Infusion of Branched-chain Enriched Amino Acid Solution in Patients with Hepatic Encephalopathy

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Hospitalized patients with hepatic insufficiency often suffer from severe catabolic states and are in urgent need of nutritional support during their acute illness. Protein intolerance, however, remains a significant problem with respect to the provision of adequate nutrition, either enterally or parenterally. The following report is an anecdotal series of 63 consecutive patients in a large urban hospital treated prospectively with nutritional support using a prototype high branched-chain amino acid solution (FO80) given by technique of total parenteral nutrition by the subclavian or internal jugular route with hypertonic dextrose. Sixty-three patients, of which 42 had chronic liver disease (cirrhosis) with acute decompensation and 17 with acute hepatic injury as well as four with hepatorenal syndrome, are the subject of this report. All required intravenous nutritional support and were either intolerant to commercially available parenteral nutrition solutions or were in hepatic encephalopathy at the time they were initially seen. The cirrhotic patients had been hospitalized for a mean of 14.5 ± 1.9 days before therapy, had a mean bilirubin of 13 mg/100 ml, and had been in coma for 4.8 ± 0.7 days despite standard therapy. Patients with acute hepatitis had been in the hospital for 16.2 ± 4.1 days before therapy, had a mean bilirubin of 25 mg/100 ml, and had been in coma 5.2 ± 1.6 days before therapy. Routine tests of liver function, blood chemistries, amino acids, EEGs, and complex neurological testing including Reitan trailmaking tests were used in the evaluation of these patients. Up to 120 grams of synthetic amino acid solution with hypertonic dextrose was tolerated in these patients with improvement noted in encephalopathy of at least one grade in 87% of the patients with cirrhosis and 75% of the patients with hepatitis. Nitrogen balance was achieved when 75 to 80 grams of synthetic amino acids were administered. Survival was 45% in the cirrhotic group and 47% in the acute hepatitis group. Encephalopathy appeared to correlate with individual amino acids differentially in the various groups and with the ratio between the aromatic and the branched-chain amino acids. Ammonia did not correlate with either the degree of encephalopathy or improvement therefrom. In 24 patients therapy for hepatic encephalopathy was limited to infusion of the branched-chain enriched amino acid solution only, with wake-up in 66% of this group. The results strongly suggest that in protein intolerant patients requiring nutritional support, infusion with branched-chain enriched amino acid solutions is well tolerated with either no worsening of or improvement in hepatic encephalopathy coincident with the achievement of nitrogen equilibrium and adequate nutritional support.

NUTRITIONAL SUPPORT has always been part of therapy for patients with liver disease. The most critical component of nutritional therapy is the protein component, especially since maintenance of lean body mass is central to nutritional support. In patients with liver disease, however, this often presents a problem with hepatic insufficiency, intolerance to protein, and resultant hepatic encephalopathy.

This report concerns itself with a prototype high branched-chain amino acid solution that has been extensively tested in experimental animals[1] and in preliminary fashion in man.[2] The theoretical basis for this approach to hepatic encephalopathy, namely, normalization of plasma amino acid pattern, has been extensively reviewed[3-5] and need only be reviewed briefly here. The "amino acid-neurotransmitter hypothesis"[6] relates hepatic encephalopathy to an imbalance of central adrenergic and indoleamine neurotransmitters, secondary in turn to abnormal transport of aromatic amino acid precursors of aminergic neurotransmitters to within the brain.[7] Although ammonia, glutamine, and abnormalities in the blood-brain barrier itself contribute to the mechanisms by which encephalopathy is presumably produced, the abnormal plasma amino acid pattern, increased aromatic amino acids, and decreased branched-chain amino acids[8-10] favor the accumulation of the toxic aromatic amino acids within the central nervous system.

