Insulin and Glucagon Levels in Liver Cirrhosis

Relationship with Plasma Amino Acid Imbalance of Chronic Hepatic Encephalopathy

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Alterations in insulin and glucagon levels might account for the plasma amino acid imbalance of cirrhotics. In order to verify this hypothesis we evaluated basal insulin, glucagon, branched-chain amino acids, aromatic amino acids, and free tryptophan in 13 controls and 37 cirrhotics divided on the basis of their mental state; in 4 patients the hormonal and amino acid patterns were sequentially studied during various stages of encephalopathy. Glucagon is high in cirrhotics and progressively increases with the worsening of the mental state. Free tryptophan and aromatic amino acids show a similar behavior and significantly correlate with glucagon levels ($r = 0.67$ and $r = 0.81$, respectively). On the other hand insulin levels, which are high in cirrhotics without encephalopathy, fall in the presence of deep coma. Insulin did not correlate with any of the plasma amino acids considered. Our data suggest that the catabolic state associated with increased glucagon levels may account for some of the alterations in the plasma amino acid profiles of cirrhotics. Portal-systemic shunting does not seem to be the common cause of both hyperglucagonemia and hyperaminoacidemia. Decreased branched-chain amino acid levels may be related to factors different from those involved in the alterations of carbohydrate homeostasis.

In cirrhotic subjects a distinctive pattern of plasma amino acids (AAs) has been observed, including high levels of both free tryptophan and aromatic AAs (phenylalanine and tyrosine) and low levels of branched-chain AAs (valine, isoleucine, and leucine) (1-5). This AA imbalance has been related to the inability of the liver to remove amines from portal blood as well as to alterations in carbohydrate homeostasis secondary to the liver failure itself (6), since the liver is the key organ in carbohydrate metabolism.

In advanced liver cirrhosis a remarkable increase in glucagon levels has been demonstrated (7), mainly when portal-systemic shunting is present (8). In spite of increased insulin levels (9), the insulin/glucagon molar ratio is decreased (10); this reduced ratio may be assumed to be a marker of a catabolic state characterized by enhanced gluconeogenesis from different sources, mainly amino acids (11). Soeters and Fischer proposed that under the catabolic stimuli of hyperglucagonemia and low insulin/glucagon ratios AAs could be released from the lean body mass or from the liver itself. Aromatic AAs (AAA), which could not be metabolized by the failing liver, would accumulate while branched-chain

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AAs (BCAA) could be used as energy sources for gluconeogenesis in organs and tissues different from the liver (6).

In order to verify this hypothesis we decided to evaluate, at the same time, plasma AA profiles as well as insulin and glucagon levels in different groups of cirrhotic patients. In the present paper we give evidence that some of the alterations in plasma AA profile of cirrhotics, with different grades of encephalopathy, significantly correlate with the hyperglucagonemia and the reduced insulin/glucagon ratio.

MATERIALS AND METHODS

Patients. Thirteen controls and 37 cirrhotic patients were studied. Control subjects (10 males and 3 females; median age 45 yrs, range 28–65) had normal liver and renal function tests and no previous history of liver disorders. In cirrhotics the diagnosis was based on clinical and laboratory data and had previously been documented by percutaneous liver biopsy and/or laparoscopy. The patients were divided into the following three groups according to their mental state as suggested by Lam et al (12) on the basis of clinical examination only, without the aid of EEG or tests for constructional apraxia:

Group 1 comprised 14 patients (9 males and 5 females; median age 53 yrs, range 34–69) with normal mental state; 5 had $HBsAg^+$ postnecrotic cirrhosis (PNC), 5 had alcoholic cirrhosis (AC), and 4 cryptogenic cirrhosis (CC). All patients had normal renal function tests.

Group 2 included 14 patients (10 males and 4 females; median age 51 yrs, range 36–71) with encephalopathy grade I-II; 4 of them had PNC, 6 AC, and 4 CC; 9 patients were on diuretic treatment and in 2 of them creatinine levels exceeding 1.5 mg/dl were also present.

