haemolysis; D. H.=distinct haemolysis.

Thus the antigen is not haemolytic at least in 500 c.mm. nor anticomplementary in four times its usual volume, but completely antigenic in 0.0004 c.cm.

The modification followed here is that in which all the following variable but known factors are used as follows. The reagents are:

The Antigen.—It is titrated as above, the acetone insoluble product containing the N₂P₁ fraction utilized; 100 c.mm. of 10 per cent. is used for each reaction.

† The units of complement and amboceptor vary a little from time to time and require to be estimated before each reaction. The complement more often varies than the amboceptor.

* A paper read at the Bombay Branch of the British Medical Association (modified).

Patient's Serum.—About 5 c.cm. of blood is drawn from the patient's median basilic or cephalic vein by a sterile platinum needle. The blood is allowed to clot, serum collected and inactivated at 56° C. for half an hour; 100 c.mm. of 50 per cent. solution, less or more in special modification, is used for a test.

Complement.—Blood is obtained from guinea-pigs, allowed to clot, serum centrifuged, and the unit enabling a unit of amboceptor to haemolyse 100 c.mm. of 10 per cent. sheep's red cells is determined; 1½ unit is used in each test. The unit varies a little from time to time and is determined as shown below.

Amboceptor.—Rabbits are inoculated with increasing quantities of washed sheep's cells till a titre of 0.0004 to 0.0001 is obtained; 1 per cent. dilution is used to determine the unit; 1½ units are used for each test.

Red Cells.—Sheep's red cells are obtained by collecting blood from the ear in saline containing 2 per cent. citrate, centrifuged and washed three times in saline; 100 c.mm. of 10 per cent. suspension is the standard taken.

Simultaneous Estimation of Amboceptor and Complement.

Five series of tubes are taken; each series contains five tubes. In the first series (1-5) put 100 c.mm. of $\frac{1}{10}$ amboceptor, in the second (6-10) 80 c.mm., in the third (11-15) 60 c.mm., in the fourth (16-20) 40 c.mm., in the fifth (21-25) 20 c.mm. of amboceptor.

To each first tube (tubes 1, 6, 11, 16, 21) add 50 c.mm. of 50 per cent. complement.

To each second tube (tubes 2, 7, 12, 17, 22) add 40 c.mm. complement.

To each third tube (tubes 3, 8, 13, 18, 23) add 30 c.mm. complement.

To each fourth tube (tubes 4, 9, 14, 19, 24) add 20 c.mm. complement.

To each fifth tube (tubes 5, 10, 15, 20, 25) add 10 c.mm. complement.

Add 100 c.mm. red cells; mix well. Make up total volume to 1 c.cm.; incubate at 37° C. for half an hour (water bath); read results. That tube containing the least amount of complement and amboceptor causing complete haemolysis is the unit of complement and amboceptor. Use 1½ units for each test.

The Reaction.—One takes two series of tubes and numbers them 1, 2, 3, 4, 5, etc. In the front row is now placed 100 c.mm. of 1 in 10 solution of antigen. Excepting tubes No. 1, put 1½ units of complement in all the tubes. In tubes No. 2, front and back rows, put 100 c.mm. of known non-syphilitic serum; in tubes No. 3 known syphilitic serum same amount; in the other tubes, Nos. 4, 5, and the rest, the serums to be tested. Mix well after each addition. Put 100 c.mm. of 10 per cent. red cells in tubes Nos. 1. Incubate at 37° C. for one hour, then add 1½ units amboceptor previously determined and 100 c.mm. of red cells, in all the tubes except No. 1; incubate at 37° C. for half an hour after careful mixing. Read results an hour after, or immediately on centrifuging. The results are as follows: No haemolysis in tubes 1 back and front, as there are only saline and antigen with corpuscles, both non-haemolytic. In tubes No. 2 complete haemolysis should occur in absence of antibody to deviate the complement which is free to produce haemolysis. In tube No. 3, front row, there is inhibition or no haemolysis, as the syphilitic antibody deviates the complement in presence of antigen in the front tube, and not in back (where there is no antigen), showing haemolysis. In the rest of the tubes—4, 5, etc.—the results will be complete haemolysis or no inhibition to slight inhibition, moderate inhibition to complete inhibition, as the case is negative, feebly, fairly and strongly positive.

