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Plasma ratio of valine, leucine and isoleucine to phenylalanine and tyrosine in liver disease

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SUMMARY The molar ratio $\frac{\text{valine + leucine + isoleucine}}{\text{phenylalanine + tyrosine}}$ was determined in the plasma of patients with liver disease of varying aetiology and severity and in an age and sex matched control group. In the control group of 58 subjects the mean ratio was 3·3 ± 0·5 (1SD). The mean ratio was significantly lowered in groups of 25 patients with alcoholic cirrhosis ($P < 0·001$), 25 patients with chronic active hepatitis ($P < 0·001$), 23 patients with primary biliary cirrhosis ($P < 0·001$), and 11 patients with cryptogenic cirrhosis ($P < 0·001$). In a group of 50 patients with cirrhosis, the ratio was significantly lowered ($P < 0·001$) irrespective of the presence of hepatic encephalopathy. A good correlation existed between the value of the ratio and the severity of the liver disease as judged histologically, with values of the ratio appearing to reflect histological change irrespective of the patient's clinical condition. There was no significant diurnal variation in the value of the ratio. Lowering of this plasma amino acid ratio appears to be secondary to liver disease and quite independent of the presence of hepatic encephalopathy.

Many metabolic abnormalities occur in hepatic coma, though none is pathognomonic (Zieve and Nicoloff, 1974). In recent years a great deal of attention has been paid to changes occurring in the neurotransmitter concentrations in the brains of experimental animals with liver failure and in the plasmas and urines of patients with both acute and chronic hepatic insufficiency. (Fischer and Baldessarini, 1971; Fischer and James, 1972; Fischer *et al.*, 1972; Baldessarini and Fischer, 1973; Lam *et al.*, 1973; Dodsworth *et al.*, 1974; Manghani *et al.*, 1975). Indeed, in 1971, Fischer and Baldessarini hypothesised that many of the manifestations of hepatic insufficiency, including hepatic coma, could be explained by replacement of the true neurotransmitters dopamine and noradrenaline, in both the central and peripheral nervous system, by false neurotransmitters such as octopamine and phenylethanolamine. These false neurotransmitters are similar in structure to the true neurotransmitters, but have only about one hundredth of their neurotransmitter potency.

An important factor in the control of neurotransmitter synthesis, especially in the adrenergic and serotonergic systems, is the brain concentration of the precursor amino acids, especially tyrosine, phenylalanine, and tryptophan. The free brain concentrations of these aromatic amino acids may, in turn, be dependent upon their plasma concentrations, although competition for active entry across the blood brain barrier does occur, especially for the three branched chain amino acids valine, leucine, and isoleucine (Orlowski *et al.*, 1974). Guroff and Udenfriend (1962) suggested that diminished noradrenaline synthesis might result from disturbances in the normal passage of neutral amino acids across the blood brain barrier, and Fernstrom and Wurtman (1972) and Fernstrom *et al.* (1974) believed that brain serotonin concentrations might be related to the ratio between tryptophan, its direct precursor, and five other amino acids competing with it for entry across the blood brain barrier. The plasma amino acid concentrations in hepatic encephalopathy could therefore be of great importance.

Specific plasma amino acid patterns have been demonstrated in patients and experimental animals with chronic hepatic insufficiency and encephalopathy. These include increased concentrations of the aromatic amino acids tyrosine, phenylalanine, and to a lesser extent free tryptophan, and decreased concentrations of the three branched chain amino acids valine, leucine, and isoleucine (Iber *et al.*, 1957;

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McMenamy *et al.*, 1965; Iob *et al.*, 1966, 1970; Mattson *et al.*, 1970; Fischer *et al.*, 1974; Morgan *et al.*, 1978).

Fischer *et al.* (1975) in attempting to relate values of plasma amino acids to the presence or absence of hepatic encephalopathy found that the molar ratio between the concentrations of the three branched chain amino acids and the two aromatic amino acids phenylalanine and tyrosine tended to be constant. In normal man or dog, the mean ratio

$$\frac{\text{valine + leucine + isoleucine}}{\text{phenylalanine + tyrosine}} \left(\frac{V + L + I}{P + T}\right)$$

was 3·0 to 3·5 whereas in animals or patients with hepatic encephalopathy the mean ratio was significantly reduced. Soeters and Fischer (1976) then put forward the hypothesis that hepatic encephalopathy might be caused by changes in the plasma concentrations of the five amino acids making up this ratio.

