The Value of Simple Laboratory Tests in the Diagnosis of Neuro-syphilis as compared with the Wassermann Reaction.[1]

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THE complement-fixation test, or, as it is known more popularly, the Wassermann reaction, was at one time considered the only reliable laboratory test for the diagnosis of neuro-syphilis. It is a complicated biological reaction, and requires expert knowledge, theoretical and practical, before any deductions can be made as to whether a patient suffers from neuro-syphilis or not. In the majority of cases the cerebro-spinal fluid has to be sent to a special pathologist attached to some general hospital, and in many cases it means sending the fluid to other towns.

For the last twelve months I have tried to compare simpler methods with the Wassermann test, to enable physicians to investigate the cerebro-spinal fluid in the hospital itself. Accurate methods are paramount, as on them depend not only the diagnosis, but also the prognosis of the case. Different pathologists have tried to simplify the complement-fixation test, but even the simplest test is very complicated, and requires special apparatus and much time, which cannot always be afforded.

The results of the methods employed were compared with the results of the Wassermann reaction in every case, and the findings are very interesting.

Four methods were used, each of which gave satisfactory results, but I consider that only by correlation of findings can information of value be obtained. A positive Wassermann reaction of the cerebro-spinal fluid is proof of syphilitic disease of the central nervous system, either interstitial (syphilis affecting primarily the meninges, blood-vessels and the neuroglia) or parenchymatous (syphilis affecting primarily the neurons—as seen in general paralysis and tabo-paresis). In stationary cases of locomotor ataxia the fluid is usually negative. The reaction may be regarded as always positive in general paralysis. A negative cerebro-spinal fluid and serum practically exclude a diagnosis of general paralysis (1). The early diagnosis of general paralysis is important, as the result of the treatment with tertian malarial blood, by producing fever, is very encouraging, and, since Pötzl discovered the means of keeping

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the malaria parasite alive for 60 hours at least, every case of general paralysis or tabo-paresis should be given a chance of treatment.

Normal cerebro-spinal fluid obtained by lumbar puncture is water-clear and limpid; its specific gravity is 1006–1008. It contains a trace of albumen, but no globulin and no fibrin. Three of the methods employed by me depend upon the presence of proteins—globulin and albumen—in the cerebro-spinal fluid, and their precipitation by certain reagents.

The first method is that of Nonne-Apelt (2). It is a very satisfactory test. Equal parts of cerebro-spinal fluid and a saturated solution of ammonium sulphate are shaken together in a test-tube. The reaction is positive if cloudiness appears within three minutes. Depending on the quantity of globulin present in the fluid it may be cloudy, opalescent, or only slightly opalescent in the same way that the Wassermann reaction may be retarded, weak, strong, or very strong. If cloudiness appears within three minutes the quantity of globulin is excessive, and the fluid may be considered positive. The Ross-Jones (3) modification of the Nonne-Apelt consists in the formation of a white ring at the junction of the saturated solution of ammonium sulphate and cerebro-spinal fluid. The white ring, really precipitated globulin, should also appear within three minutes.

The second method is that described by Weichbrodt (4). Three parts of a solution of corrosive sublimate (1 in 1000) are mixed with seven parts of cerebro-spinal fluid (·3 c.c. 1 in 1000 corrosive sublimate plus ·7 c.c. cerebro-spinal fluid). Results should be noted immediately. Fluid may be clear, opalescent, or cloudy. Cloudy fluid is very suggestive of neuro-syphilis.