In infusing a branched-chain amino acid enriched

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(FO80), 24% glucose parenteral nutrition solution, several purposes are intended:

1. Decreased efflux of aromatic amino acids from muscle by decreasing catabolism (11–13).
2. Normalization of plasma amino acid pattern by decreasing efflux of aromatic amino acids from muscle and by incorporation of the aromatic amino acids within muscle protein by protein synthesis with an appropriate supply of protein and calories. Because fat calories may not be utilized in patients with liver failure, dextrose calories are to be preferred.
3. The branched-chain amino acids may have a regulatory role in the control of muscle amino acid efflux.[14,15] Infusing BCAA decreases efflux of the aromatic amino acids from skeletal muscle.
4. Since the neutral, aromatic, and branched-chain amino acids all compete for entry across the blood-brain barrier by a common carrier system,[16] increasing BCAA decreases penetration of aromatic amino acids.

Other investigators working in Japan[17] and Western Europe[18,19] have reported on their experiences with various branched-chain enriched amino acid solutions given either peripherally or centrally with or without hypertonic dextrose respectively. Without exception, all reported beneficial effects in hepatic encephalopathy. In one study, postmortem analysis of brain in patients who awakened following BCAA therapy and suddenly exsanguinated revealed correction of indoleamine derangements.[20]

This report is an unselected anecdotal series of 63 patients with various liver diseases in a large urban hospital who required parenteral nutritional support, were in hepatic encephalopathy or were intolerant to commercially available amino acid mixtures. Since this is an uncontrolled trial in which consecutive patients were treated at request of their primary physicians, the only common thread is that they required nutritional support. It is obviously impossible to speak of efficacy of therapy with respect to survival as patients were not randomized. (A randomized prospective double-blind controlled trial is currently in progress.)

However, several points require comment:

1. In patients with severe liver disease large amounts of amino acids were administered with either no worsening or, in most cases, improvement in hepatic encephalopathy.
2. Nitrogen balance was achieved in patients in whom 80 gm of amino acids could be administered daily.
3. In patients who received no other form of therapy for hepatic coma, the administration of the branched-chain amino acid mixture was associated with wake-up.
4. The results are promising enough to clearly call for a randomized prospective trial in which branched-chain amino acids are randomized against standard therapy.

Materials and Methods

Sixty-three patients admitted to the Massachusetts General Hospital from July 1976 to June 1978, manifesting hepatic encephalopathy or coma at some time during their hospital course, form the basis for this report.

Criteria for inclusion in this study were the presence of hepatic encephalopathy (HE) greater than Grade I and/or the need for parenteral nutrition (PN) in patients with either acute or chronic liver disease and the development of intolerance to commercially available amino acid formulations. Patients were divided into three groups based on the etiology of their liver disease. Group I consisted of 42 patients with chronic liver disease and cirrhosis, in whom an acute insult such as gastrointestinal bleeding, sepsis, or overdiuresis triggered the development of acute onset hepatic encephalopathy or coma. Group II consisted of 17 patients with acute hepatitis with no history of previous liver disease. In this group were included patients with viral hepatitis, drug-induced hepatitis (halothane), acute alcoholic hepatitis, and hepatic decompensation of unknown etiology (some of those being severely septic with acute liver damage). Group III patients four patients were suffering from the hepatorenal syndrome and will be discussed separately because of the grave prognosis. Diagnosis was based on history, physical exam, liver biopsy (when possible), and autopsy. All patients remained under the care of their primary physician with the nutritional support team serving as a consultant. Informed consent was obtained in all cases for this protocol which was reviewed by the Human Studies Committee of The Massachusetts General Hospital.

Patients with chronic cirrhosis (CC) and acute onset hepatitis (AH) behave differently clinically, their plasma amino acid pattern is different, and their treatment modalities and clinical outcomes tend to be different. Therefore, we will discuss these two groups separately.

Before and during treatment with FO80 the following studies and determinations were performed: routine clinical chemistry tests (BUN, glucose, electrolytes, creatinine, calcium, phosphorus, magnesium), "liver function" tests (total protein, albumin, globulin, bilirubin, SGOT, alkaline phosphatase), ammonia (venous blood), plasma amino acids (AA), plasma octopamine (OCT) and phenylethanolamine (PEA), prothrombin time, partial thromboplastin time, platelet count, hemoglobin, hematocrit, white blood count. Determinations were performed before starting infusion with FO80, daily for the first 3 days of treatment with FO80

and every other day or twice weekly later on during treatment. Electroencephalograms were performed on a similar schedule.