Special Modification.—In cases of doubt, as in feeble reactions, and in cases under treatment where the quantitative reaction is to be followed (to ascertain the units of syphilitic antibody, as done by Noguchi¹ in following a case), one employs three or more rows of tubes, a third, etc., in front of the second. In this last row one puts 200 c.mm. of inactivated serum, and the results are then expressed as follows:

Strongly Positive:

- ++++ when both front tubes show complete inhibition.
- +++ when the third front row shows complete, and second front row incomplete inhibition or slight haemolysis.

Feeble or Weak:

- ++ when the third front row shows good inhibition, and second front row only slight inhibition.
- + when the third front row shows good inhibition, and second front row nil.

The Results.

The following is a summary of results obtained by me in 102 cases in which blood serum, and cerebro-spinal, pericardial, and hydrocele fluids were examined at the Northcote Police Hospital, including a series of 14 reactions taken in persons under observation prior to certification as to their state of mind at the Northcote Police Hospital observation cells, by special request of Dr. A. Powell, Police Surgeon, Bombay.

Control Cases.—Blood serum was examined in 6 cases from amongst the laboratory and the hospital staff. Negative results were obtained in all cases—all normal.

Diseases other than Syphilis.—Forty-nine cases were examined in which conditions other than syphilis prevailed (a list of these cases is appended); 46 cases, or nearly 94 per cent., gave negative results. Of the 3 positive, 1 was of nodulo-anaesthetic leprosy, 1 rat-bite fever, and 1 of mixed infection, which subsequently turned out to be syphilitic. In the latter the earliest in which the reaction was found positive seven days after the appearance of the papule. Noguchi gives the fifth day as the earliest. The results on the negative side compare favourably with the claim of the Wassermann reaction as excluding conditions and diseases other than syphilis.

Diseases other than Syphilis.

Diseases.	No. of Cases Examined.	Results.	
		Positive.	Negative.
*Malaria	5†	0	5
Glandular enlargement (elbow) ...	1‡	0	1
Neuritis	1	0	1
††Pericardial fluid	2	0	2
Tubercle	3§	0	3
Epididymitis (gonorrhoea) ...	1	0	1
Gonorrhoea and soft sore ...	2	0	2
Dysentery and soft sore ...	1	0	1
"Brown rash all over body 2 years" (Dr. D.'s case)	1	0	1
Leprosy	2¶	1	1
*Rat-bite fever	1	1	0
Corneal ulcer	2	0	2
Traumatic ulcer, foot (shoe-bite) ...	1	0	1
Traumatic ulcer, heel	1	0	1
Ulcerative stomatitis	1	0	1
Small-pox	1	0	1
Typhoid fever	2	0	2
Diarrhoea (amocbae not found) ...	1	0	1
Malingering	3	0	3
Soft sores, multiple sores, bubo groin	9	1	8
††Hydrocele fluid	1	0	1
Guinea worm (eosinophilia 32 per cent.)	1	0	1
Catarrhal jaundice	1	0	1
Asthma	1	0	1
Convalescent pneumonia ...	1	0	1
Pyorrhoea alveolaris	1	0	1
§§Kala-azar (by special request of Dr. Row)	1	0	1
Testicular enlargement 3 years (by special request of Lieutenant-Colonel Street, I.M.S.)	1	0	1
Total	49	3	46

* In all cases the malarial parasites were demonstrated in smears, a routine examination of all fever cases admitted being made. The results are in accord with the findings of Fletcher (On the Wassermann Reaction in Malaria at Kuala Lumpur, *Lancet*, June 13th, 1914). All cases of true malaria, unless complicated with syphilis, yield negative results.

