THE NEW METHOD OF DETECTING LATENT SYPHILIS.

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If we bleed a guinea-pig and mix some of its serum with the red blood corpuscles of a rabbit, these cells undergo no alteration. Inject this guinea-pig with the red blood corpuscles of a rabbit on three or four occasions at ten-day intervals; the serum of this animal will now have the property of laking the red blood corpuscles of a rabbit, that is, of causing them to part with their haemoglobin.

The serum of this guinea-pig which has received subcutaneous injections of rabbit's blood is found to consist of two constituents:—

No. 1 Substance.—Alexin, also known as cytase or complement. This is destroyed by heating the serum at 55° C. for half an hour. It is not specific and is present in the blood of any animal.

No. 2 Substance.—Sensibilisatrice, also known as “fixateur,” “amboceptor,” “immune body,” or “anti-body.” It withstands heating at 55° C. for half an hour, by which it is freed from the No. 1 substance, or alexin, present with it. This No. 2 substance is strictly specific, and has the peculiar property of being absorbed by the red blood corpuscles of a rabbit and by none other.

Hence to cause the dissolution of the rabbit’s red blood corpuscles we require (a) No. 1 substance, or alexin, which may be derived from the blood of a normal rabbit, or guinea-pig, or from any other animal, including man; (b) No. 2 substance, or sensibilisatrice, or “anti-body,” which can only be obtained from the blood of another species of animal after its inoculation with rabbit's red blood corpuscles. Haemolysis will not occur if only one of these constituents be present. Thus rabbit’s blood mixed with human serum which contains No. 1 substance, or alexin, but no No. 2 substance, remains unchanged; no laking is produced. Also rabbit’s red blood corpuscles mixed with No. 2 substance, or “anti-body” alone, retain their haemoglobin, but the corpuscles absorb the “anti-body” readily, and in this condition, when added to any fluid containing No. 1 substance, or alexin, rapidly undergo haemolysis and at the same time use up No. 1 substance, or alexin, in the process. We are, therefore, now in possession of a reagent, namely, these specially prepared rabbit red blood corpuscles, which
will detect the presence or absence of No. 1. substance, or alexin, in a fluid. They may be called "sensitive corpuscles."

We then proceed to another experiment. The blood of a normal guinea-pig has little effect on cholera vibrios. Inject this animal with an emulsion of this microbe on three or four occasions, at ten-day intervals. We shall thus obtain a serum which has the power of converting into granules and destroying cholera vibrios. As before, we analyse this serum and find that it is composed of two constituents:

No. 1 Substance.—Alexin, cytase, or complement, destroyed by heating at 55° C. for half an hour; not specific—that is, it is contained in the blood of any animal.

No. 2 Substance.—Sensibilisatrice, also known as "fixateur," "amboceptor," "immune body," or "anti-body," which resists heating to 55° C. for half an hour. Any of the No. 1 substance which may have been present with it is thus eliminated. This No. 2 substance, or "anti-body," is strictly specific, and can only be obtained from an animal immunised with the cholera vibrio. It has the property of being absorbed by this microbe and by no other organism.

 Destruction of the cholera vibrios does not occur if only one of the above constituents be present in a serum, but when the cholera commas are suspended in a fluid containing No. 2 substance, or cholera "anti-body," alone, they readily absorb it, and are then in a condition to take up No. 1 substance, or alexin, from any fluid to which they may be added. Add them to such a liquid. The vibrios are seen to undergo destructive changes. At the same time the No. 1 substance, or alexin, disappears. The disappearance of this No. 1 substance, or alexin, is therefore an index of the destruction of the vibrios. We can accordingly ascertain without the aid of the microscope whether transformation of the cholera vibrio has taken place or not, by testing the mixture for the absence or presence of No. 1 substance, or alexin. If No. 1 substance is found, the vibrios are unchanged. If No. 1 substance is absent, the vibrios are destroyed.