The third method is that by Boltz (5). This test probably depends upon the presence of minute quantities of cholesterol in the cerebro-spinal fluid of patients suffering from neuro-syphilis. It is a colour test, and the result of it in every way coincided with the results of the Wassermann reaction. The technique is very simple, 1 c.c. of fluid is placed in a test-tube; to it ·3 c.c. of pure acetic anhydride is added drop by drop. The mixture is gently shaken; to it is added ·8 c.c. of pure concentrated sulphuric acid, and the whole gently shaken. Within five minutes the colour of the fluid is examined against a white background. A blue-pink or lilac shade shows the fluid to be positive. If the fluid remains clear or yellowish in colour, the fluid is negative. Boltz seems to be of opinion that it is not a cholesterol reaction, as the addition of sulphuric acid alone does not give the characteristic change of colours as in the Salkowski (6) reaction. Although the theoretical explanation of the colour test is not the object of this paper, I think

that it is very likely a cholesterol reaction after all, being due to minute quantities of cholesterol kept in solution by the protein in the fluid of a patient suffering from neuro-syphilis. Cholesterol is found in almost every part of the body. It is insoluble in water, but a little of the white of an egg mixed with water gave the characteristic colour of the acetic anhydride sulphuric acid test. Boltz's test is really a slight modification of the Liebermann-Burchard (6) reaction.

The fourth method consists in examining and estimating the cells in the spinal fluid. They should be made as soon after obtaining the fluid as possible. The Fuchs-Rosenthal (7) counting chamber (made by Hawksley) is undoubtedly the most satisfactory. The chamber is divided into sixteen large squares, each of which is again divided into 16 small squares ($\frac{1}{400}$ sq. mm.). The height of the chamber is ·2 mm., therefore the volume of fluid in the chamber is 3·2 c.mm. To disintegrate any red cells and to aid in differentiating white cells the spinal fluid is diluted with a stain in the ratio of 10 parts of fluid to one part of stain. One of the best stains is the following mixture:

Sat. alc. sol. methyl violet . .	15 c.c.
Glacial acetic acid . . .	50 ,,
Distilled water . . .	100 ,,

Count the cell contents in all the squares, divide by 3·2 and then multiply by $\frac{11}{10}$. The kind of cells is important. Small and large lymphocytes are the predominating cells in syphilitic involvement of the central nervous system. Normally there are 2–3 cells per c.mm. of fluid; if more than 10 lymphocytes per c.mm. are present, neuro-syphilis should be suspected (8).

Sixty cases were examined, including 28 cases of general paralysis and one case of locomotor ataxia. In every case of general paralysis the Wassermann reaction was positive; the Nonne-Apelt, Weichbrodt and acetic anhydride sulphuric acid tests were positive. There was a varying leucocytosis. The Wassermann reaction was negative in the case of locomotor ataxia, as were the Nonne-Apelt, Weichbrodt, and acetic anhydride sulphuric tests. The remainder consisted of cases of epilepsy, psychosis due to arterio-sclerosis, senile dementia, dementia præcox, cases of melancholia, mania, and psychosis due to alcoholism. In these the Wassermann reaction was negative. The Nonne-Apelt, Weichbrodt, and acetic anhydride, sulphuric acid tests were also negative.

Conclusions.

1. The laboratory diagnosis of neuro-syphilis can be simplified by using the methods mentioned above.

2. A few graduated 1 c.c. pipettes and a few test-tubes is the whole apparatus required for the first three methods, and the whole laboratory investigation takes only about 30 minutes.

3. The reagents are easily obtainable and cheap.

4. The tests were found to coincide with the findings of the Wassermann reaction.

5. In every case of general paralysis the reactions were positive and there was an increase in the number of lymphocytes in the cerebro-spinal fluid.

6. The more advanced and active the syphilitic condition of the nervous system, the more positive were the Nonne-Apelt, Weichbrodt, and acetic anhydride sulphuric tests.

It is worth mentioning Pötzl's technique for transporting tertian malarial blood for inoculating patients suffering from general paralysis.

Ten to 15 c.c. of venous blood are withdrawn from a patient suffering from tertian malaria, and allowed to run into a sterile glass flask of 30 c.c. capacity, containing 10 to 15 small glass sterile beads. The flask is closed with a sterile cork and the whole gently shaken for ten minutes, at the end of which time the blood becomes defibrinated and partially hæmolyzed. The serum is then gently poured into a sterile test-tube, corked, and placed either in an ice-chest or a thermos flask containing ice at 0° C. The amount of blood to be injected into a patient varies from 2 to 6 c.c., either subcutaneously or intra-muscularly.

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