A METHOD OF TESTING ANTIBACTERIAL SERA, WITH SOME OBSERVATIONS ON THE IMMUNISING BODIES IN THEM.

By W. M. CROFTON, M.B.

(*From the Pathological Laboratory, the Medical School, Cecilia Street, Dublin.*)

THE treatment which Dr A. E. Wright has advocated of certain chronic infections, by the injection of sterilised emulsions (vaccines) of the micro-organism causing the disease, is now well known, but a brief statement of its principles may make the following observations more clear.

Wright has found that the injection of such vaccine increases the phagocytic power of the patient's leucocytes, and that this power of phagocytosis is due to the increase of a substance in the patient's serum which is destroyed by heating to a temperature of 60° C., for ten minutes. This substance is supposed to prepare the micro-organism for phagocytosis, and in its absence the phenomenon of phagocytosis cannot take place. This substance Wright calls "opsonin," and the power of the serum to prepare the micro-organism for phagocytosis is termed the "opsonic power" of the serum. This opsonic power is estimated in the following manner. Equal quantities of the patient's serum, of an emulsion in normal saline of the micro-organism, and of washed leucocytes are mixed together in a capillary pipette, and incubated for a definite time at 37° C. A stained preparation is then made of the incubated mixture, and the average number of micro-organisms in the polynuclear leucocytes is estimated. The result of this estimation is compared with the result obtained from a similar preparation made from one's own serum, which is taken as a standard.

While estimating the opsonic power of the blood of some coccus-infected patients whom I have been treating by Wright's method, it occurred to me that a similar technique would furnish a simple and

https://doi.org/10.1017/S0022172400006744 Published online by Cambridge University Press

efficient test for the quality and power of antibacterial sera in general. Accordingly I obtained some "polyvalent" anti-streptococcus serum and proceeded to test it, adopting Wright's technique.

In all the experiments I used my own washed leucocytes. The streptococci were obtained from a scalp wound in which the suppuration showed a marked tendency to spread. The serum was dated Feb. 1st, 1905, and was first tested on Feb. 6th, 1905.

Experiment I.

Tube I contained a mixture of anti-streptococcus serum, emulsion of cocci and leucocytes.

Tube II contained a mixture of my own serum, emulsion of cocci, and leucocytes.

The tubes were incubated for 30 minutes at 37° C., and the average number of cocci in the leucocytes estimated. The following were the results.

Tube I. Average number of cocci in 30 polynuclear leucocytes = 11

Tube II. „ „ „ „ = 12·3

This shows that the opsonic power of the anti-streptococcic serum of this particular strain of streptococcus was less than that of my own serum.

That this power was very rapidly lost after opening the phial is shown by the fact that on repeating the experiment next day the tube corresponding to tube I gave an average of 4 cocci per polynuclear leucocyte. A fresh phial of the same serum obtained on Feb. 24th gave an average of 0 cocci per polynuclear leucocyte.

These experiments demonstrate the progressive loss of opsonin in serum which is kept for any length of time, and if the opsonic hypothesis of immunity from bacterial infections is correct, it is clear that the serum, in order to be of benefit to the patient, would have to be quite recently drawn off. This would mean that the treatment of streptococcal and other bacterial infections by means of antibacterial sera is outside the range of practical therapeutics, since to obtain the maximum effect the serum would have to be almost directly transferred from the immunised animal to the patient.

Considering this, it seemed possible that the opsonic hypothesis might be brought into line with the hypothesis of haemolysis and bacteriolysis, and that the opsonin, which is heat labile (disappearing on heating to 55° C.) and also time labile, might have similar characteristics to complement or alexin. If this were so there might be in the inactive serum a body, heat stable and time stable, corresponding to immune body, copula, or substance sensibilisatrice, and the inactive serum could be reactivated just as an inactive haemolytic serum can be reactivated.

To investigate this point the following experiments were undertaken.

https://doi.org/10.1017/S0022172400006744 Published online by Cambridge University Press

Experiment II.

In this experiment the first sample of anti-streptococcic serum was again used. Equal parts of my own freshly drawn serum and anti-streptococcic serum were mixed, and used as the serum element in tube I. The serum element in tube II was made by mixing equal parts of my own fresh serum and normal saline solution. In each case cocci and leucocytes were added as usual, and the mixtures incubated for a quarter of an hour at a temperature of 37° C.