Group 3 comprised 9 cirrhotics (2 PNC, 5 AC, and 2 CC) with encephalopathy grade III-IV; 6 were males and 3 females; their median age was 56 yrs (range 41–71). In 5 patients renal failure was present. The precipitating cause of encephalopathy was gastrointestinal bleeding in two of them; the remaining patients were on diuretic treatment which might have played a role in the worsening of their mental state.

No attempt was made to measure the degree of portal-systemic shunting in the three groups of patients.

In all subjects, except group 3 patients, blood samples were taken after 12 hours' fasting. In group 3 blood samples were taken as soon as they entered the ward, before any therapy for hepatic encephalopathy started; relatives reported that these patients had eaten no food for at least 3 hr.

In 4 patients the hormonal and AA pattern could be sequentially studied during various stages of hepatic coma, one of them during severe encephalopathy of unknown origin and, during several weeks when mild encephalopathy and later normal mental state were achieved. Three patients were studied before and during encephalopathy caused by progressive liver failure; in these subjects mild encephalopathy progressed to deep coma and death in about one month.

Blood was taken for glucose, insulin, glucagon and AA determination as well as for routine analysis. Samples for insulin, glucagon, and AA assay were stored at −20°C until analyses were carried out.

Methods. Blood glucose was immediately determined by means of a glucose-oxidase method. Albumin levels and prothrombin time were measured by standard techniques. Insulin (IRI) was determined by radioimmunoassay using Insulin Kit (Dow Lepetit, Milano). Blood samples for glucagon (IRG) assay were collected in chilled tubes containing 500 units Trasylol and 1.2 mg Na_2 EDTA/ml of blood in a total volume one tenth the expected volume. IRG was then measured using 30 K Unger's antibody and [^{125}I]glucagon (CNTS, Orsay) (13). The insulin/glucagon molar ratio (IRI/IRG) was calculated as suggested by Muller et al (14) with the following formula:

$$\frac{\text{IRI}}{\text{IRG}} = \frac{\text{IRI } (\mu\text{U/ml})}{\text{IRG (pg/ml)}} \times 23.33$$

which is based on a molecular weight of 6000 and biologic activity of 25 units/mg for insulin and a molecular weight of 3500 for glucagon.

Amino acid profiles were assayed on the deproteinized portion of plasma after treatment with 4% sulfosalicylic acid. The supernatants were applied to a preprogramed Carlo Erba 3A 27 Automatic Aminoanalyzer and peaks of free AAs were read for both identity and quantity. Free tryptophan (TRY) was evaluated with a spectrofluorometric method according to Denckla and Dewey (15) in an ultrafiltrate obtained from 2 ml of plasma centrifuged at 800 g in an Amicon cone CF 50A for 45 min at room temperature (16).

Statistical analysis. Differences between mean values were tested for significance by means of Student's t test for unpaired data. Values and significance of linear correlation coefficients were calculated by methods described in a standard text.

RESULTS

Glucose, Insulin, Glucagon, and IRI/IRG Molar Ratio. Blood glucose levels (means ± SEM) were, respectively, 79 ± 3, 86 ± 4, 91 ± 5, and 96 ± 12 mg/dl in controls and the three groups of cirrhotic patients. Although higher blood glucose levels were observed in cirrhotics, mainly in the presence of hepatic encephalopathy, no significant differences were observed between Groups.

IRI levels (11.6 ± 0.8 μU/ml in controls) were significantly elevated in groups 1 and 2 (15.7 ± 1.7 and 15.0 ± 1.4, respectively; $P < 0.05$), while in group 3, although wide variations could be observed, they were in the normal range of controls (12.7 ± 3.5).