Patients were evaluated daily by a member of the hyperalimentation team, by a neurologist (JL), and by a gastroenterologist (JD). All patients in this study were seen and evaluated by the same physicians. Encephalopathy grades followed the classification of Adams and Foley.[21] Reitan trailmaking tests[22] were performed 1 to 2 times a week depending on the patient's neurologic status.

Total urinary nitrogen was determined daily by the Kjeldahl method and nitrogen balances calculated. Plasma amino acid determinations were carried out by a Beckman MB-121 amino acid analyzer on the supernatant of plasma rendered protein free by precipitation with 5% sulfosalicylic acid.

Octopamine and phenylethanolamine were determined by a modification of the Molinoff technique.[23] FO80 was infused as a 4% amino acid solution (Table 1) containing 40 grams amino acids, 240 grams of dextrose, electrolytes, and vitamins in a total volume of 1000 ml through a catheter inserted into the subclavian or internal jugular vein, the tip located in the superior vena cava. Protocol for insertion of these lines, management, and maintenance of sepsis followed the same guidelines used for any other form of central hyperalimentation in this hospital. Solutions were infused at a constant rate over 24-hour periods. Infusions were begun at a rate of 40 to 60 ml/hr depending primarily on fluid and glucose tolerance and on renal function. The infusion rate was increased by 10 to 20 ml/hr every 24 to 48 hours according to tolerance up to a maximum of 3000 ml/day (120 grams of amino acids and 3000 calories/day).

In patients with impaired renal function a lower dose of amino acids with a higher caloric density (2.2% amino acid solution of FO80 mixed in 35% glucose) was used.

Other treatment modalities commonly used in the treatment of hepatic encephalopathy such as neomycin, lactulose, cathartics, and the like were continued or discontinued depending on the primary physician's decision. As the study progressed there was an increased tendency to stop other modes of therapy when starting FO80. Among this group of 63 patients there were 24 patients who were treated with FO80 only. This group will be discussed separately.

All the clinical and laboratory data, liver function tests, ammonia, amino acids, octopamine, phenylethanolamine, nitrogen balances, electroencephalograms, trail tests, amount of FO80 infused, patient response, outcome, and complications were stored on punchcards. Data was analyzed by a computer, using the Statistical Package for the Social Sciences (SPSS) for condescriptive, frequency distributions, t test, Pearson correlations, nonparametric correlations, regression analysis, and cross-tables.

TABLE 1. *Composition of the Synthetic Amino Acid Formulation FO80 Used in This Study (g/1000 ml Infused Solution)*

L-Essentials	
Isoleucine	4.50
Leucine	5.50
Lysine	3.05
Methionine	0.50
Phenylalanine	0.50
Threonine	2.25
Tryptophan	0.33
Valine	4.2
Nonessentials	
L-Alanine	3.80
L-Arginine	3.00
L-Histidine	1.20
L-Proline	4.00
L-Serine	2.50
Glycine	4.50
L-Cysteine HCl-H_2O	>0.1
Dextrose	240
Total Nitrogen	5.6
Electrolytes	As needed
Vitamins	B, C, D, E, A

Results

Results will be discussed separately for the group of patients with acute-on-chronic cirrhosis and encephalopathy (42 patients) and patients with acute onset hepatitis and encephalopathy (17 patients). The very small group of patients with the hepatorenal syndrome will be mentioned whenever relevant (4 patients).

In the chronic cirrhosis (CC) group there were 62% males and 38% females. Their mean age was 58.3 ± 2 years, 60% of the group being in the 6th and 7th decade. In the acute hepatitis group (AH) there were 59% males and 41% females. They tended to be younger with a mean age of 43.3 ± 3.9 years, 70% being in the third, fourth, and fifth decade of life (Fig. 1). The small hepatorenal failure group consisted of 75% (three) males and 25% (one) female, their mean age 52 ± 14 years.

