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Citrulline as a Diagnostic Parameter and a Major Amino Acid Constituent of Cerebrospinal Fluid in Hepatic Coma

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Summary: Free amino acids of cerebrospinal fluid and serum in hepatic coma have been studied. Citrulline was found to form about 80% of the total free amino acids in cerebrospinal fluid, whereas serum contained slightly higher levels of tyrosine, methionine, phenylalanine and glutamine. The higher level of citrulline in cerebrospinal fluid may be attributed to the inhibition of argininosuccinic acid synthetase in this disease.

Citrullin als diagnostischer Parameter und wichtiger Aminosäure-Bestandteil des Liquors im Leberkoma

Zusammenfassung: Die freien Aminosäuren in Liquor und Serum bei Leberkoma wurden bestimmt. 80% der gesamten freien Aminosäuren im Liquor war Citrullin. Im Serum zeigten sich gering erhöhte Konzentrationen von Tyrosin, Methionin, Phenylalanin und Glutamin. Die höheren Konzentrationen von Citrullin im Liquor könnten auf einer Hemmung der Argininosuccinatsynthetase bei Leberkoma beruhen.

Introduction

The free amino acids of cerebrospinal fluid (CSF) and serum in hepatic coma have been studied previously. *Walshe* (1) observed abnormal amino acid metabolism in this disease. Other scientists showed amino acids after hepatectomy (2, 3). Furthermore, *Whitehead* and *Whittaker* (4) reported that the glutamine content of the CSF is increased in hepatic coma. Recently *Khatra* et al. (5) have demonstrated inhibition of the *Krebs-Henseleit* enzymes in liver cirrhosis.

Our object is to develop a simple and rapid test which can aid the early diagnosis of this disease.

Materials and Methods

Eight patients having hepatic coma were included in this study. They were examined clinically and the relevant liver function tests were performed as shown in table 1. Samples of sera and CSF were obtained early in the morning and were analysed for free amino acids. The analysis was done by using one and two dimensional thin layer chromatography, as well as paper chromatography. The thin layer plates were coated with Merck Silica Gel G (0.5 mm) and chromatograms were developed in two different solvent systems; *n*-butanol-acetic acid-water (volumes, 40 ml + 10 ml + 50 ml) and *m*-cresol-phenol (volumes, 10 ml + 10 ml). The same solvent systems were used for paper chromatography. Standard amino acids were included as references in both techniques. Similarly normal CSF and serum samples were also examined. Quantitative measurements of free amino acids were performed using an automatic scanning machine (chromoscan MK 11, double beam recording and integrating densitometer; serial No. 1127; Joyce Loebl & Co. Ltd.; Princesway, Team Valley, Gateshead 11, England), after staining with 0.5% ninhydrin in acetone. The maximum possible error was found to be ± 2%.

Results

The clinical findings as well as the results of the important liver function tests are shown in table 1. Only these clinical data could be obtained with our limited facilities. The concentrations of the free amino acids in sera and CSF of patients with hepatic coma and those of normal persons are shown in table 2. The pattern of amino acids

Tab. 1. Clinical features and liver function tests of eight patients in hepatic coma.

Patients	1	2	3	4	5	6	7	8
Age	43	47	65	60	45	32	41	24
Sex	♂	♂	♂	♀	♂	♂	♂	♂
Coma grade[1])	4	4	4	2	2	2	2	4
Jaundice	+	+	+	+	+	+	+	+
Liver[2])	2 cm	4 cm	–	–	2 cm	4 cm	8 cm	–
Spleen[2])	2 cm	3 cm	4 cm	–	6 cm	–	–	6 cm
Ascites	+	+	+	+	+	+	+	–
Flapping tremor	–	–	–	–	+	+	+	+
Clinical diagnosis (predisposing to coma)	All the patients have liver cirrhosis							
Serum bilirubin (mg/dl)	10.0	14.3	15.4	4.9	3.5	3.25	16.3	9.4
Serum alkaline phosphate (King-Armstrong units)	20.2	22.5	X	7.3	6.3	27.60	16.7	18.0
Serum alanine aminotransferase (*Reitman-Franklin* Units)	63.0	84.0	54.0	36.4	X	29.8	120.0	78.5
Prothrombin (s)	26.0	32.0	25.0	X	22.0	40.0	X	31.0
Serum albumin (g/dl)	2.6	2.3	2.41	2.4	2.4	X	2.4	3.58
Serum globulin (g/dl)	3.1	3.8	2.86	4.72	3.63	X	3.61	4.22

[1]) Coma grading out of 4.
[2]) Liver and spleen palpable in centimeters below right and left costal margin respectively.
X not done.

Tab. 2. Amino acid pattern in hepatic coma (Cerebrospinal fluid and serum, mg/dl).