(*a*) Tube I. Average number of cocci in 30 p.n. leucocytes = 8·7
Tube II. „ „ „ „ = 6·6

The next day the experiment was repeated with an exactly similar result. On Feb. 24th, with the new phial of anti-streptococcic serum, the result was as follows:

(*b*) Tube I. Average number of cocci in 30 p.n. leucocytes = 16·2
Tube II. „ „ „ „ = 11·3

The larger number of cocci taken up by the polynuclear leucocytes in this experiment was due to the fact that the emulsion used contained more cocci per unit volume than in the former experiment.

What then are the conclusions to be drawn from these experiments? If nothing corresponding to the immune body were present I expected to find that the results obtained from the two tubes would be similar, whereas if such a substance were present tube I would give a larger average per polynuclear leucocyte than tube II. This actually proved to be the case, and the fact would tend to show that there is present a substance corresponding to the immune body, or cupola of Ehrlich's hypothesis, or the substance sensibilisatrice of Bordet.

These experiments furthermore do not support either of the two rival hypotheses of Wright and Neufeld. According to Wright the substance in the serum which prepares the micro-organism for phagocytosis is opsonin, an unstable body, which in his view directly attacks the micro-organism, and neutralizes whatever may be in it which prevents it from serving as food for the leucocyte. Neufeld's view is directly opposed to that of Wright. He worked with a highly immune serum, obtained from rabbits, by injecting a very virulent strain of streptococcus. If an emulsion of these cocci, either in serum previously inactivated by heating, or in salt solution, were mixed with some of this immune serum which was also inactivated, and white corpuscles added, the whole being then incubated, Neufeld found that the polynuclear leucocytes took up enormous numbers of these cocci. It will be seen that anything of the nature of opsonin or complement was carefully excluded by heating the immune serum, by using inactivated serum or salt solution for making the emulsion of the cocci, and by washing the corpuscles.

https://doi.org/10.1017/S0022172400006744 Published online by Cambridge University Press

The conditions of my experiments with the anti-streptococcic serum were practically identical with those of Neufeld's experiments. The serum was inactive, the emulsion of cocci was made with normal saline solution, and the corpuscles were washed. His ingredients were mixed in a hanging drop, mine in a capillary tube, but in no case in my experiments did any phagocytosis take place unless there was some heat labile substance (opsonin, complement) present. I cannot see any explanation of this divergence of results, unless the mode of action of a highly immune serum differs from that of a less highly immune serum, an unlikely hypothesis.

Neufeld contends that Wright's results ought not to be compared with his, since Wright was working with weak normal serum, while he was working with highly immune serum. The following experiments show, however, that the immunising substances in my own serum and in the serum of a patient in the early stages of immunisation, are similar to those in the immune sera obtained from animals.

Experiment III.

The test organisms were staphylococci. In tube I the serum element consisted of equal parts of my own freshly drawn serum and of my own serum heated for a quarter of an hour to 60° C. The serum element in tube II consisted of my own fresh serum and of the serum of a patient which had been heated for a quarter of an hour to 60° C. I expected to find that if no substance such as an immune body were present the results obtained from the two tubes would be similar, while if such a substance were present the results obtained from the two tubes would differ considerably.

Tube I. Average number of cocci in 20 Polynuclear leucocytes = 12·9
Tube II. „ „ „ „ = 8·7

Experiment IV.

Tubes I and II made as in the last experiment.
Tube I. Average number of cocci in 20 Polynuclear leucocytes 8·3
Tube II. „ „ „ „ 4.8

Experiment V.

The conditions being again similar.

Tube I. Average number of cocci in 20 Polynuclear leucocytes 4·0
Tube II. „ „ „ „ 4·4

The patient whose serum was used in this experiment was approaching the end of her treatment, whereas in experiments III and IV the patients were in the early stages of the treatment.

https://doi.org/10.1017/S0022172400006744 Published online by Cambridge University Press

The above experiments show that there is a substance in my own and slightly immune sera which is heat stable, and which has a decided influence in the opsonic power of the serum, and consequently, is presumably of the same nature as the substance in the highly immune sera obtained from animals, in fact is the immune body.

To sum up the results. According to Wright the substance in the serum which prepares the micro-organism for phagocytosis is heat labile; according to Neufeld it is heat stable; while the above experiments seem to show that an interaction of a heat labile with a heat stable substance is necessary for the process to take place.