We, however, had noted changes in the plasma concentrations of these five amino acids in many patients with liver disease who did not have encephalopathy (Morgan *et al.*, 1978). We therefore calculated this plasma ratio for a large number of patients with liver disease of varying aetiology and severity and compared the values that were obtained with those found in control subjects. In addition we evaluated the ratio as an index of hepatocellular damage, by correlating its value with several indicators of liver damage including histology. We studied fasting and post-prandial values and looked for diurnal variation in the ratio. Finally, we measured the ratio in several patients sequentially over several months, and compared the values obtained with changes in the patient's clinical condition and standard liver function tests.

Methods

AMINO ACID ANALYSIS

The measurement of the plasma amino acid concentrations was standardised. Venous samples of blood were taken between 09:00-10:00 under non-fasting conditions and placed in heparinised tubes. The plasma was separated and deproteinised in a final concentration of 3% sulphosalicylic acid. Norleucine was added to the supernatant after deproteinisation to a final concentration of 0·25 mmol/l, to act as an internal standard. All samples were stored at −20°C before analysis with a Technicon TSM amino acid analyser, using a six hour taped programme for the two column method for physiological solutions. The overall reproducibility of results was consistent within ±5%. The $\frac{V + L + I}{P + T}$ ratio was calculated for each plasma sample.

PATIENT STUDIES

In four control subjects (two male, two female) and six patients with liver disease (three male, three female) the plasma $\frac{V + L + I}{P + T}$ ratio was measured on several occasions during one day. All 10 patients had taken a standard 80 g protein, 2000 calorie diet for 10 days before and on the study day.

To assess the effect of hepatic encephalopathy on the plasma ratio two patient groups and a control group were studied.

(1) The first group comprised 106 patients with liver disease of varying aetiology and severity, none of whom had clinical or electroencephalographic (EEG) evidence of hepatic encephalopathy. Of the 106 patients, 22 were alcoholics with abnormal liver function tests but with only minimal changes on liver biopsy, 25 had alcoholic cirrhosis, 25 had chronic active hepatitis (CAH), 23 had primary biliary cirrhosis (PBC) with grade 3 or 4 histological change on liver biopsy, and 11 had cryptogenic cirrhosis.

(2) The second group comprised 50 patients with cirrhosis of varying aetiology (25 alcoholic, 15 PBC, 10 CAH) in 20 of whom there was both clinical and EEG evidence of hepatic encephalopathy, while in the remaining 30 there was not.

The age and sex matched control group of 58 comprised 20 laboratory staff, 20 hospital inpatients with general medical disorders, and 18 with gastrointestinal disorders. All had normal liver and renal function tests.

A mean plasma $\frac{V + L + I}{P + T}$ ratio was calculated for the patients in each separate liver disease category, for the cirrhotics with and without hepatic encephalopathy and for the control group. Student's *t* test was used for statistical analysis.

To assess the value of the $\frac{V + L + I}{P + T}$ ratio as an index of hepatic dysfunction a group of 40 patients with alcohol related liver disease was studied. In each patient the prothrombin time was measured and the plasma assayed for aspartate transaminase, alkaline phosphatase, bilirubin, total protein, albumin and bile acids and amino acids both fasting and two hours post-prandially. Plasma analyses were carried out using standard laboratory

techniques. All 40 patients had recently had a liver biopsy and the degree of histological damage and derangement had been assessed as mild, moderate, or severe by two independent observers. The plasma amino acid ratio as calculated was correlated with the various indices of hepatic damage for each of the 40 patients studied.

Long-term follow-up studies were undertaken in six patients with acute type A hepatitis and in six patients with cirrhosis during treatment for chronic hepatic encephalopathy. The clinical condition, standard liver function tests, and the plasma $\frac{V + L + I}{P + T}$ ratio were monitored at frequent intervals.

Results

In the four control subjects and the six patients with liver disease in whom the ratio was measured on several occasions in one day after stabilisation on the same diet, there was no significant diurnal variation in the value of the ratio (Fig. 1). In particular there was no significant variation between fasting and non-fasting values.