But we have already prepared a reagent for the detection of No. 1 substance, or alexin, namely, the "sensitive" red blood corpuscles of the rabbit. These remain unchanged if the No. 1 substance, or alexin, has vanished, but part with their haemoglobin if alexin be free. We are now in a position to test the properties of an unknown serum. For example, we are called upon to determine whether a certain serum contains cholera No. 2 substance,
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or cholera "anti-body." We first heat the serum to 55° C. for half an hour to destroy any No. 1 substance, or alexin, it may contain. We introduce cholera vibrios, and after a time a known quantity of No. 1 substance, or alexin, derived from the fresh blood of any normal animal. If the cholera No. 2 substance, or "anti-body," is present, the No. 1 substance, or alexin, will be consumed, and its absence recognised by the addition to the mixture of "sensitive" blood cells, our alexin detector. If these remain undissolved, alexin is absent. We then know that the alexin we added has been used by the cholera vibrios. This is possible only through the intervention of cholera No. 2 substance, or "anti-body," which, therefore, was present in the unknown serum. The principle portrayed is universal. A microbe in conjunction with its No. 2 substance, or "anti-body," specific to itself, takes up and fixes No. 1 substance, or alexin.

By this procedure we have accomplished means by which a specific No. 2 substance, or "anti-body," can be demonstrated. The method will strike the reader as being extremely ingenious. Bordet and Gengou ¹ were the talented experimenters who first devised it, and it is usually called their "alexin-fixation reaction." It has been widely adopted, and has proved of great value and exactness in the detection of specific No. 2 substances, or "antibodies," in many infections, such as plague, typhoid and paratyphoid fevers, dysentery, and notably in tubercle and gonorrhoea. By a modification of it, it is possible to determine the presence of traces of blood so minute as to escape notice altogether by other tests, however delicate.

We will now apply our knowledge to the diagnosis of syphilis. From the foregoing it is obvious that in a mixture containing the following in suitable proportions: No. 1 substance, or alexin; No. 2 substance, or "anti-body," specific for the Spirochaeta pallida; No. 3, S. pallida; the alexin will be combined with the S. pallida through the intervention of the No. 2 substance, or syphilitic "anti-body." On utilising our alexin test—"sensitive blood corpuscles"—no alexin will be discovered—that is, the "sensitive cells" will remain unchanged; their hemoglobin will not be discharged. If the No. 2 substance, or syphilitic serum, in the above mixture be replaced by normal serum, the alexin will remain free, since the S. pallida is unable to combine with it through the absence of specific No. 2 substance, or syphilitic "anti-body."

¹ J. Bordet et O. Gengou, Annales de l'Institut Pasteur, May, 1901, p. 289.
Haemolysis of the "sensitive" blood cells will therefore result on adding our alexin reagent. So by the addition of *S. pallida* and No. 1 substance, or alexin, to an unknown serum, we can discover whether the specific No. 2 substance, or syphilitic "anti-body," is contained in that serum. Also it is plain that if the *S. pallida* be absent in the above mixture, the alexin will have nothing on which to fix itself and will remain free. If, then, to an unknown secretion, or exudation, we add in proper proportions (a) No. 1 substance, or alexin, and (b) specific No. 2 substance, or "anti-body," namely, syphilitic serum, we discover the presence of the *S. pallida* in that secretion or exudation by the alexin becoming fixed. Its disappearance is put in evidence by the alexin test. The "sensitive" blood cells remain unaffected.

The details of the technique adopted by Marie and Levaditi, the latest experimenters, are here given. To prepare their "sensitive" red blood corpuscles, or "alexin test," they gave several injections of sheep's blood to rabbits. After some weeks they bled them and heated the serum at 56° C. for half an hour. This gave them their No. 2 substance, or "anti-body," specific for sheep's red blood corpuscles. Sheep's red blood corpuscles were washed in physiological salt solution and made with it into a 5 per cent. emulsion. This emulsion was treated with No. 2 substance, or sheep's blood "anti-body." The sheep's blood corpuscles were thus rendered "sensitive," and were now their "alexin detector." For No. 1 substance, or alexin, they used fresh guinea-pig's blood serum.

Since it is impossible to obtain cultures of *S. pallida*, emulsions of liver and spleen of infants which had succumbed to hereditary syphilis were used. These viscera were proved, microscopically, to be rich in *S. pallida*, and were then finally minced and emulsified in physiological salt solution, 20 grammes in 100 cc., and 0.5 per cent. phenol added. The supernatant fluid, after centrifuging, was used as the emulsion of *S. pallida*. The source of their specific No. 2 substance, or syphilitic "anti-body," was the serum of a patient suffering from secondaries, or of a baboon which had received inoculations of syphilitic virus.

