

THE APPLICATION OF IMMUNITY REACTION TO THE CEREBRO-SPINAL FLUID.*

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Time does not allow of a preliminary explanation and discussion of the various theories of immunity by which modern investigators have sought to explain the changes which occur in the serum of an animal which has been inoculated with some foreign cell or toxin.

Suffice it to say that the hypotheses of Ehrlich have so well withstood the assaults of critical experimentation that they have come to be tacitly accepted as, at least, a convenient working basis until chemistry in its rapid strides shall have come to our aid and simplified what are now merely to be expressed as properties possessed by a serum into definite colloid or proteid formulas. In the following paper the use of technical terms has been avoided so far as possible, but such as do occur are among those which were introduced by Ehrlich and are still in constant use.

One of the ingenious applications of an immunity reaction to diagnosis has been the so-called "complement-fixation test" introduced by Bordet and Gengou in 1901.¹ The immune property of a serum, e. g., the immunity which a typhoid patient's serum acquires against the typhoid bacillus, by reason of which it agglutinates and destroys the bacilli in the Widal test, is known to consist of two parts. One part, called antibody, represents that peculiar property of the serum which makes it immune to that organism and to no other. This antibody is thermostabile, that is, it is not affected by heating the serum to 56 C. The other part, complement, is a constituent of all sera whether normal or immune. It is thermolabile and is driven off by heating to 56 C., but can be restored by simply adding a small quantity of fresh, unheated, normal serum from the same or from another suitable species. So, if we first heat the typhoid patient's serum and then add it to an extract made from a broth culture of typhoid bacilli, the organisms will not be destroyed, for the serum has been inacti-

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vated by the loss of its native complement. But by adding a few drops of serum, from, let us say, a guinea-pig, the patient's serum is at once re-activated and the lysis of the bacilli takes place.

If we have a patient on whom we wish to make a specific diagnosis of typhoid, we have the direct method of demonstrating the bacilli in the patient's blood or excreta, usually a difficult task, or the indirect method of determining the existence of antibody to typhoid in the patient's serum. For the latter procedure we may do the well-known Widal test, or resort to the complement-fixation test, which is somewhat as follows: In a test-tube which we will call tube No. 1, we place a suitable amount of the serum to be tested and of an extract of typhoid bacilli. Next we add a small quantity of fresh guinea-pig serum as complement. If the complement in this guinea-pig serum is absorbed, we can tell that the patient was suffering from typhoid and that an antibody to the typhoid bacillus had formed in his serum, otherwise the complement would remain free, for it is bound only where the serum is specifically immune to the organism present. But there is nothing in the appearance of the fluids in the test-tube to indicate what has taken place; how are we to prove that complement has been absorbed? For this we use, as an indicator, an immune reaction which also requires complement for its fulfillment, but which is easily visible to the naked eye—namely, hemolysis. A rabbit is injected repeatedly with increasing quantities of sheep blood from which the serum has been washed and the corpuscles suspended in physiological salt solution. As a result of this there is generated in the serum of the rabbit an antibody for sheep red-blood cells—a fact which can be demonstrated by bringing the two together, when the suspension of sheep erythrocytes will change in appearance from a bright, opaque red to a clear, transparent cherry color, due to the destruction of the corpuscles and the liberation of their hemoglobin. A small quantity of this rabbit's serum is heated to drive off its complement and placed in a test-tube, which we will call tube No. 2, together with a suspension of sheep red-blood-cells. This gives the potential for hemolysis, complement alone being needed. If the contents of tube No. 1 be added to that of tube No. 2 and hemolysis occurs in the latter, it indicates that free complement existed in tube No. 1 and means that the patient's serum did not contain typhoid antibody. On the other hand, if

hemolysis does not occur, we can say that typhoid antibody did exist in the patient's serum and, acting specifically on the extract of typhoid bacilli, caused the absorption of complement. This is known as the complement-fixation or complement-absorption test.

In syphilis, however, we deal with an organism which has not been cultivated and concerning whose very morphology we are not yet clear. In 1906, Wassermann, Neisser and Bruck¹ published their method of using the complement-fixation test in the diagnosis of syphilis. For an analogue of the extract of typhoid bacilli they used a watery extract of the liver and spleen of a syphilitic foetus, after first ascertaining by smears that the organs contained large numbers of spirochaetes. They added to this the heated serum of a syphilitic and found that complement was absorbed. Conversely, when they used serum from a person who did not have syphilis, complement was not absorbed. Later in the same year Wassermann and Plaut² performed the test in general paralysis and tabes, using, instead of the blood-serum, the cerebro-spinal fluid. They obtained the same results as with syphilitic serum, with the same negative controls. The blood serum in these diseases also sometimes gave positive reaction but in a very much smaller per cent than the spinal fluid. Thus it appeared that we were about to be given a new diagnostic criterion for general paralysis and a proof of its specific relationship to syphilis.

It was soon found, however, by other investigators, that an extract from normal livers could be used just as satisfactorily as that from syphilitic organs and that an alcoholic extract did quite as well as a watery one. So it is now known that the presence of syphilitic organisms in the Wassermann extract had nothing to do with the reaction, but that the latter depended upon certain lipoid bodies which are abundant in the liver.

