

THE SIGNIFICANCE OF AMINO-ACIDS IN NEURO-PSYCHIATRIC DISEASES.

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THE relationship of neuro-psychiatric disorders to electro-physiology and biochemistry has a vast literature, review of which is not within the scope of this preliminary report. The report itself concerns a study of an aspect of this relationship based on 30 unselected neuro-psychiatric cases. Each case had an electroencephalographic (E.E.G.) and a cerebrospinal fluid (C.S.F.) investigation, the latter with reference to the presence of amino-acids.

Before details of the investigations are given, the techniques and criteria of evaluation should be outlined. The majority of the E.E.G. records were taken with a 6-channel Ediswan machine, using eight varieties of montages (21 cases). Overventilation was carried out in all and photic stimulation in nine cases; nine of the records were obtained with the 4-channel Ediswan "portable" at Redhill County Hospital, Surrey. As to the evaluation of the records, a three-point scale of Normal, Abnormal and Severely Abnormal has been adopted, but it must be borne in mind that any differentiation between normal and abnormal records is arbitrary, and is based on obtained correspondences without a known theoretical basis. The records in this study have been evaluated as abnormal when there was a relative excess of medium to high amplitude theta, a marked fast activity and/or sharp waves; severely abnormal records denote in this evaluation those showing delta focus and severely disorganized or paroxysmal activities.

The assessment of amino-acids from the C.S.F. demands a more detailed description, since the method used might be subject to criticism. The paper chromatography method was employed, and the technique followed was that recommended by C. E. Dent (1951). Three ml. of C.S.F. was deproteinized by precipitating the proteins with ethanol; the centrifuged fluid was treated with three times its volume of chloroform and the deproteinized fluid separated. The fluid then was desalted, in the Shandon electrolytic apparatus, then passed through a No. 4 sintered glass filter to remove insoluble matter formed during desalting. One ml. of the fluid was evaporated and the amino-acids were extracted using three separate 0.1 ml. volumes of distilled water. This was then applied in 0.02 ml. amounts to the Whatman No. 3 mm. filter paper, for 2-dimensional chromatography. The amino-acids on the paper were treated

in one direction with a saturated solution of water in phenol, dried and then treated in the other direction with n-butyl-alcohol with acetic acid and water. The dried paper was sprayed with ninhydrin to develop the separated amino-acids. The developed amino-acids were identified by their relative position, by their colour, and by calculating the R_F (ratio factor). In comparison with the control chromatograms the R_F , however, whilst reliable within one chromatogram, showed a considerable range within the whole group of chromatograms. The same fact was also observed by R. H. Horrocks and G. B. Manning (1949) when studying sugar chromatograms. They demonstrated that the differences were due to changes in temperature. The R_F , however, is only one means of identification, and identification within one chromatogram remains unaffected.

SUBJECTS AND RESULTS.

The material consisted of 20 male and 10 female patients, all adults. Their average age was 51.2 (range 20-78). For the purpose of this preliminary investigation their diagnostic distribution has been grouped as follows:

Group A: Neuro-psychiatric conditions having a well-defined pathology and showing both psychiatric and neurological symptoms; such were cerebral tumours, general paralysis of the insane, disseminated sclerosis with psychosis, etc., 9 cases.

Group B: Neuro-psychiatric conditions having a well-defined pathology and showing predominantly psychiatric symptoms. Such were senile dementias, including arteriosclerotic dementias, with no previous history of mental breakdown, 8 cases.

Group C: Neuro-psychiatric conditions, showing psychiatric symptoms only, and having no defined pathology. Such were chronic and sub-acute schizophrenics, neuroses, etc., 13 cases.

To begin with, the E.E.G. records were taken and evaluated. Then the C.S.F.'s were obtained and the presence of amino-acids ascertained. The latter investigation showed that various amino-acids were present, and that the variety of acids increased in number in certain cases. Four acids were identified in all cases: glutamine, glutamic acid, alanine and glycine. Tryptophane and valine were identified in the majority of cases. The remainder of the cases showed seven or more amino-acids present, some of which we were unable to identify. Thus inspection of the tables suggested that on the basis of the number of amino-acids present the patients should be divided into two groups, one group having various acids up to and including six in number and the other showing the presence of acids over that number.

The relationship between number of amino-acids present and abnormality of E.E.G. is set out in Table I. This shows that there is a definite correspondence between the occurrence of amino-acids in excess of six, and abnormal E.E.G. findings. The correspondence between severely abnormal E.E.G. findings and the presence of more than six amino-acids is even more definitely marked (see Table II), than when the E.E.G. findings are merely divided into normal and abnormal records.

