The cause of this resistance has been made the subject of further study. After testing the saponin and eel serum on a variety of red cells, the supernatant fluid was pipetted off, and tested on normal red cells. It was found that the fluid which had been in contact with resistant cells was least hemolytic, and vice versa. It is conceivable that the resistant erythrocytes may either absorb a disproportionate quantity of the hemolysin, or may contain a neutralizing substance.

It has been customary in human pathology to judge of the resistance of red cells according to their vulnerability in anisotonic solutions of salt. The above described experiments indicate that specific resistance to a circulating toxin may be associated with marked loss of resistance to anisotonic solutions of salts. The red cells in advanced cases of cancer have been shown (Lang and others) to possess a greatly increased degree of resistance to anisotonic solutions. The demonstration of a hemolysin in the circulating blood of cancerous cases, and of an increased resistance thereto on the part of the red cells, has made it possible to prove that the resistance is specific to this hemolysin, and only accidental and occasional for the anisotonic solutions.

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The butyric reaction for syphilis in man and in the monkey.

By **HIDEYO NOGUCHI.**

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In a preliminary communication[1] I stated elsewhere that an increase in certain protein constituents of the blood serum and of the cerebro-spinal fluid of patients suffering from active or latent syphilis or parasyphilitic affections is a constant occurrence. I wish to describe here briefly the technique of employing butyric acid for the detection of this increase of protein.

Cerebro-spinal fluid. — One or two parts[2] of spinal fluid[3] are mixed with five parts[4] of 10 per cent. butyric acid solution[5] and are

[1] Noguchi: *Jour. of Exp. Med.*, 1909, xi, p. 84.

[2] 0.1 or 0.2 c.c. are sufficient and convenient.

[3] Must not contain blood.

[4] 0.5 c.c. for the quantities above specified.

[5] Best in 0.9 per cent. salt solution.

heated over a flame to a brief boiling. One part[1] of normal solution of NaOH is then added quickly to the heated mixture and the whole is boiled once more for a few seconds. The presence of an increased content of protein in a spinal fluid is indicated by the appearance of a granular or flocculent precipitate which gradually settles under a clear supernatant liquid. The intensity of the reaction varies greatly according to the amount of the protein which a given specimen contains, but the granular appearance of the precipitate means a positive reaction for syphilis or parasyphilitic affections.

With normal or non-specific specimens there will be a slight opalescence or sometimes a marked turbidity which, however, does not settle out in several hours or even in 24 hours.

Blood serum. One part[2] of clear serum is mixed with nine parts[3] of half saturated solution of ammonium sulphate. Upon complete precipitation, the mixture is centrifugalized and the compact deposit (globulin fractions) is separated from the supernatant fluid by decantation. The deposit is then redissolved in ten parts[4] of 0.9 per cent. salt solution, in which it easily dissolves. The globulin solution thus obtained is ready for the acidification with butyric acid. This is done by mixing one part of the solution with an equal part of 10 per cent. butyric acid solution. It is my custom to take 0.5 c.c. of each solution for mixing. On standing, prompt and dense turbidity begins to appear in the tubes containing the fractions of the serum of syphilitic or certain non-syphilitic patients, while those from normal serum remain quite clear after several hours, or show only slight opalescence without precipitation.

A few words may be added here as to the results of investigations made with the above methods. About 250 specimens of cerebro-spinal fluid, mostly of parasyphilitics, and about 300 specimens of the blood of syphilitic and parasyphilitic patients, together with many control specimens derived from patients with non-syphilitic diseases and normal persons, have been studied.

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[1] Namely, 0.1 c.c. in this case.

[2] Usually 0.5 c.c. is sufficient and convenient.

[3] Namely, 4.5 c.c. in this instance.

[4] 5 c.c. in this instance.

Spinal fluid derived from parasyphilitic cases gives a typical reaction, becoming granular in a few minutes and sedimenting in from 10 to 15 minutes. Cerebro-spinal fluid from cases of congenital, tertiary or secondary syphilis gives quite constantly a positive reaction, but the intensity is usually less and two hours may be required before the characteristic granular appearance becomes manifest. Cerebro-spinal fluid from cases of cerebral or spinal syphilis gives invariably a positive reaction. Negative reaction was obtained with the spinal fluid from cases of acute anterior poliomyelitis, epilepsy, alcoholic psychosis, dementia precox, senile dementia, spastic paraplegia, lobar pneumonia and typhoid fever. On the other hand, an abundant flocculent precipitate was usually formed with the spinal fluid from cases of tubercular meningitis, influenza meningitis, or epidemic cerebro-spinal meningitis.[4] Cerebro-spinal fluid collected from two cases of hydrocephalus also gave abundant precipitation. In all of these acute inflammatory cases, except one of hydrocephalus, the Wassermann reaction was, however, negative. A number of post-mortem spinal fluids were examined with such results that it seems desirable to use the method as a routine diagnosis for syphilis or parasyphilitic affections at autopsy. In the spinal fluid of two monkeys with active experimental syphilitic lesion at the site of inoculation, which persisted about 6 months, the reaction was positive.

Referring to the results of examinations of the blood serum, it appears that the reaction is non-specific for syphilis, because a similar reaction can be obtained in certain cases of tuberculosis, carcinoma and Hodgkin's disease.

In view of the constancy with which an abnormally high globulin content attends the florid stage of syphilis and appears to be present in an early primary stage, and is present in the late secondary and tertiary stages of imperfectly treated cases, one is thus enabled to follow the course of an anti-syphilitic treatment. Moreover the butyric acid test is a more delicate indicator than the Wassermann reaction, for the latter is very frequently negative in this latter class of cases. Under conditions of adequate treatment, the globulin fraction of the blood serum is not increased. A negative re-

[4] These acute inflammatory conditions are quickly and perfectly excluded by clinical and usual microscopical methods of diagnosis.

action with the butyric acid test indicates either the absence of syphilitic infection or a successful cure of the disease. There is no necessary relation between the Wassermann test and the quantity of globulins in the luetic serum.

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The quantitative separation of leucin from valin.

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Of the known amino-acids determined in semi-quantitative estimations of final proteolytic products, leucin and its relatives, isoleucin and valin, have proven unusually difficult to prepare pure in even approximately quantitative amounts. The separation of these substances, because of their close physical and chemical similarity, has offered almost insurmountable difficulties to previous investigators. The acids form isomorphous mixtures which are absolutely inseparable by crystallization; and their esters have so nearly the same boiling points that they cannot be fractionated by distillation. Because of these difficulties, most investigators have not attempted to separate the mixture, but have reported the entire mass as leucin. Fischer[1] states that all the figures reported from his laboratory for leucin in protein hydrolyses refer to this mixture. Ehrlich[2] has recently reported a method for separating the three substances, but it involves a long process, large losses, and the racemization of the isoleucin and valin.

We have been able to separate the leucin isomers readily from valin in quantitative amounts. The method, which is very simple, rests on the fact that if a molecular lead acetate solution is added to an ammoniacal solution of the leucin-valin mixture, the leucins are precipitated as analytically pure $Pb(C_6H_{12}O_2N)_2$. If too great an excess of lead acetate is added, a portion of the valin may also be precipitated. Consequently, the mixture is first analyzed, an estimate of the proportion of leucin calculated from the carbon content, and 20 per cent. excess of the theoretical amount of lead

[1] Fischer: Unters. über Aminos., Polypeptide, und Proteïne, p. 67.

[2] Ehrlich: *Bioch. Zeitschr.*, 8,'399, 1908.