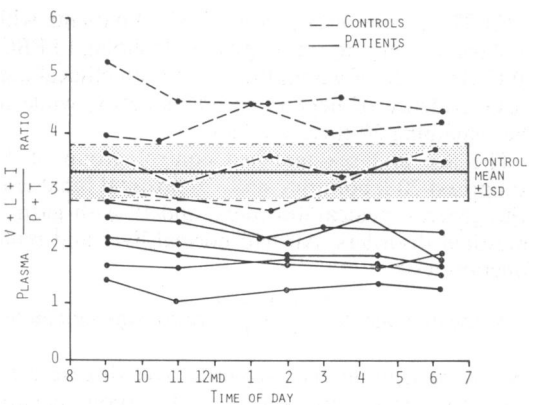

Fig. 1 *Diurnal variation in the plasma* $\frac{V + L + I}{P + T}$ *ratio in control subjects and patients with liver disease.*

PATIENTS WITH LIVER DISEASE WITHOUT ENCEPHALOPATHY (Fig. 2)

The mean plasma $\frac{V + L + I}{P + T}$ ratio in the control group was 3·3 ± 0·5 (1SD). The mean ratio in the group of 22 alcoholics with minimal liver damage was not significantly different, 3·2 ± 0·6. However, the mean ratio in all the other patient groups was highly significantly lowered when compared with the control mean ($P < 0.001$). The mean ratio for the 25 patients with alcoholic cirrhosis was 2·1 ± 0·7, for the 25 patients with CAH 2·0 ± 0·6, for the 23 patients with PBC 1·7 ± 0·6, and for the 11 patients with cryptogenic cirrhosis 1·4 ± 0·7.

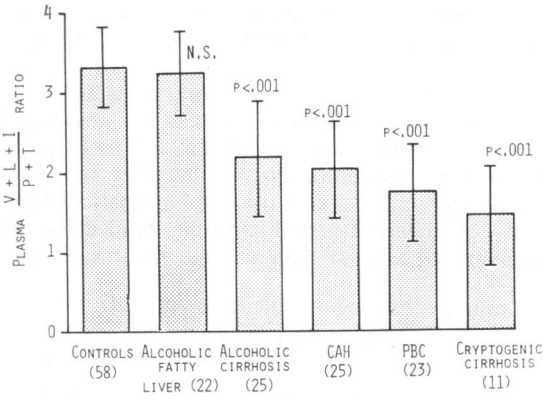

Fig. 2 *The plasma* $\frac{V + L + I}{P + T}$ *ratio in control subjects and patients with liver disease but without hepatic encephalopathy.* ‡ *mean ratio ± 1SD. NS: not significant.* P: *Student's t test.*

PATIENTS WITH LIVER DISEASE AND ENCEPHALOPATHY (Fig. 3)

There was a highly significant lowering of the mean plasma ratio in both the group of cirrhotics with hepatic encephalopathy, 2·2 ± 0·6 ($P < 0.001$) and the group without, 2·1 ± 0·6 ($P < 0.001$) when compared to the control mean. There was no significant difference between the mean plasma ratio in the two groups.

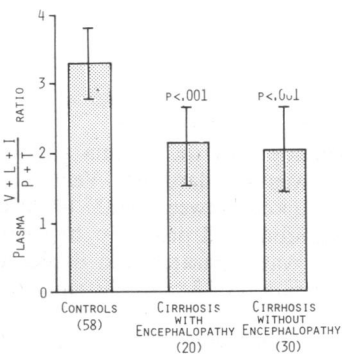

Fig. 3 *The plasma* $\frac{V + L + I}{P + T}$ *ratio in control subjects and in patients with cirrhosis with and without hepatic encephalopathy.* ‡ *mean ratio ± 1SD.* P: *Student's t test.*

All six patients with cirrhosis who were monitored during treatment for chronic hepatic encephalopathy, showed similar results (Table 1) which are well exemplified by the patient illustrated in Fig. 4. This patient with alcoholic cirrhosis and severe hepatic encephalopathy showed a dramatic clinical improvement when given a low protein diet and lactulose. His EEG improved from a mean frequency of 4 to 8·5 cycles per second (c/s) (normal > 8·9 c/s) during the time period illustrated, and his standard liver function tests also showed some improvement. His plasma amino acid ratio did not change significantly.