The relationship of diagnostic status to the physiological findings is displayed in Table III. This demonstrates that Group A is characterized by the excess

number of amino-acids and Group C by the lesser number, to a statistically significant extent. On the other hand, there is no statistically significant difference between Groups A and B and Groups B and C, in respect of the number of amino-acids present.

Finally Table IV demonstrates the correlations between the diagnosis and the severely abnormal E.E.G. findings. It shows that in this respect each group differs from the other to a statistically significant degree.

TABLE I.

Amino-acids.	E.E.G.		Totals.
	Normal.	Abnormal.	
Less than 7 . . .	11	6	17
Seven and more . . .	3	10	13
Totals . . .	14	16	30

$$p = .03.$$

p denotes the probability of getting by pure chance the obtained frequencies or frequencies showing a more extreme contrast. (Fisher, R. A., 1950.)

TABLE II.

Amino-acids.	E.E.G.		Totals.
	Normal.	Severely abnormal.	
Less than 7 . . .	11	1	12
Seven and more . . .	3	6	9
Totals . . .	14	7	21

$$p = .01.$$

TABLE III.

Amino-acids.	Diagnosis.			Totals.
	A.	B.	C.	
Less than 7 . . .	2	5	10	17
Seven and more . . .	7	3	3	13
Totals . . .	9	8	13	30

$$A \text{ vs. } B \ p = .12.$$

$$A \text{ vs. } C \ p = .02.$$

$$B \text{ vs. } C \ p = .89.$$

TABLE IV.

E.E.G.	Diagnosis.			Totals.
	A.	B.	C.	
Normal . . .	0	3	11	14
Severely abnormal . . .	6	1	0	7
Totals . . .	6	4	11	21

$$A \text{ vs. } B \ p = .03.$$

$$A \text{ vs. } C \ p = < .001.$$

$$B \text{ vs. } C \ p = .03.$$

COMMENT.

The investigations show that the number of amino-acids in the C.S.F. averages five, all being quite distinctly recognizable and that this number increases in certain neuro-psychiatric illnesses. The establishment of any possible qualitative relationship between the various amino-acids and neuro-psychiatric symptomatology demands the investigation of large homogeneous groups of cases. The study also reveals a parallelism between an increased number of amino-acids and E.E.G. abnormalities. One is fully aware, that despite the use of the analyser, the evaluation of an E.E.G. record is to some degree subjective, and its validity would be strengthened if a similar evaluation were given by two or three independent interpreters. At least the subjective error here was constant ; moreover, the category of severely abnormal E.E.G. is outside the margin of subjective judgment.

From the clinical point of view, of course, the relationship of the physiological findings to the diagnostic categories is of primary importance. One does not expect to find an answer to the problems presented by a single diagnostic category in a piloting experiment, based on 30 cases only. The shortcomings of the arbitrary diagnostic groups (A, B and C) are not only due to their relatively small numbers but, to some degree to their dyshomogeneous nature. In the eight cases of Group B, for instance (the senile group), five patients showed an abnormal E.E.G. and only one a severely abnormal E.E.G.; a comparatively high incidence of abnormal records. Group C, on the other hand, was a mixture of nine schizophrenias, three neuroses and one depression ; they had two abnormal records, and no severely abnormal record at all, as Table IV shows. This is somewhat below the expected number of abnormal records within the schizophrenic group. Clearly it would be desirable to operate with carefully defined homogeneous groups in order to ascertain more accurate quantitative and qualitative interrelations between the physiological and clinical findings.

The question of homogeneous groups, however, raises the problem of psychiatric diagnosis, and the uncertainties, for example, of the various subclassifications of schizophrenia. On the other hand, in the present sample, the patients in Group A, all showed clinically a marked intellectual deterioration corresponding to the presence of excess acids and severely abnormal E.E.G.'s. Group B, from the point of view of intellectual deterioration occupied an intermediate position, but its position was less clear in regard to the number of amino-acids. These clinical and physiological findings suggest that investigations should be attempted that take as their correlative classification kinds and degrees of cognitive disturbance rather than the usual diagnostic entities. The present findings encourage us to undertake such investigations on a larger scale.

SUMMARY.

1. Various amino-acids were identified in the cerebrospinal fluid of 30 unselected neuro-psychiatric cases.
2. A statistically significant relationship was found between the presence of amino-acids in excess of six and the occurrence of an abnormal E.E.G.

3. The patients with an increased number of amino-acids in their C.S.F. and a severely abnormal E.E.G. showed clinically a marked intellectual disorganization.

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