Although the specificity of one element in this so-called Wassermann reaction has been thus exploded, the test remains a valuable one, for a very high percentage of blood-sera from syphilis and spinal fluids from metasymphilitic diseases evince this peculiar property of absorbing complement in the presence of lipoids, while sera and spinal fluids from other disorders are practically always negative in this regard. The sharing of this characteristic by that group of disorders is significant of their relationship.

The technique of the Wassermann reaction as applied to the cerebro-spinal fluid consists in placing a certain quantity (.1 — .2 cc.) of the specimen to be tested in a tube and adding to it the proper amount (1 cc.) of extract of syphilitic or normal organs. 1 cc. of a 10 per cent solution of complement in the shape of guinea-pig serum is then added and the whole incubated at 37 C. for 30 minutes to give time for the complement to be bound. Then the hemolytic system, consisting of 1 cc. of a 5 per cent suspension of red-blood-cells of a sheep and two units, or twice the quantity necessary for complete hemolysis, of the heated serum of a rabbit immunized to sheep erythrocytes, is added. This rabbit serum will cause hemolysis of the sheep blood-cells if there is free complement in the tube, so the tube is again placed in the oven and observed at frequent intervals for several hours. If hemolysis occurs it shows that complement was not bound by the first ingredients and constitutes a negative reaction. The non-occurrence, or blocking, of hemolysis similarly would constitute a positive reaction, since it indicates that complement was absorbed in the first instance. Numerous controls are of course set out for each test.

It will be seen that the process is a laborious one, necessitating as it does the immunizing and bleeding of animals, the making of extracts of organs, the washing of blood-cells, etc. One of the chief objections is that the ingredients are used in a fluid form. In this state they deteriorate steadily and have to be standardized, itself a time-consuming procedure, at nearly every series of experiments. In short, the test was possible only in a well-equipped laboratory and by trained workers.

After a series of experiments, Noguchi⁴ of Rockefeller Institute, has succeeded in so modifying and simplifying the technique that it bids fair to become almost a bed-side test. In the first place, he substituted for sheep blood in the hemolytic system, the blood from a human being. Thus, instead of requiring fresh sheep blood for each repetition, it is only necessary to prick the ear or finger of some individual who is known to be free from syphilis, let a few drops fall into a small tube of physiological salt solution, and we have our blood suspension. For the other components in the reaction he has succeeded in impregnating absorbent paper with the liver extract, with the serum of the rabbit which was immunized to human blood and with fresh guinea-pig serum or complement.

Complement, especially, is very unstable and is active only for about three days in the fluid state, while when dried on paper it will retain its strength for months. These various papers are standardized in the laboratory so that small squares of certain dimensions represent, each, the required dose of that particular solution in the test. In paper form the substances will keep for long periods and, of course, are infinitely easier of manipulation. The substitution of the anti-human for the anti-sheep hemolytic system is a great advantage in that it does away with the difficulty which often arises from the presence in human blood-serum of a natural hemolysin for sheep blood.

The possibility of preparing this paper form of the various components in the Wassermann reaction in large laboratories by pharmaceutical houses and its distribution by them, is apparent, and an extremely valuable diagnostic test for syphilis and general paralysis may thus be brought within the reach of any practising physician who possesses a little technical skill.

With regard to the reliability of this reaction in the diagnosis of general paralysis, there have been some widely varying results by different workers. The majority, however, attest to its occurrence in from 60 to 75 per cent of all cases of that disease. In a communication about to be published, Noguchi and the writer have compared the Wassermann reaction with the butyric and other tests as applied to the spinal fluid. The Wassermann reaction, while not giving as high a percentage of positive results as the butyric acid reaction and the cell-count, is of undoubted value, and by eliminating some of the opportunities for error which now exist its efficiency will be much increased. In the series just mentioned, which consisted of about 200 cases, the Wassermann reaction gave 73 per cent of positive findings in general paralysis against 90 per cent which showed the butyric acid reaction and increased cell-content. A negative Wassermann reaction, then, is relatively of much less significance than the other tests, and doubtful reactions are very common. But a definitely positive result can be accepted without much question as meaning a syphilitic or metasyphilitic disorder of the central nervous system. Even in cases of acute inflammatory disease of the meninges in which the proteid and cell-content are very high, the Wassermann reaction is always negative. In two cases of psychoses other than general

paralysis a positive reaction was obtained, but syphilis could not be excluded in their histories. Only four cases of spinal fluid from active lues without nervous involvement were examined, but all were negative. A certain number of such cases, however, have been found in the experience of others to give positive results, and this opens the question of whether these are the persons who, in later life, are prone to develop general paralysis. If such cases could be followed it would help much to clear up the subject of whether the nervous system is invaded at the time of the luetic attack or is subsequently involved.

We may conclude, then, that in general paralysis and tabes, and in lues of the central nervous system, there exists in the cerebro-spinal fluid, besides an increase in cells and proteids, a characteristic which enables it to bind complement in the presence of certain lipoids. That this property is possessed also by syphilitic serum but appears to be absent in other conditions. That the detection of this substance not only aids in the diagnosis of general paralysis, but also is strong presumptive evidence of the relationship of this disease to syphilis.

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