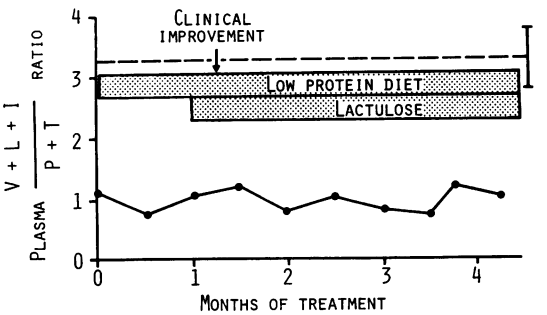

Fig. 4 *Patient J.C. with alcoholic cirrhosis and chronic hepatic encephalopathy.* ‡ *mean control* $\frac{V+L+I}{P+T}$ *plasma ratio ± 1SD.*

RELATIONSHIP OF PLASMA $\frac{V+L+I}{P+T}$ RATIO TO HEPATIC DYSFUNCTION

In the 40 patients with alcohol related liver disease who were studied, no consistently significant correlation was found between the value of the amino-acid ratio and the prothrombin time, plasma aspartate transaminase, alkaline phosphatase, bilirubin, total protein, albumin, or bile acids. However, as shown in Fig. 5, a highly significant correlation existed between the ratio and the severity of the liver damage as judged histologically ($r = 0{\cdot}74$, $P < 0{\cdot}001$).

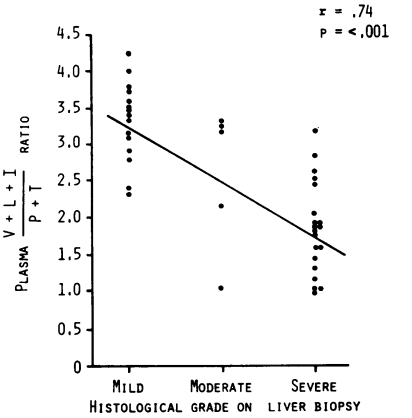

Fig. 5 *Correlation between the plasma* $\frac{V+L+I}{P+T}$ *ratio and liver histology in a group of 40 alcoholics. r: regression coefficient.* P*: Student's t test.*

All six patients with acute type A hepatitis who were followed serially showed similar results (Table 2) which are well exemplified by the patient illustrated in Fig. 6. This patient became clinically well six weeks after the onset of jaundice and the standard liver function tests became consistently normal at nine weeks. The amino acid ratio remained abnormal for almost 16 weeks at a time when the liver biopsy still showed minor abnormalities.

Table 2 *Details of follow-up of six patients with acute type A hepatitis*

Patient	*Age (yr)*	*Sex*	*Clinically well (weeks)*	*LFTs normal (weeks)*	$\frac{V+L+I}{P+T}$ *normal (weeks)*
E.U.	40	M	6	9	16
A.E.	51	M	10	15	24
M.C.	30	M	16	20	36
L.S.	31	F	8	10	14
J.M.	20	M	10	10	12
M.M.	19	F	12	16	22

LFTs: liver function tests.

Table 1 *Details of six patients with cirrhosis and chronic hepatic encephalopathy treated for four months with a low protein diet and lactulose*

Patient	*Age (yr)*	*Sex*	*Aetiology of cirrhosis*	*Pre-treatment* $\frac{V+L+I}{P+T}$	*Post-treatment* $\frac{V+L+I}{P+T}$	*Clinical improvement*	*EEG improvement*
J.C.	63	M	Alcoholic	1·1	1·1	Yes	Yes
J.K.	61	M	Alcoholic	1·6	1·8	Yes	Yes
R.T-H.	54	M	Alcoholic	1·2	1·2	Yes	No
G.D.	60	M	Cryptogenic	1·5	1·3	No	No
L.C.	55	F	PBC	1·3	1·5	Yes	Yes
R.R.	67	F	PBC	1·2	1·4	Yes	Yes

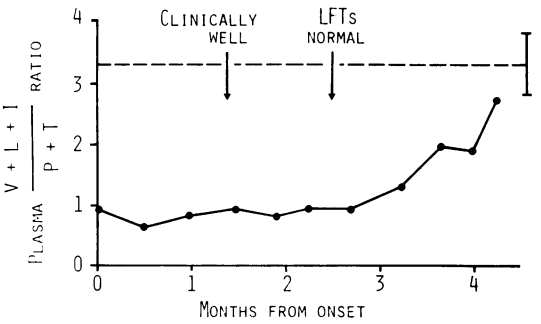

Fig. 6 *Patient E.U. with acute type A hepatitis.* ‡ *mean control* $\frac{V + L + I}{P + T}$ *plasma ratio* ± *1SD. LFTs: standard liver function tests.*