This is the detail of one of Marie and Levaditi's experiments, by which they demonstrated the presence of syphilitic No. 2 substance, or "anti-body," in the cerebro-spinal fluid of a general paralytic.

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They mixed:

(1) 0.1 cc. of fresh guinea-pig's blood serum—No. 1 substance, or alexin.

(2) 1 cc. of cerebro-spinal fluid suspected to contain syphilitic "anti-body."

(3) 0.1 cc. of liver extract—S. pallida emulsion.

The mixture was incubated at 36° C. for two hours. They then employed their alexin detector, which was prepared by mixing:

(1) 0.1 cc. sheep's blood—No. 2 substance, or "anti-body."

(2) 1 cc. of 5 per cent. emulsion of washed sheep's red blood corpuscles.

This mixture was added to the first and the whole incubated for one hour at 36° C. No hemolysis was observed. This proved that the alexin they had added had disappeared. This disappearance could only be rendered possible by the S. pallida fixing it through the intervention of No. 2 substance, or "anti-body," specific for the Spirochaeta, contained in the cerebro-spinal fluid. They controlled their results by observing complete hemolysis—that is, presence of free alexin, when they employed the fresh guinea-pig's blood serum and cerebro-spinal fluid only, without any liver extract, and when they mixed guinea-pig's blood serum and liver extract without any cerebro-spinal fluid. Moreover, they proved that the reaction was negative—that is, the alexin was not fixed—when they substituted normal infant's, in place of the Spirochaeta-containing, liver, and when they made use of normal cerebro-spinal fluid instead of that derived from syphilitics or paralytics.

The following are abstracts of all observations published up to the present time.

Neisser, Bruck and Schucht¹ examined 163 persons presenting symptoms of active syphilis, and by means of Bordet-Gengou "alexin-fixation reaction," proved the existence of the S. pallida in exudations or discharges from, or the syphilitic "anti-bodies" in, the blood serum of 70 per cent. In 58 per cent. of ninety-nine syphilitic patients who bore no signs of the disease at the time of examination the results were positive.

Wassermann and Plaut² investigated the cerebro-spinal fluid of forty-one general paralytics and detected the syphilitic "anti-bodies" in 88 per cent. The reaction quite failed when the cerebro-spinal


fluid of twenty-two other persons were used as controls. The latter list included some cases of cerebro-spinal meningitis. They showed that the cerebro-spinal fluid of the general paralytics was richer in the "anti-body" than the serum of the same patients. They inferred that this excess was caused by the local invasion of the S. pallida on the cerebro-spinal nervous system. Marie and Levaditi have confirmed Wassermann and Plaut’s researches. They discovered the syphilitic "anti-body" in the cerebro-spinal fluid in 73 per cent. of thirty-nine general paralytics, and in 66 per cent. of the cases of tabes examined.

L. Detre¹ found the syphilitic "anti-body" in the blood serum of two syphilics. He also detected the presence of S. pallida in the liver, pancreas and discharges from condylomata.

It seems that this method of diagnosing latent syphilis can be adapted for use in the United Kingdom, where it is illegal to obtain a drop of blood from the ear of a rabbit or guinea-pig by the prick of a needle, but where we incur none of the penalties of the law if we thus vivisect ourselves. We should require a serum hemolytic for human blood obtained by injecting washed red blood corpuscles of man into an animal on several occasions. The alexin test would then be composed of our own washed red cells, rendered "sensitive" by being treated with this serum. Our own fresh blood serum would provide us with the No. 1 substance, or alexin. The liver of a syphilitic foetus rich in Spirochaetæ, if desiccated, retains its properties for a long period, and would give us a convenient supply of Spirochaeta virus. The serum of a patient in the active secondary stage would be the source of our specific No. 2 substance, or "anti-body."

It is universally agreed that the technique is difficult and must be controlled in every way, or fallacious conclusions may be reached. Moreover, as in most other laboratory procedures, positive results afford good grounds for positive opinions, but negative observations do not necessarily warrant us to express the contrary.