IRG levels (67 ± 4 pg/ml in controls) were significantly increased in group 1 (117 ± 6; $P < 0.001$) as well as in the two groups of comatose patients (183

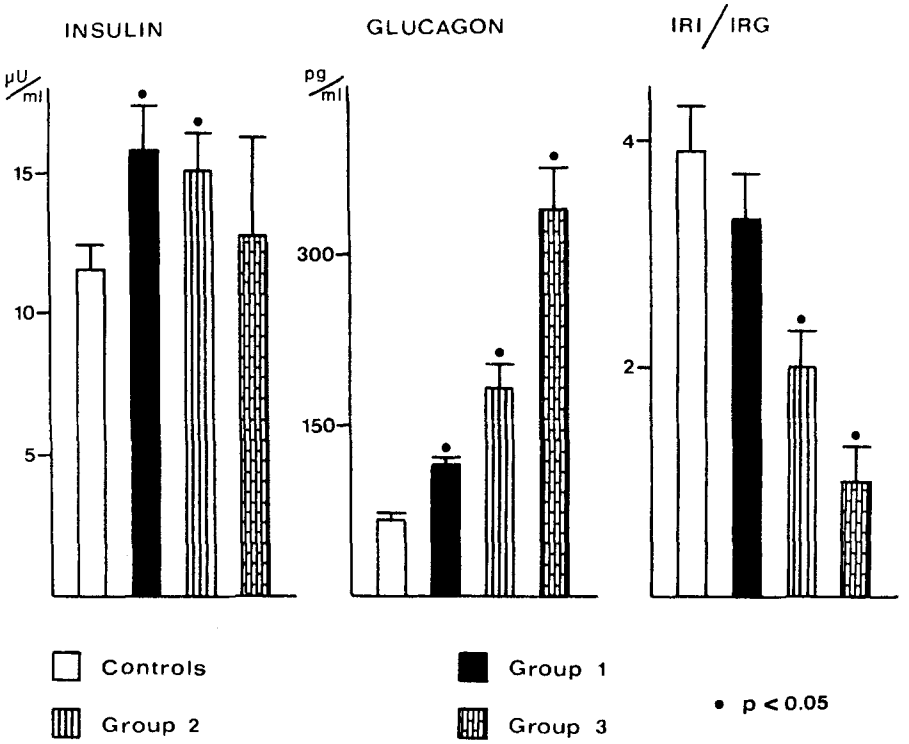

Fig 1. Insulin, glucagon, and insulin/glucagon molar ratio in the controls and the three groups of cirrhotics. The results are expressed as means ± SE. The statistical differences between controls and each group of cirrhotic patients are reported.

± 22 and 340 ± 37, respectively). The differences between group 1 and group 2 and between group 2 and group 3 were both statistically significant ($P < 0.01$ and $P < 0.001$, respectively).

IRI/IRG was not significantly reduced in group 1 when compared to controls (3.3 ± 0.4 vs 3.9 ± 0.4). In group 2 and group 3 patients, on the contrary, IRI/IRG was significantly reduced (2.0 ± 0.3 and 1.0 ± 0.3) when compared to controls as well as to group 1 ($P < 0.005$). A significant difference ($P < 0.02$) was also noticed between group 2 and group 3.

Insulin, glucagon, and insulin/glucagon molar ratio in controls and cirrhotics are summarized in Figure 1.

Plasma Amino Acid Profiles. Table 1 shows the plasma AA levels (means ± SEM) and the ratio BCAA/AAA and TRY/BCAA + AAA of the controls and the cirrhotics.

TRY and AAA were high in cirrhotics and correlated with the encephalopathy grade since they pro-

TABLE 1. PLASMA AMINO ACIDS IN CONTROLS AND IN THREE GROUPS OF CIRRHOTICS*

		Cirrhotics					
		Group 1		*Group 2*		*Group 3*	
	Controls	*P value*†	*Mean*	*P value*	*Mean*	*P value*	*Mean*
TRY (nmol/ml)	5.5 ± 0.5	○	8.5 ± 0.7	+	15.8 ± 3.1	+	24.8 ± 2.0
AAA (nmol/ml)	121 ± 7.0	+	168 ± 14	+	217 ± 19	+	362 ± 63
BCAA (nmol/ml)	453 ± 52	○	254 ± 32	NS	263 ± 25	NS	281 ± 35
BCAA/AAA	3.9 ± 0.6	○	1.7 ± 0.2	NS	1.5 ± 0.1	+	1.0 ± 0.1
TRY/BCAA + AAA	0.010 ± 0.0006	○	0.019 ± 0.001	○	0.029 ± 0.003	+	0.044 ± 0.007