Amino acid	Patient subjects[1])		Normal subjects[2])	
	CSF	Serum	CSF	Serum
Citrulline	*5.2* ± 0.40	0.25 ± 0.12	0.00	0.00
Arginine	0.2 ± 0.10	0.15 ± 0.05	0.20 ± 0.02	1.30 ± 0.33
Tyrosine	–	*0.20* ± 0.15	0.15 ± 0.05	1.00 ± 0.50
Methionine	0.15 ± 0.05	*1.40* ± 0.10	0.10 ± 0.03	0.35 ± 0.10
Phenylalanine	0.05 ± 0.01	*1.90* ± 0.20	0.25 ± 0.05	1.00 ± 0.35
Glutamine	0.10 ± 0.02	11.00 ± 2.00	1.50 ± 0.25	8.00 ± 2.20
Alanine	–	3.00 ± 0.51	0.50 ± 0.10	3.50 ± 0.25
Cystine	–	2.00 ± 0.25	0.30 ± 0.15	1.20 ± 0.25
Lysine	–	1.50 ± 0.33	0.50 ± 0.20	2.50 ± 0.20
Aspartic acid	0.50 ± 0.10	3.00 ± 0.22	–	–
Serine	–	1.00 ± 0.20	0.05 ± 0.20	1.20 ± 0.30
Leucine	–	1.70 ± 0.50	0.25 ± 0.05	1.50 ± 0.50
Tryptophan	–	1.10 ± 0.15	0.22 ± 0.10	1.20 ± 0.21
Proline	0.21 ± 0.05	3.50 ± 0.25	0.45 ± 0.05	2.00 ± 0.55
Total	6.40	33.70	4.47	24.75

[1]) Patients with hepatic coma.
[2]) Control.
In italics: Significant at 1% level of probability.

in CSF is shown in figures 1 and 2. In sera the concentration of tyrosine, phenylalanine, methionine and glutamine are slightly increased. In CSF citrulline was found to form the major component (about 80% of the total amino acids). Citrulline was also increased in the sera of the same patients.

Identification of citrulline was carried out by comparing R_f values of standard citrulline with the corresponding spot of the samples. This was done by using single dimensional thin layer chromatography and paper chromatography in the two different solvent systems. These values were found to be identical. In addition, a definite identification was made by co-chromatography with standard citrulline employing a two dimensional thin layer chromatography. Furthermore, a specific staining reaction with *Ehrlich*'s aldehyde reagent gave a pinkish yellow colour with the citrulline spot.

Discussion

The results proved clearly (tab. 2, fig. 1) that citrulline is the major amino acid component in CSF and sera of patients with hepatic coma. It forms about 80% of the total amino acids in CSF. On the other hand, no citrulline was detected in normal samples (fig. 2). Citrulline is the substrate of argininosuccinic acid synthetase in the *Krebs-Henseleit* cycle. Obviously inhibition of this enzyme results in accumulation of citrulline. Reduced activities of that enzyme as well as other urea cycle enzymes have recently

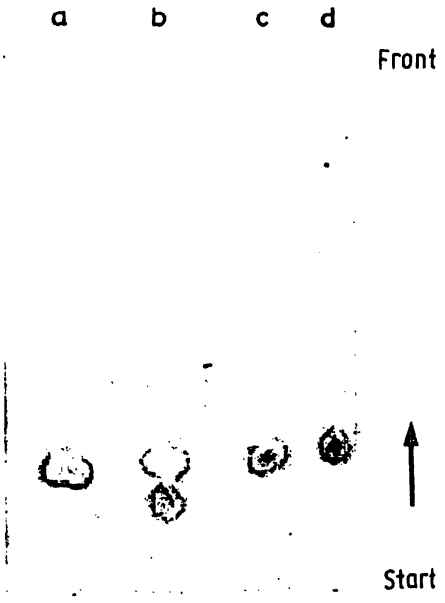

Fig. 1. Thin layer chromatography of:
a) CSF from patient with hepatic coma plus standard citrulline.
b) CSF from patient with hepatic coma plus standard arginine.
c) CSF from patient.
d) Standard citrulline.
The chromatogram was developed in *n*-butanol-acetic acid-water (volumes, 40 ml + 10 ml + 50 ml).

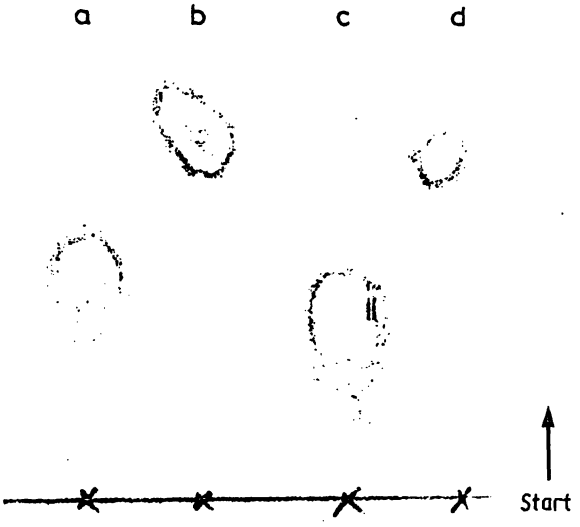

Fig. 2. Thin layer chromatography of:
Normal CSF (a and c).
Standard citrulline (b and d).
The solvent system is the same as in Figure 1.

been demonstrated in patients with liver cirrhosis (5). These results support the earlier finding (6, 7) that citrulline accumulates in blood due to a defect in argininosuccinic acid synthetase in mental retardation. This phenomenon could also occur in CSF due to accumulation of ammonia which may inhibit the same enzyme (8).

The slight elevation of tyrosine, methionine, phenylalanine and glutamine is in a good agreement with the observation of *Mattson* et al. (9) in surgically induced hepatic coma. Moreover these amino acids were found to act as false neurotransmitters in this disease (10).

Whenever possible, our studies are being extended to include other patients, in order to ascertain the complete reliability of this result. It is evident, however, that the result described will be of value in the early diagnosis of hepatic coma, since the technique used is rapid, simple, requires no expensive equipment, and should be applicable in routine clinical investigation.

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