† Four benign (parasites in blood) tertian; one malignant tertian.

‡ No filaria in blood.

§ One C.S.F., tubercle in lung; one paraplegia, tubercle in spine; one tubercle in left apex.

¶ One nodulo-anaesthetic, positive reaction; one anaesthetic (*B. leprae* in smear from patch), negative reaction.

** By kind permission of Captain Jones, M.D., I.M.S. No signs nor symptoms nor history of syphilis.

†† Complement fixation with tubercle (kindly lent by Dr. Row) also negative.

‡ Fluid obtained *post mortem* from control accident cases (otherwise healthy).

§§ Complement fixation with the patient's serum and kala-azar antigen was done by the writer by special request of Dr. Row, who describes the test elsewhere in detail.

Now as to *lucetic cases*: 31, including manifest, latent, and doubtful cases of syphilis, were examined. Of these, 24 gave a positive reaction.

Out of a total of 14 cases with sores (5 clinically Hunterian), 4 yielded positive results. One negative case

with a receding papule had two injections of neo-salvarsan before the test. In a positive case with mixed infection the reaction was obtained on the seventh day. The percentage of findings is rather large, as all the cases came directly under observation without previous treatment. I do not consider the reaction as essential at this stage, though it may help to differentiate chancroidal conditions and urethral chancres.¹ The finding of *Spirochaeta pallida* at this stage is more easy and equally reliable, according to Major Harrison. Of the other 9 cases of multiple sores, buboes, and gonorrhoea, 8 gave negative results. Of 17 cases of secondary syphilis (including 2 latent cases with positive reactions), 14 yielded positive results, or 87 per cent.; 8 cases of tertiary syphilis were examined, 4, or 50 per cent., gave positive results, 4 negative; of the latter, 1 was a chronic alcoholic, had one injection of salvarsan and took mercury; 2 had been under mercury for a long time, more than four years. Thus the results depend on the stage in which the patient is and the treatment the patient has had.

Persons under observation at the Northcote Police Hospital Cells.

Diseases.	No. of Cases Examined.	Results.	
		Positive.	Negative.
Cerebral syphilis	1	1	0
Tabo-paresis	1	1	0
Uncertifiable (no signs of syphilis)...	1	1	0
Melancholia	1	1	0
Delusional insanity	1	1	0
Senile dementia	2	0	2
Toxic insanity (Ganja)	3	0	3
Acute mania	1	0	1
Malingering	2	0	2
Homicide	1	0	1
Total	14	5	9

Out of 14 cases examined amongst persons under observation at the Northcote Hospital cells prior to the certification of their mental condition, 5 gave positive reactions—that is, 35.7 per cent. One positive case showed early signs of cerebral syphilis, loss of memory and inco-ordinate speech. There was a history of syphilis contracted five years ago. Of the remaining 4 positive cases, 1 was a tabo-paretic with perforation of the septum and Romberg's sign and Argyll Robertson pupil and delusions; the other 3 presented evidences, such as general glandular enlargement, scar on the penis. This shows that the prevalence of syphilis is large amongst the class of people who eventually become residents of asylums or gaols. The writer hopes to pursue his observations on this class of patients.

Lastly, I may mention a case sent by Dr. D. for an opinion, as the patient wanted to marry:

A. S., aged 22, a Jew, gave the following history: Had primary sore October, 1912; secondaries, March, 1913. Had three injections of salvarsan—0.4, 0.6, 0.6 gram—under the late Dr. Jan Mohamad, M.D.Lond., at intervals of fifteen days. He continued mercury after this from May, 1913, to a month prior to the examination, when he presented no signs or symptoms. He gave a negative reaction.

The case in question demonstrates the importance of the Wassermann reaction, not only for diagnosis but for the more important point of prognosis in syphilis.

I thank Dr. A. Powell, Police Surgeon, Bombay, and during his temporary absence on leave his successors, Captain D. Jones, I.M.S., and Major Murison, I.M.S., for kindly placing at my disposal clinical material at the Northcote Hospital, Bombay, during the six months over which the observations extend.