*Plasma amino acids and molar ratios BCAA/AAA and TRY/BCAA + AAA in controls and in the three groups of cirrhotic patients. Results are expressed as means ± SE. The statistical differences between the groups are reported.
†+ $P < 0.05$; ○ $P < 0.005$; NS = not significant.

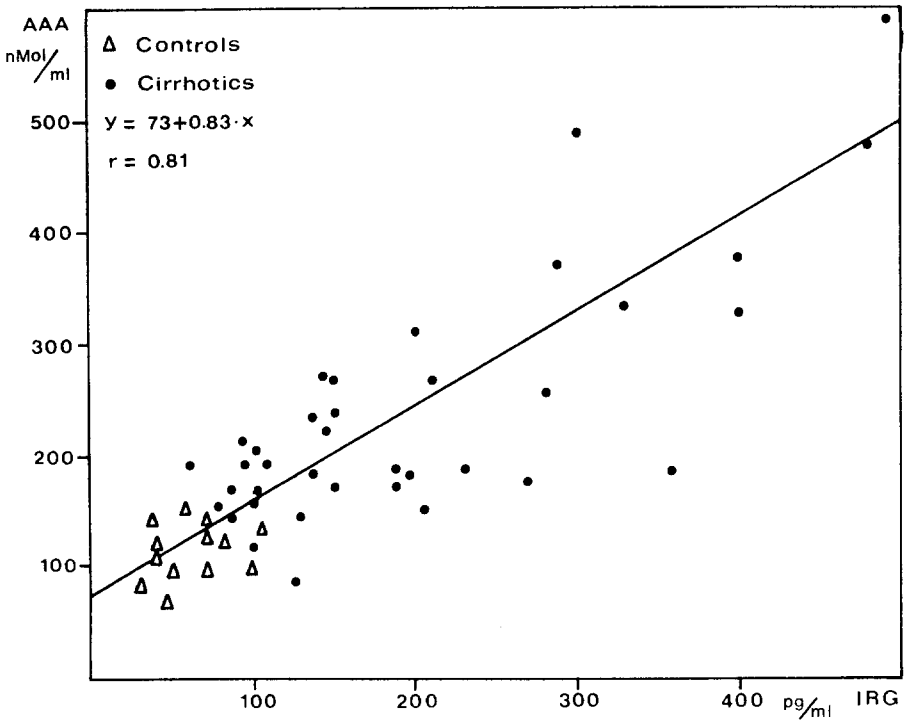

Fig 2. Correlation between aromatic amino acid and glucagon levels in both controls and cirrhotics. When the coefficient was calculated in cirrhotic patients alone, the equation of the curve was: $y = 73 + 0.85x$; $r = 0.79$.

gressively increased with the deterioration of the mental state.

BCAA and the molar ratio BCAA/AAA were significantly reduced in group 1. With the worsening of the mental state BCAA increased (although no statistical differences were noted), while BCAA/AAA further decreased, mainly in group 3 patients, where mean BCAA/AAA was significantly lower when compared to group 1 and group 2.

The molar ratio TRY/BCAA + AAA was high in cirrhotics and progressively increased in patients with hepatic encephalopathy.

Relationship Between Hormonal and AA Imbalance. The r coefficients of linear regression analysis were calculated comparing IRI, IRG, and IRI/IRG to plasma AA levels and to molar ratios between AAs in both controls and cirrhotic patients. When coefficients were calculated in cirrhotic patients alone, r values were only just inferior to those reported and P values remained statistically significant ($P < 0.001$).

