Alpha-amino nitrogen in cerebrospinal fluid and its alterations in meningitis, multiple sclerosis, and some other neurological disorders

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SYNOPSIS The concentration of alpha-amino nitrogen (a measure of total amino-acids) was estimated in 58 samples of cerebrospinal fluid in which other findings were normal. Values for lumbar and ventricular cerebrospinal fluid in adults, and lumbar cerebrospinal fluid in children, are reported. A relationship was found between the concentrations of alpha-amino nitrogen in lumbar cerebrospinal fluid and in plasma.

Alpha-amino nitrogen was also estimated in 79 samples of fluid from patients with meningitis, cerebral tumour, multiple sclerosis, and other neurological disorders. High concentrations were found in meningitis, spinal block, and xanthochromic fluids. In cerebral tumours, the concentrations were mostly normal. Low concentrations were found in children, in many cases of multiple sclerosis, and in benign intracranial hypertension. In some cases, the changes cannot yet be explained.

Mestrezat (1912) reported that the amino-acid nitrogen level of cerebrospinal fluid was raised in a case of meningitis. Since this time, there have been many investigations of amino-acids in normal and pathological cerebrospinal fluid, but though numerous abnormalities have been reported, results have often been conflicting (*e.g.*, Knauff, 1958; Logothetis and Bovis, 1961; 1962), and no information of useful diagnostic value has been obtained. These disappointing results might have been contributed to by technical difficulties, including the use of non-specific methods for estimating amino nitrogen in the older work, and more recently, the extensive use of the tedious technique of quantitative paper chromatography, which is apt to give different results in the hands of different investigators, and is unsuitable for the detection of minor changes. This paper describes a preliminary survey of the concentrations of alpha-amino nitrogen in cerebrospinal fluid and plasma in control samples and some pathological conditions, using a recently developed and relatively specific colorimetric ninhydrin method. The method does not measure individual amino-acids but gives a reliable estimate of the total amino-acid concentration.

MATERIALS AND METHODS

Samples of cerebrospinal fluid (C.S.F.) were obtained from patients in the following groups: (1) controls: in these, who were undergoing investigation for neurological or psychiatric disorders, the cerebrospinal fluid was normal with respect to total protein, cell count, Pandey, Nonne-Apelt, and Lange tests; (2) cases of meningitis; (3) cases of intracranial tumour; (4) cases of multiple sclerosis; (5) a miscellaneous group of other neurological disorders, details of which are given below.

Cerebrospinal fluid was centrifuged and the supernatant taken for analysis. Heparinized blood samples were drawn at least four hours after the last meal, and the plasma analysed. Samples were stored deep-frozen until analysis. Analyses for alpha-amino nitrogen (α -NH₂N) were made by the colorimetric ninhydrin method (Matthews, Muir, and Baron, 1964; Williams, Donaldson and, Matthews, 1965). In calculating the concentrations of α -NH₂N in cerebrospinal fluid, water, and plasma water, it was assumed that the water content of normal cerebrospinal fluid was 985 g./l. (Levinson, 1929) and the water content of plasma was calculated from the formula of Eisenman, Mackenzie, and Peters (1936), assuming a protein concentration of 7 g./100 ml. In a few cases, two-dimensional paper chromatography of amino-acids was carried out in butanol-acetic and phenol-ammonia solvents after deproteinization with 9 volumes of hot ethanol, a volume equivalent to 0.25 ml. Cerebrospinal fluid was applied to the paper. No attempt was made at quantitative estimation of the individual amino-

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acids. The significance of differences between means was assessed by the *t* test.

RESULTS

The concentrations of α -NH₂N in control samples and in the majority of the pathological conditions studied are summarized in Table 1.

CONTROLS The mean α -NH₂N in lumbar cerebrospinal fluid from adults aged 16-75 (mean age 46) was 1.23 mg./100 ml., and that in ventricular cerebrospinal fluid from adults aged 17-62 (mean age 37) was 1.13 mg./100 ml.; the difference was significant ($P < 0.05$). The mean value in lumbar cerebrospinal fluid from children under 5 (1.09 mg./100 ml.) was lower than that in adults ($P < 0.01$). There was no relationship between α -NH₂N and age within the adult group. In 14 cases, α -NH₂N was estimated in lumbar cerebrospinal fluid (mean 1.21 mg./100 ml. or 1.23 mg./100 ml. cerebrospinal fluid water) and also in plasma (mean 4.3 mg./100 ml. or 4.6 mg./100 ml. plasma water). The mean ratio of the concentration of α -NH₂N in cerebrospinal fluid water to that in plasma water (*R*) was 0.27. There was a positive correlation between the concentrations of α -NH₂N in plasma and cerebrospinal fluid (Fig. 1). There was no relationship between total cerebrospinal fluid protein and the concentration of α -NH₂N. Chromatography (six samples) showed glutamine, glycine, alanine, valine, leucine, tyrosine, histidine, and small amounts of serine, threonine, and glutamic and aspartic acids, a similar pattern to that reported by Walker, Telles, and Pastore (1955) and Westall (1962).