REFERENCES.

¹ Noguchi: *Serum Diagnosis of Syphilis*. ² F. H. Thiele and D. Embleton: Method of Increasing the Accuracy and Delicacy of the Wassermann Reaction, *Lancet*, vol. i, p. 526, February 21st, 1914. ³ McDonagh: *Salvarsan in Syphilis and Allied Disorders*. ⁴ F. H. Thiele and D. Embleton: A Method of Increasing the Accuracy and Delicacy of the Wassermann Reaction, *Lancet*, vol. i, p. 1032, April 11th, 1914.

INSECTS AND WAR:

XIII.—THE MOSQUITO (*Anopheles maculipennis*).

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(Continued from p. 331.)

Gnats are unnoted wheresoe'er they fly,
But eagles gaz'd upon with every eye.

SHAKESPEARE, *Rape of Lucrece*.

The Egg.

THE eggs of the mosquito are deposited in fresh water, and at first they are white, but they very rapidly darken until they assume a polished black appearance. Each egg is 0.72 mm. in length and its greatest breadth, which is somewhere about its middle, is 0.16 mm. The egg is boat-shaped and one end, as is usual in boats, is slightly deeper and fuller than the other. The under surface is fluted and is marked by a minute network. The upper surface has a coarser reticulation which divides the surface into nearly equal hexagonal areas. The rim of the "boat" is thickened, and these thickenings are regularly ribbed: they extend over about the median third of the egg and recall the rounded float which runs along the edge of a lifeboat, and indeed they serve the same purpose, for they are composed of air cells, and their function is to keep the boat-shaped eggs right side upward. Soon after the egg has been laid it is of a greyish-black colour, but after a certain amount of attrition an outer membrane splits off—the membrane which has given the egg its reticulated appearance. This membrane scales off in fragments, and is of a grey colour. The egg beneath it is glistening black, as shiny and as black as patent leather.

One curious fact that Professor Nuttall and I noticed in the life-history of the egg is that when it is drawn by capillary action a little way out of the water on to the leaf of a water plant or some other half-submerged object, the blunt end always points downwards. Now the blunt end is the head end, and thus, should hatching take place whilst the egg is suspended half in the water and half in the air, the larva will emerge into its proper element and not into the atmosphere.

Like other objects floating on the surface, the mosquito egg slightly indents the surface. The number of eggs seems to vary. According to Grassi, each female deposits about one hundred eggs, whilst Howard puts the number as varying from forty to one hundred. According to the former writer, the eggs of *A. maculipennis* lie side by side like the bridges of boats which span the Rhine, whilst those of *A. bifurcatus* arrange themselves with their ends in contact, forming star-like patterns. Unlike the eggs of the gnat (*Culex*), the eggs of *Anopheles* do not adhere together, and the result is they are very readily scattered by the wind. But in sheltered places, like a laboratory aquarium, if undisturbed, Grassi found that they tended to congregate together, as indeed do most minute objects floating on the surface of the water. Our observations did not entirely confirm those of Grassi. In Cambridge, at any rate, we found the eggs in our aquaria always scattered. Very frequently empty eggshells were met with, but they too were scattered. As a rule, the ova are deposited in water rich with algae or other vegetable life, and are more frequently in shallow than in deep water, the temperature of shallow water being naturally somewhat higher.

On the second or third day after oviposition (and this depends upon the temperature), the young larva leaves the egg and begins to swim in the water. The egg hatches by the detachment of a cap-like portion of the anterior end of the eggshell. There is no visible ring indicating the limits of this operculum, but the cap is usually more or less of the same size. Opinions differ as to how far desiccation interferes with development of the larva in the eggshell. They do not seem to be able to stand more than forty-eight hours of drought. There is no evidence that they can survive throughout the winter period. Everything that we know indicates that the egg must pass this period within the mother's body, and that they only attain maturity in early spring, when the weather becomes warmer.