Glucagon levels significantly correlated with BCAA/AAA ($r = -0.48$) and TRY/BCAA + AAA ($r = 0.63$), but more closely with TRY and AAA levels alone ($r = 0.68$ and $r = 0.81$, respectively) (Figure 2). IRI/IRG more closely correlated with the molar ratio BCAA/AAA ($r = 0.57$) and with TRY/BCAA + AAA ($r = -0.67$) (Figure 3). No statistical correlation was demonstrated between IRI and the plasma AA levels considered.

Glucagon plasma levels did not correlate with albumin or prothrombin time ($r = -0.33$ and -0.07, respectively), which were considered to be indexes of hepatic function.

Sequential Studies of Hormonal and AA Patterns. In the patients studied before and during encephalopathy (A, B, and C of Figure 4), as well as in the single patient in whom a reversal of the altered mental state could be observed (D), the hormonal and AA patterns in the various stages of encephalopathy were similar to those demonstrated in the unpaired groups of cirrhotics. With the worsening of the mental state, a progressive increase in IRG levels was observed, which strictly correlated with TRY and AAA ($r = 0.79$ and $r = 0.85$). The IRI and BCAA levels did not show statistically significant differences during the different stages of the disease.

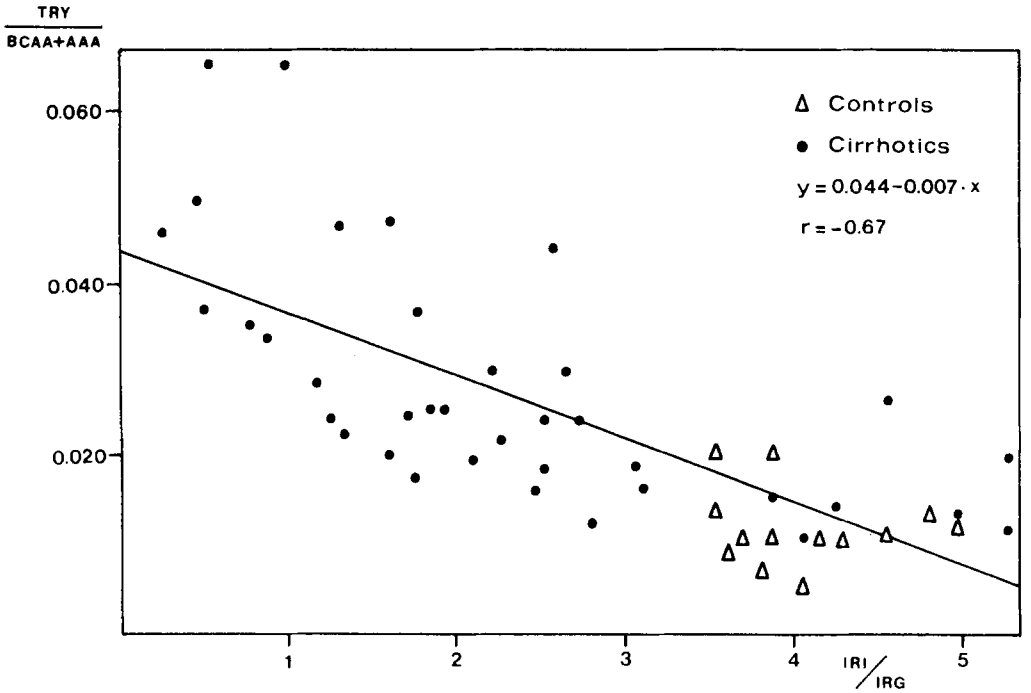

Fig 3. Correlation between the molar ratio TRY/BCAA + AAA and IRI/IRG in both controls and cirrhotics. The equation of the curve, calculated in cirrhotic patients alone, was: $y = 0.043 - 0.006x$; $r = -0.64$.

