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Changes in the Free Amino Acid Composition of Cerebrospinal Fluid in Liver Disease. (27597)

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Disturbance in protein metabolism in patients with liver disease has been known since Frerichs(1) established the presence of leucine and tyrosine crystals in urine of patients with acute yellow liver-atrophy. Since then, the amino acid content of serum and urine of such patients has been extensively studied. Far less quantitative data on the free amino acid content of cerebrospinal fluid are available(2,3,4,9).

In the following study, an attempt is made to clarify the following questions which have arisen in the study of cerebrospinal fluid of liver disease patients: (a) Is there any significant difference in the free amino acid composition of cerebrospinal fluid of healthy individuals and patients with liver disease? (b) Is there any relationship between degree of hepatic parenchymal damage and changes in the free amino acid composition of the cerebrospinal fluid? (c) Do these changes play any part in the pathogenesis of hepatic coma?

Materials and methods. This study entailed detailed clinical and laboratory examination of 25 metabolically healthy adults between the ages of 20 to 42 years and of 28 patients with liver disease. Of the latter, 5 were suffering from obstructive jaundice due to malignant tumors and 18 from cirrhosis of the liver. Of the cirrhotics 11 were alcoholics; 1 was associated with cardiac failure; 1 was a result of biliary infection; and 5 followed hepatitis. In 22 of these 28 cases the clinical diagnosis was later confirmed either by peritoneoscopy, biopsy, or autopsy. In most cases clinical diagnosis was also supported by liver function tests such as bromsulphthalein clearance, galactose tolerance, and serum transaminase level as well as by results of serum electrophoretic analysis and by measurement of prothrombin time, serum bilirubin, nonprotein nitrogen, and free alpha-amino-N in plasma. The methods of analysis of the deproteinized cerebrospinal fluid, obtained by lumbar puncture, have been previously described(5). Altogether 35 cerebrospinal fluids from the 28 patients were analyzed. The average cell count ranged between 0-22 cells, and protein content ranged from 22-53 mg %. The free alpha-amino-N (Moore and Stein(6)), indican (Shivaram and Müting(7)), as well as free and combined phenolic bodies (Schmidt(8)) were also measured.

Results. The results are shown in Table I. In 12 patients without hepatic coma, the free alpha-amino-N in cerebrospinal fluid ranged between 0.94-2.07 mg %, the normal being 0.75 mg % ($\pm$ 0.24). Among the free amino acids which most increased were tyrosine, tryptophane, phenylalanine, leucine, isoleucine, and especially methionine, glycine, and glutamine. The increase in 14 amino acids is statistically significant.

In 16 cases with hepatic coma, free alpha-amino-N of the cerebrospinal fluid ranged between 2.45 and 3.90 mg % which is 3 to 4 times the normal value (Table I). Of these patients, 3 with the most severe attack from the clinical point of view also showed the highest values. Breakdown products of methionine such as methionine sulfoxide were demonstrable in 4 cases. In addition to the sulfur containing amino acids, glutamine also showed an increase in cerebrospinal fluid to an extent as high as 10 times normal.

TABLE I. Free Amino Acids in Cerebrospinal Fluid of Patients with Liver Disease, with and without Hepatic Coma.

	Normal adults (25)*		Liver disease patients			
			Without hepatic coma (12)*		With hepatic coma (16)*	
	M	Calculated as α-amino-N	M	P	M	P
Aspartic acid	.13 ± .03	.0137	.21 ± .09	.01	.35 ± .22	<.001
Glutamic "	.27 ± .13	.0257	.32 ± .14	n.s.	.59 ± .21	<.001
Lysine	.31 ± .15	.0345	.42 ± .23	"	1.00 ± .49	<.001
Arginine	.20 ± .06	.0162	.21 ± .10	"	.35 ± .18	<.01
Histidine	.26 ± .08	.0234	.32 ± .14	"	.47 ± .21	<.001
Tyrosine	.18 ± .06	.0139	.39 ± .17	<.001	.91 ± .50	<.001
Tryptophane	.20 ± .06	.0275	.51 ± .35	<.001	.82 ± .36	<.001
Phenylalanine	.19 ± .06	.0161	.35 ± .16	<.001	.67 ± .31	<.001
Proline	.27 ± .08	.0329	.36 ± .15	<.05	.60 ± .23	<.001
Cystine	.24 ± .07	.0280	.22 ± .09	n.s.	.40 ± .14	<.001
Methionine	.13 ± .03	.0122	.25 ± .10	<.001	.45 ± .25	<.001
Taurine	.32 ± .12	.0360	.56 ± .37	<.01	1.31 ± .52	<.001
Leucine	.23 ± .05	.0244	.57 ± .32	<.001	.72 ± .39	<.001
Isoleucine	.12 ± .03	.0127	.27 ± .14	<.05	.33 ± .17	<.001
Valine	.26 ± .11	.0432	.49 ± .24	<.05	.88 ± .46	<.001
Glycine	.39 ± .17	.0728	1.05 ± .41	<.001	2.47 ±1.12	<.001
α-Alanine	.34 ± .10	.0533	.64 ± .33	<.001	1.22 ± .32	<.001
Serine	.28 ± .11	.0372	.24 ± .08	n.s.	.56 ± .19	<.001
Threonine	.18 ± .06	.0212	.45 ± .19	<.001	.63 ± .20	<.001
Glutamine	.45 ± .18	.0864	1.09 ± .63	<.001	3.60 ±1.26	<.001
α-Amino-N	.75 ± .24	.6340	1.45 ± .31	<.001	2.97 ± .50	<.001

M = Mean ± stand. dev., all values given in mg %.
P = Statistical significance.
n.s. = no significance.
* No. of patients.