Discussion

Fischer *et al.* (1975) showed that the plasma ratio of the three branched chain amino acids valine, leucine, and isoleucine to the two aromatic amino acids phenylalanine and tyrosine was significantly reduced in animals and patients with hepatic encephalopathy. The hypothesis was then made by Soeters and Fischer (1976) that hepatic encephalopathy might, in fact, result from changes in the plasma concentrations of the amino acids making up this ratio. Our studies have shown, however, that the $\frac{V + L + I}{P + T}$ ratio is lowered in patients with chronic liver disease irrespective of the presence of hepatic encephalopathy. So it would seem that alterations in the plasma concentrations of these five amino acids are not a cause of, and do not result from, hepatic encephalopathy: rather they appear to occur simply as the result of liver damage.

Although no consistent correlation was observed between the value of this plasma ratio and the prothrombin time or the concentrations of the plasma aspartate transaminase, alkaline phosphatase, bilirubin, total protein, albumin, or the bile acids, a good, consistent, and significant correlation existed between the ratio and the severity of the liver damage as judged histologically. Our long-term studies show that the value of the ratio often reflected histological liver damage independently of the patient's clinical condition. In the patients with acute, reversible liver disease, the ratio remained abnormal while there was still histological liver damage, often at a time when the standard liver function tests had become consistently normal and the patients were clinically well. In patients with cirrhosis complicated by hepatic encephalopathy, the ratio remained abnormal even though the patients showed clinical and often EEG improvement when treated with dietary protein restriction and lactulose.

Although changes were seen in the plasma concentrations of the five individual amino acids throughout the day, no significant diurnal variation occurred in the value of the ratio in either the control subjects, or in the patients with liver disease. The value of the ratio is therefore reliable and reproducible.

The plasma concentrations of the five amino acids making up the ratio change in the presence of liver damage. The liver is the main site of catabolism for phenylalanine and tyrosine, so that the plasma concentrations of these amino acids are dependent upon their handling by the liver. The increased plasma concentrations of phenylalanine and tyrosine seen in patients with liver disease may therefore occur because the failing liver cannot catabolise them and they accumulate in the plasma.

The ability of the liver to handle the branched chain amino acids, however, is strictly limited. As other tissues—in particular, kidney and skeletal muscle—have a considerable capacity for the transamination and subsequent oxidation of the branched chain amino acids to provide utilisable ATP, the plasma concentrations of these amino acids are largely controlled by their peripheral tissue metabolism (Miller, 1962). The uptake of the branched chain amino acids into these tissues is promoted by insulin, so that their plasma concentrations are considerably affected by the amount of circulating insulin (Felig *et al.*, 1969). The liver is responsible for extracting 40 to 60% of insulin from the circulation (Kaden *et al.*, 1973). It is not therefore surprising that cirrhosis is commonly associated with hyperinsulinaemia reflecting its impaired extraction by the damaged liver (Creutzfeldt *et al.*, 1970). Hyperinsulinaemia, if present, might be responsible for the low plasma concentrations of branched chain amino acids.

The distinct pattern of raised aromatic amino acids and reduced branched chain amino acids seen in chronic liver disease is therefore consistent with loss of hepatic control over certain amino acids—for example, phenylalanine and tyrosine—together with excessive removal of branched chain amino acids by peripheral tissues due to hyperinsulinaemia.

However, significant lowering of the plasma values of the branched chain amino acids can be found in patients with very little liver damage as exemplified by mild type A hepatitis or minimal alcoholic fatty change (Morgan *et al.*, 1978). This may reflect increased peripheral utilisation of these amino acids by the body tissues to offset the liver's inability to maintain glucose homeostasis, resulting in a fall in

circulating branched chain amino acid concentrations. This suggests that a decrease in the liver's capacity for gluconeogenesis is an early consequence of liver damage.

We have, as a result of our studies, been unable to find support for the suggestion that hepatic encephalopathy results from changes in the plasma $\frac{V + L + I}{P + T}$ ratio.

We have, however, shown that lowering of this plasma ratio occurs solely as a result of liver damage.

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