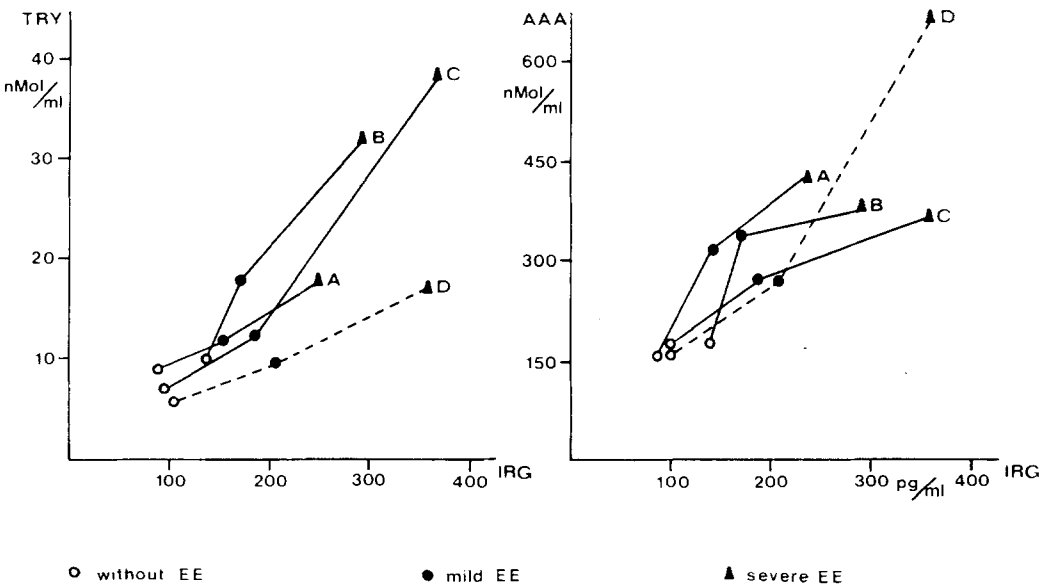

Fig 4. Correlation between glucagon levels and free tryptophan (left) and between glucagon and aromatic aminoacids (right) in the four patients sequentially studied before and during (patients A, B, and C) and during and after (patient D) the various stages of hepatic coma. The equations of the curves, calculated on the 12 points reported, were, respectively: $y = -0.86 + 0.08x$; $r = 0.79$ (left) and $y = 56.0 + 1.26x$; $r = 0.85$ (right). These equations are similar to those calculated for the unpaired data in the three groups of cirrhotics.

DISCUSSION

When we planned this work we faced the problem that we could not starve cirrhotic patients with deep coma for 12 hr as we did for the other groups examined nor could we delay therapy. So we decided to take blood samples in group 3 patients as soon as they entered the ward. Morgan et al (17) have recently shown that there are no significant diurnal variations in the value of the molar ratio BCAA/AAA, but both BCAA and AAA, and to a greater extent TRY and TRY/BCAA + AAA, may be reduced by glucose ingestion (18). Moreover a glucose meal may induce significant alterations in IRI, IRG, and IRI/IRG levels (19); therefore a careful evaluation of the postabsorptive state might be relevant in comparing the hormonal and AA patterns. No differences in glycemia levels were present in the three groups of cirrhotics examined; because of it we assume that a recent carbohydrate ingestion might not have altered either IRI and IRG levels or plasma AA profiles.

As previously shown (9) we found elevated insulin levels in cirrhotics. This hyperinsulinemia is probably due to decreased hepatic degradation of insulin secondary to both liver cell damage and portal-systemic shunting (20). With the worsening of the mental state, insulin levels failed to further increase in cirrhotics, in sharp contrast with that observed during the encephalopathy of fulminant hepatic failure where a tenfold increase in insulin levels has been reported (21). The relative fall in insulin, in the presence of possibly increased liver cell damage and portal-systemic shunts, can be only explained on the basis of reduced secretion, just as it happens in other catabolic states such as prolonged starvation (22).