FIG. 1. The relationship between the concentration of α -NH₂N in plasma and lumbar cerebrospinal fluid. The calculated regression line is shown. ($r = 0.55$; $P < 0.05 > 0.02$).

MENINGITIS In all cases, the values were raised, ranging from 1.9 to 5.4 mg./100 ml. There was no correlation between the α -NH₂N and total protein or white cell count. In one case, the cerebrospinal fluid concentration (5.4 mg./100 ml.) substantially exceeded the plasma concentration (3.7 mg./100 ml.). In another, the α -NH₂N level was only slightly raised (1.9 mg./100 ml.) while other changes were very gross (total protein 900 mg./100 ml., white cell count 10,000/c. mm., sugar 5 mg./100 ml.). Ten days later, when the condition was responding to treatment, the α -NH₂N level had risen to 5.0 mg./100 ml. while the protein had fallen to 100 mg./100 ml. and the white

TABLE I
ALPHA-AMINO NITROGEN CONCENTRATIONS IN CEREBROSPINAL FLUID IN CONTROLS AND
IN VARIOUS PATHOLOGICAL CONDITIONS

Condition	Source of Cerebrospinal Fluid	No.	α -NH ₂ N (mg./100 ml.)	
			Mean $\pm$ S.E.	Range
Controls (adult)	Lumbar	42	1.23 $\pm$ 0.017	0.96—1.47
	Ventricular	8	1.13 $\pm$ 0.046	0.97—1.36
Controls (children under 5)	Lumbar	8	1.09 $\pm$ 0.061	0.92—1.14
Meningitis, pyogenic	Lumbar	6	3.3 $\pm$ 0.70	1.9—5.4
Meningitis, tuberculous	Lumbar	1	4.9 —	—
	Lumbar	1	1.12 —	—
Gliomas	Lumbar	1	3.7 ¹ —	—
	Ventricular	6	1.13 $\pm$ 0.09	0.76—1.40
	Ventricular	1	2.9 ² —	—
Meningiomas	Lumbar	2	1.28 —	1.27—1.30
	Ventricular	2	1.04 —	—
Secondary carcinomas	Ventricular	4	1.04 $\pm$ 0.20	0.69—1.50
Acoustic neuroma	Ventricular	1	1.16 —	—
Cerebellar haemangioblastoma	Ventricular	1	1.09 —	—
Multiple sclerosis	Lumbar	33	1.01 $\pm$ 0.085	0.54—1.60
Benign intracranial hypertension	Lumbar	6	1.03 $\pm$ 0.076	0.85—1.36

¹Extensive subarachnoid spread

²Fluid xanthochromic

cell count to 170/c.mm. Chromatography (four samples) showed a generalized increase in amino-acids, including glutamine.

CEREBRAL TUMOURS In the majority of cases, the values for α -NH₂N were within the control ranges for lumbar or ventricular cerebrospinal fluid. In three, the values were unusually low; in one (0.76 mg./100 ml.) the tumour was a medulloblastoma, and in the other two (0.69 and 0.71 mg./100 ml.) it was a secondary carcinoma. Gross elevations of cerebrospinal fluid α -NH₂N were found in a case with xanthochromic fluid, and in a glioma with widespread subarachnoid dissemination.

MULTIPLE SCLEROSIS In 10 out of 33 cases (30%), the concentration of α -NH₂N was below the lower limit of the control range, and the mean concentration (1.01 mg./100 ml.) was significantly lower than in controls ($P < 0.01$). The α -NH₂N level was estimated in cerebrospinal fluid and plasma in six cases; in these the mean concentration in cerebrospinal fluid was 1.1 mg./100 ml. (1.12 mg./100 ml. cerebrospinal fluid water) and the mean concentration in plasma 4.2 mg./100 ml. (4.5 mg./100 ml. plasma water $R = 0.25$). Chromatography of three samples with subnormal α -NH₂N suggested a decrease in all amino-acids.

MISCELLANEOUS CONDITIONS In five out of six cases of benign intracranial hypertension, the level of α -NH₂N in lumbar cerebrospinal fluid was below the lower limit of the control range, and the difference between the mean value and that in controls was highly significant ($P < 0.001$). Four cases of infected hydrocephalus in children had high concentrations similar to those found in meningitis in adults. In a case of spinal block due to a myxopapillary ependymoma, the α -NH₂N level in lumbar cerebrospinal fluid was 4.3 mg./100 ml. In a case of haemangioma of the cord, the lumbar cerebrospinal fluid, which was xanthochromic, had an α -NH₂N concentration of 3.0 mg./100 ml. In the remainder of the conditions, including viral encephalitis (2), cerebrovascular insufficiency (4), motor neurone disease (1), and acute infective polyneuritis (1), values were normal.