Discussion. The increase in free amino acid content of cerebrospinal fluid in liver disease could be a result of decreased blood-brain-barrier or change in brain metabolism of specific amino acids. The first view is supported by the fact that a definite relationship existed between the level of free alpha-amino-N in plasma and cerebrospinal fluid of our 28 liver disease patients as shown in Fig. 1. This should also hold for individual aromatic and sulfur containing amino acids whose oxidative breakdown has been greatly reduced because of serious hepatic parenchymal damage. This view was supported by our finding that patients loaded with 2 g of methionine *per os,* as suggested by Müting(10), had abnormal excretion of free methionine and free sulfate in 24-hour urine during and after the load. While 30 healthy individuals after loading excreted an average of 125 mg unmetabolized methionine, the 28 patients with liver disease, after loading with 2 g methionine *per os,* excreted an average of 310 mg. Pathological breakdown products such as methionine sulfoxide and methionine sulfone might be expected under such conditions(3). In regard to hepatic coma, it is of interest that Campanacci(13,14) has shown in animal experiments that methionine sulfoxide can cause changes in the brain similar to those seen clinically, as well as EEG changes. Methylmercaptan, according to Challenger and

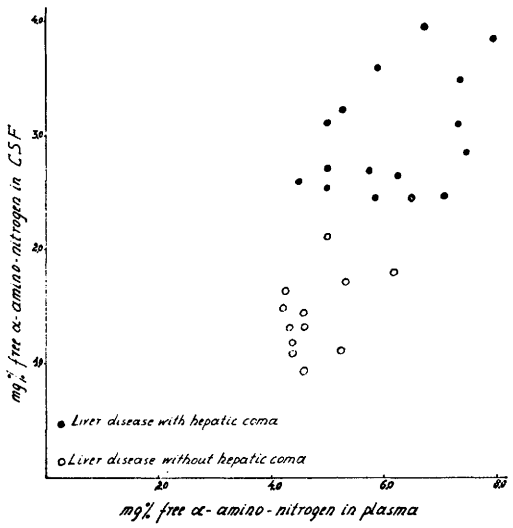

FIG. 1. Relation between free α-amino-nitrogen in plasma and CSF in liver disease.

Walshe(11), is another breakdown product of methionine associated with foetor hepaticus.

On the other hand the high glutamine increase in C.S.F. cannot be explained by its corresponding rise in serum level(12). A disturbance in brain metabolism related to high blood ammonia and its detoxication could be regarded as one cause of the high glutamine level.

Related to the deterioration in hepatic metabolism of tyrosine and tryptophane, Müting and Shivaran observed that free indol and phenol derivatives increased 5 to 10 times normal values(12).

Therefore, it would appear that the changes described here in the amino acid content of C.S.F. from patients with liver disease probably play an important role in the pathogenesis of hepatic coma.

Summary. The amino acid composition of deproteinized cerebrospinal fluid was quantitatively determined by paper chromatography in 25 healthy adults, 28 patients with hepatic disease, (16 with hepatic coma, and 12 without coma). A significant increase of all free amino acids in the cerebrospinal fluid of those with hepatic coma was found. There was also an increased concentration of 14 free amino acids in the fluid of patients with liver disease without coma. Methionine sulfone and methionine sulfoxide were also found as pathologic breakdown products in the fluid of those with hepatic coma. A decrease in oxidative properties of the liver is suggested as a possible cause.

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Stimulated Glycolysis of KB Cell Cultures by Guanidine Derivatives and Other Compounds Affecting Respiration.* (27598)

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Glucose utilization and lactic acid production by cell cultures have been found to increase with concentration of glucose in the medium(1,2). Similar stimulation has resulted from anaerobic conditions(3,4) and from treatment of cell cultures with a variety of agents including insulin(5), thyroxine(6), HCN(2), azide(7), and 2,4-dinitrophenol (8).

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The present report concerns the stimulation of glucose utilization, lactic acid production, and inhibition of growth of cultured cells by a variety of guanidine derivatives and other compounds that may affect respiration.

Materials and methods. Two procedures were utilized for appraisal of effects of compounds on cell cultures. In the first procedure an evaluation was made of the effects of test compounds on KB cell cultures during an extended period of growth in a complete medium containing dialyzed bovine serum. One