As to glucagon levels, previous studies have shown that a hyperglucagonemia may be demonstrated in cirrhotics only when portal-systemic shunting is present (8), although a more recent study has reported hyperglucagonemia as a feature of hepatocellular damage (23). The high glucagon levels of cirrhotics seem to be due to an altered secretion and sensitivity to glucagon rather than to a decreased hormonal catabolism since the kidney is the major site of glucagon degradation (24). Sherwin et al have recently postulated that liver failure may alter the negative feedback signal which could lead to glucagon secretion suppression (25). In our study we could demonstrate a significant increase in glucagon levels also in group 1 patients, where no evidence of encephalopathy was present. However, hyperglucagonemia in cirrhotics closely correlates with encephalopathy grades since it progressively increases with the deterioration of the mental state.

As far as plasma AAs are concerned, the levels of both TRY and AAA showed a progressive increase with the worsening of the mental state similar to that observed for glucagon levels. We found highly significant correlations between glucagon and these amino acids, the better correlation existing with AAA being probably explained on the basis of the well-known influence of albumin and free fatty acid plasma levels on the bound/free tryptophan ratio (26).

We have no direct evidence that a causal relationship exists between glucagon and these amino acids; however, many data may support this hypothesis. First of all glucagon acts as a catabolic hormone, stimulating gluconeogenesis in man (27) and promoting ureagenesis (28) and protein catabolism in the liver (29) and possibly on skeletal muscle (30), although contrasting data have been reported (31, 32). Secondly the administration of oral glucose, in an effort to suppress glucagon secretion, has proven effective in reducing the levels of both TRY and AAA in man (18).

Alternatively the levels of both glucagon and amino acids could be influenced by liver failure or portal-systemic shunting. Hepatic function, as assessed by plasma albumin levels and prothrombin time, seems not to be the link between hyperglucagonemia and hyperaminoacidemia since glucagon levels in cirrhotics do not correlate with hepatic function. A careful evaluation of the degree of collateral circulation is needed in order to assess the relevance of portal-systemic shunting in the pathogenesis of both the hormonal and AA imbalance of cirrhosis. As far as our data are concerned, the results of the sequential studies we performed in patients A, B, and C could fit with the hypothesis that shunting of blood might play a major role in the alterations observed; however, in patient D, who could be followed from a stage of deep coma to normal mental state, the fall in AA and IRG levels can hardly be explained on the basis of a reduced collateral circulation. So we believe that shunting is not the only cause of all the changes, and it is possible a causal relationship between hormonal imbalance and altered AA pattern may exist.

We also evaluated IRI/IRG according to Unger's hypothesis of a bihormonal modulation in maintaining blood glucose levels as well as in directing food-

stuff metabolism towards an anabolic or a catabolic pathway (11). Previous studies have shown that IRI/IRG, following portacaval anastomosis in dogs (33) and in the course of liver cirrhosis in man (10), is reduced only when neurologic symptoms and signs occur. In both animals and humans the fall of IRI/IRG is due to increasing glucagon levels as well as to a relative fall in insulin levels which return to the normal range of controls. In our patients we could demonstrate a good correlation between IRI/IRG and both the increase of TRY and AAA and the molar ratio BCAA/AAA and particularly TRY/BCAA + AAA which this and previous work (3) have shown to be representative of the mental state of cirrhotics.

We did not find any correlation between BCAA levels and insulin, glucagon, or IRI/IRG. Munro et al (34) first related BCAA with insulin in cirrhotics and later Soeters and Fischer (6) tried to explain decreased BCAA levels with increased incorporation into muscle, or preferably adipose tissue under the anabolic stimulus of insulin. Our data are in contrast with this hypothesis since no correlation could be found between increasing insulin and decreasing BCAA levels. Thus factors different from those involved in the maintenance of carbohydrate homeostasis probably account for the decrease in BCAA levels, which seems to be a sensitive alteration of plasma AA profile of liver cirrhosis quite unrelated to chronic hepatic encephalopathy.

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