There remains the practical question as to whether an inactive immune serum is of any use in the treatment of a patient. Experiment II *a* and *b* show that it is, provided that the necessary complement is present in the patient's plasma. It would seem that the effect of this heat stable substance is to concentrate so to speak the inimical complement on the invading micro-organism. In some severe infections, where complement may be almost or completely absent, the injection of inactive or feebly active serum would be useless. To meet this difficulty Wassermann has suggested the injection at the same time of some normal serum, in order to provide complement. There seems to be no reason why this should not be done, since the difficulties would be no greater than in the method of transfusion for the loss of blood.

The next serum tested was some antistaphylococcus "aureus" serum which was sent to me for trial.

Experiment VI.

In tube I was a mixture in equal quantities of the antistaphylococcus serum, emulsion of cocci and corpuscles.

In tube II the serum element was my own fresh serum. Both tubes were incubated for twenty minutes at 37°C.

Tube I. Average number of cocci in 20 Polynuclear leucocytes = 0·0
Tube II. „ „ „ „ = 9·5

Repetition gave an exactly similar result, and later I obtained another sample of the serum and repeated the experiment with the following result.

Tube I. Average number of cocci in 30 Polynuclear leucocytes = 0·0
Tube II. „ „ „ „ = 8·4

https://doi.org/10.1017/S0022172400006744 Published online by Cambridge University Press

Experiment VII.

A similar method was adopted with this serum as with the antistreptococcic serum, the serum being reactivated with my own freshly drawn serum. In tube I the serum element consisted of my own freshly drawn serum and the anti-staphylococcic serum in equal parts. In tube II the serum element was my own fresh serum and salt solution in equal parts.

Tube I. Average number of cocci in 20 Polynuclear leucocytes = 4·2
Tube II. „ „ „ „ = 7·2

This experiment gives a result the exact reverse of the similar experiment with the antistreptococcic serum (Experiment II (*a*) and (*b*),) showing the presence of some substance having a distinctly inhibitory influence on my serum. That this substance was heat resistant is shown by the following experiment.

Experiment VIII.

Tube I was made up as in Experiment VII except that the antistaphylococcic serum was previously heated for a quarter of an hour to 60° C. Tube II was as before.

Tube I. Average number of cocci in 30 Polynuclear leucocytes = 4·3
Tube II. „ „ „ „ = 7·0

I then tried to find out on which of the elements in my serum this substance was acting. The following experiment shows that the substance present was antagonistic to the complement.

Experiment IX.

The cocci used in both tubes were first incubated with my inactavited serum for half-an-hour at 37° C. To tube I was added a mixture of my own fresh serum and antistaphylococcic serum in equal parts, they having been previously incubated together for a quarter of an hour at 37° C. In tube II the mixture consisted of my own fresh serum and salt solution in equal parts and similarly incubated together. The final mixture in each tube was completed by the addition of corpuscles.

Tube I. Average number of cocci in 30 Polynuclear leucocytes = 3·8
Tube II. „ „ „ „ = 6·3

Repetition of the experiment gave a similar result.

The question remains how this anti-complement is to be accounted for. I am informed by the manufacturers that the serum used was that of a horse which had been immunised by increasing doses of sterilised broth cultures of the staphylococcus, given at intervals of four days, and that the serum was drawn off on the eleventh day after the last injection.

https://doi.org/10.1017/S0022172400006744 Published online by Cambridge University Press

It has been suggested to me that the phenomenon was due to the presence of some preservative added to the serum by the manufacturers. I find on enquiry that a small quantity of trikresol is added to all the sera they supply, so that if the effect was due to the presence of this substance, it ought to have been present in the case of the anti-streptococcic serum. Possibly the explanation may be that the horse had attained a condition of hypersusceptibility.

Conclusions.

1. That there is in inactive immune serum a substance corresponding to immune body.
2. That a like substance is present in my own serum and in the serum of patients in the early stages of immunisation.
3. That the micro-organism is prepared for phagocytosis by the interaction of two substances, one heat labile (complement), the other heat stable (immune body).
4. That an inactive immune serum is of use in treatment provided that complement is present in the patient's plasma.

Whether the above conclusions prove to be right or wrong, I trust the original object of the experiments has been attained, and a simple method of testing antibacterial sera demonstrated.

I am greatly indebted to Professor McWeeney for his kind assistance and keen criticism, both of my experiments and their results.

https://doi.org/10.1017/S0022172400006744 Published online by Cambridge University Press