DISCUSSION

Very few determinations of cerebrospinal fluid amino-acid nitrogen have been made by ninhydrin methods, which are the most specific available. The present results agree well with six normal values obtained by the standard gasometric ninhydrin technique (Christensen, Cooper, Johnson, and Lynch, 1947). The normal values in the older litera-

ture, which are still widely quoted, were obtained by less specific methods (formol titration, nitrous acid, or Folin's colorimetric method) and are much higher than the present figures, ranging up to 4 mg. amino nitrogen per 100 ml., with a mean of about 2 (e.g., Weichmann and Dominicki, 1926; Lickint, 1929; Levinson, 1929; Katzenelbogen, 1935).

The correlation between α -NH₂N concentrations in control samples of cerebrospinal fluid and plasma is of physiological interest. It has previously been shown that amino-acid levels in cerebrospinal fluid are raised when plasma amino-acids are pathologically increased (e.g., Lickint, 1929; Walshe, 1953; Müting, 1962) but it cannot be assumed that under all conditions the cerebrospinal fluid concentration of a given substance will be related to its concentration in the blood. Thus, the potassium concentration in cerebrospinal fluid is independent of that in plasma (Bradbury, Stubbs, Hughes, and Parker, 1963). The present findings emphasize the importance of estimating plasma amino-acids when altered amino-acid concentrations are found in the cerebrospinal fluid. In many studies this has not been done.

The physiological factors influencing the concentrations of amino-acids in cerebrospinal fluid are undoubtedly complex (Davson, 1956), and in pathological states so many more variables may be involved that a consistent relationship between a particular alteration in amino-acid levels and a given pathological state cannot be expected in every instance. There is, however, complete agreement that the total amino-acid concentration is raised in meningitis, both pyogenic and tuberculous (e.g., Weichmann and Dominicki, 1926; Lickint, 1929; Levinson, 1929; Katzenelbogen, 1935; Schöenberg, 1954; Schiavini and Nebbia, 1957). The rise is usually assumed to result from increased permeability of the blood-brain barrier, though proteolysis, tissue destruction, and decreased tissue uptake may also be involved. The present finding that the α -NH₂N concentration in cerebrospinal fluid may be appreciably greater than that in the plasma suggests that increased permeability is not always the sole cause.

Lickint (1929) reported raised concentrations of cerebrospinal fluid amino nitrogen in cerebral tumours, whereas Logothetis and Bovis (1962) found that individual amino-acids were normal in five cases. In the present series, the level of α -NH₂N was usually normal. The occasional low values may have resulted from uptake of amino-acids by rapidly growing tumour tissue. High values were associated with neoplastic meningitis and with xanthochromia. In the xanthochromic fluid, proteolysis may have been an important factor in the rise.

Walker *et al.* (1955) and Bauer and Boesche (1957) reported that cerebrospinal fluid amino-acids were normal in multiple sclerosis. The present work confirms the claims of Logothetis (1955) and Logothetis and Bovis (1962) that in some cases their concentrations are low. The plasma estimations did not suggest that this was the result of low plasma concentrations, a point of interest in view of the suggestion (Shatin, 1964) that malabsorption of amino-acids might be concerned in the pathogenesis of the disease. The permeability of the 'blood-cerebrospinal fluid barrier' appears to be increased in multiple sclerosis (Haever, Tourtellotte, Richard, Gustafson, and Bryan, 1964) and this might be expected to increase amino-acid concentrations in the cerebrospinal fluid. No explanation of the reduction in amino-acids can be offered at the present stage. The concentration of B₁₂ in the cerebrospinal fluid of cases of multiple sclerosis is also unaccountably low (Basil, Brown, and Matthews, 1965). The low α -NH₂N concentrations in cases of benign intracranial hypertension are similarly unexplained.

The results of this and other work may be summarized as follows. The α -NH₂N concentration in the cerebrospinal fluid is normally about 30% of that in plasma, and is related to the plasma concentration. It is raised in meningitis (probably in all forms) and when the concentration in plasma is abnormally high. High values may also occur in spinal block and when blood has entered the cerebrospinal fluid. Low concentrations are normally found in children. They also occur in some cases of multiple sclerosis, of benign intracranial hypertension, and occasionally in association with cerebral tumours. Some of these changes are as yet quite unexplained, and merit further investigation.

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