XLIV. THE OCCURRENCE OF DIASTASE IN THE CEREBRO-SPINAL FLUID.

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THE earliest investigations into the chemistry of cerebro-spinal fluid were carried out on fluid obtained from cases of hydrocephalus and spina bifida, and so we find that as early as 1891 Toison and Lenoble [1891] reported that they found the cerebro-spinal fluid of a hydrocephalic to have a slight amylolytic power. Several years later Cavazzani [1896] observed similar results in two such cases. Galletta [1908], who examined eleven normal cases, only found amylase in two of them. More recently Mestrezat [1912] stated that in two normal fluids he found just a trace of diastase, and in his book he comes to the conclusion that normal cerebro-spinal fluid has no amylolytic power or only very slight traces of it. Stocks, however, has stated [1916] that he had succeeded in demonstrating amylase in every fluid of the body which he had examined, including the cerebro-spinal fluid.

It is important to note that the term "normal" as applied to cerebro-spinal fluid must be considered merely relative, for the mere fact of there existing a reason for withdrawing some of the fluid from the living person makes the case a pathological one. Again, post-mortem fluid is known to differ from that in the living, so that observation on post-mortem cerebro-spinal fluid cannot be considered normal. It is only by considering a series of fluids taken during life and post-mortem that it is possible to arrive at some conclusion as to what the normal is likely to be. Both Galletta and Mestrezat (*vide supra*) define their "liquides normaux" as obtained from "sujets chirurgicaux."

The cases in the investigation about to be described fall into two groups. Firstly those in which the cerebro-spinal fluid was taken during life and secondly those in which the fluid was taken at known intervals after death. In many of the cases in the latter group a specimen of blood was taken at the same time for purposes of comparison.

Method. The method used for estimating the diastatic content of the cerebro-spinal fluid was a slight modification of the method for other body fluids, described in conjunction with Dodds [1924].

The following solutions are required:

1. Buffer solution. This is made by mixing 15 cc. of Sørensen's phosphate solution A with 85 cc. of solution B. The resulting solution should have a p_H of 6.1. Solution A: 11.876 g. of $Na_2HPO_4 \cdot 2H_2O$ dissolved in 1 litre of boiled

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distilled water. Solution B: 9.078 g. of KH_2PO_4 dissolved in 1 litre of boiled distilled water. The solutions are kept in paraffin-coated bottles, and a drop of toluene is added to prevent the growth of moulds.

2. Standard dextrin solution: 0.75 g. of pure erythro-dextrin is dissolved in a small volume of boiled distilled water, and the volume is made up to 100 cc.

3. Iodine solution:

<i>N</i> /10 iodine in potassium iodide solution	...	20 cc.
Saturated ammonium sulphate solution	...	500 „
Distilled water		1000 „

Procedure. 1.5 cc. of cerebro-spinal fluid are pipetted into a tube and 6 cc. of buffer solution are added. The mixture is well shaken. 0.5 cc. of this buffered mixture is pipetted into a test-tube, graduated at 30 cc., and 1.0 cc. of the standard dextrin solution added. After thorough shaking the tube is incubated in a water-bath at 37° for half-an-hour. Whilst the incubation is proceeding the standard is prepared. This is made up by pipetting 1.0 cc. of standard dextrin into a tube graduated at 30 cc.

When the incubation period is complete the tube is removed from the water-bath, and 15 cc. of the iodine solution are added to it and to the standard. The volume of each is then made up to the mark, mixed and compared in the colorimeter, the standard being set at 20 mm.

Small-scale curve to show relation between units of diastase and grams of dextrin used up.

The amount of dextrin present in the unknown is calculated by the familiar colorimetric ratio—that is: $\frac{20}{X} \times Y$; where X is depth in millimetres of unknown, and Y is the quantity of dextrin in the standard. Since 1 cc. of 0.75 % dextrin

solution—that is, 0.0075 g. of dextrin—was present before incubation, the amount of dextrin used up can be calculated by subtracting from this the figure obtained in the above calculation. By reference to the curve this can be converted into Wohlgemuth units.

Group I. The cerebro-spinal fluid in this group was taken during life. Twenty-eight cases were examined with the following conditions:

Tabes	6
Syphilis (primary and secondary)	5
Uraemia	3
General paralysis of the insane	2
Tuberculous meningitis	2
Cerebral tumour	2
Cerebral syphilis	2
Encephalitis lethargica	1
Neuritis	1
Meningo-myelitis	1
Acute anterior polio-myelitis	1
Cerebro-spinal meningitis	1
Infective endocarditis	1

In addition, three samples of cerebro-spinal fluid were examined which were obtained by collecting the fluid drawn off before the injection of a spinal anaesthetic.

In the uraemic cases the urea content of the fluids was estimated for diagnostic purposes. These figures will be referred to later.

Results. In all the thirty-one cases except two not a trace of diastatic enzyme was detected. In one case of tabes and one of secondary syphilis only a trace (less than one unit) of diastase was found.

Group II. In the thirty-six cases comprising this group the spinal fluid was collected at known intervals post-mortem. Blood was taken at the same time. Urea estimations were carried out on the specimens to show the presence, if any, of nitrogen retention, for it is reasonable to expect to find in cases showing a high urea content accumulation of other substances, including perhaps the enzyme diastase.

Results. Only seven of these cases were observed to contain diastase in the cerebro-spinal fluid. From the table given below it will be seen that four of the seven show marked urea retention:

Case	Hours after death	Blood		Cerebro-spinal fluid	
		Urea mg. per 100 cc.	Diastase units	Urea mg. per 100 cc.	Diastase units
1	16	284	8	261	8
2	7	90	7	82	2
3	24	44	5	37	3
4	8	104	4	91	2
5	11	101	30	91	3
6	21	28	3	26	4
7	10	—	4	—	1

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That the cause of the urea retention is not associated with the appearance of the diastase in the cerebro-spinal fluid will be seen when it is stated that the urea figures for the uraemia cases in Group I were 101, 102 and 143 mg. per 100 cc. No trace of diastase was found in these latter cases where the fluid was withdrawn during life.

DISCUSSION.

It appears that during life, whatever the condition is, it is rare to find diastase in the cerebro-spinal fluid. It will be admitted that the nearest approach to normal fluid was obtained from cases punctured for spinal anaesthesia, and it is very significant that none of these contained any diastase. Moreover, twenty-six of the cerebro-spinal fluids derived from ante-mortem sources yielded similar results, and only two were found to contain any diastase at all, this being under one unit. It is very unlikely, therefore, that diastase is a normal constituent of cerebro-spinal fluid. In all the cases taken within 24 hours after death about 20 % were found to contain appreciable quantities of diastase. The presence of the enzyme was not observed in any case until about 7 hours after death, quite independently of the occurrence of nitrogen retention. It is therefore a post-mortem effect.

SUMMARY.

1. The presence of diastase in the cerebro-spinal fluid of human beings during life is extremely rare.
2. Approximately 7 hours after death roughly 20 % of cerebro-spinal fluids were found to contain definite amounts of the enzyme.
3. The appearance of diastase in the cerebro-spinal fluid is a post-mortem effect occurring after about 7 hours.

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