Hospital Stay Before Therapy with FO80

The CC group of patients was hospitalized for a mean of 14.5 ± 1.9 days and the AH group for 16.2 ± 4.1 days before treatment with FO80 was initiated. Sixty percent of patients with CC and 43% of patients with AH were in the hospital for up to 2 weeks before starting on FO80. Seventeen percent and 25% respectively were in the hospital for 3 weeks and 24% and 31%

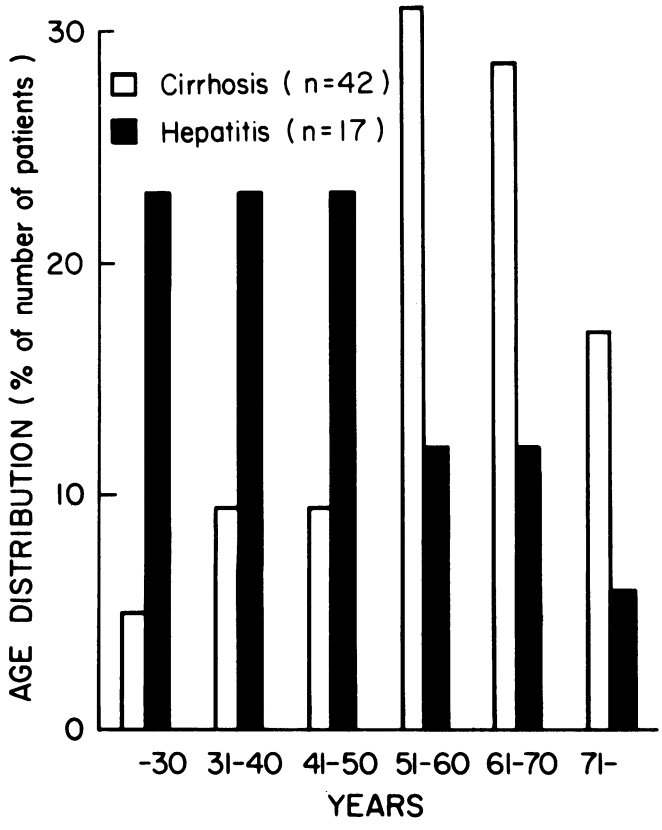

FIG. 1. Age distribution of patients with cirrhosis or hepatitis. Patients with cirrhosis tend to form the larger group and are distributed with most of the patients being in the 6th and 7th decade. The patients with acute hepatitis, of which many have acute alcoholic hepatitis, tend to be younger with most of the patients in their third, fourth, and fifth decade.

respectively were hospitalized for more than 3 weeks before starting on FO80.

Duration of Encephalopathy or Coma Before Starting Treatment with FO80

In the CC group, 76% of the patients started treatment with FO80 within 7 days of onset of encephalopathy or coma (mean 4.8 ± 0.6 days), while in the AH group 80% of the patients were started within a week of coma onset (mean 5.2 ± 1.6 days).

Period of Treatment with FO80

In the CC group of patients treatment with FO80 was carried out for 1 week in almost one third of the patients, for 2 weeks in another third and for 3 weeks in the last third (mean 13 ± 1 days). In the AH group, 82% of the patients received FO80 for 4 to 14 days (mean 13.2 ± 3.8 days) (Fig. 2). The hepatorenal group was treated with FO80 for a mean of 17.2 ± 11.3 days.

Post-treatment with FO80 Hospital Stay

After starting treatment with FO80, 39% of patients with CC and 41% of patients with AH stayed up to 2 weeks in the hospital, 39% and 24% respectively stayed 2 to 4 weeks, and 22% and 35% respectively stayed longer than 4 weeks.

The CC Group had a mean post-FO80 hospital stay of 23.6 ± 3.8 days compared with a significantly longer stay of 33.3 ± 7.8 days in the AH group.

Clinical Improvement—Hepatic encephalopathy

Before treatment the encephalopathy grades of the groups were distributed as follows:

	CC	AH
STAGE I	13%	44%
STAGE II	23%	11%
STAGE III	37%	22%
STAGE IV	23%	22%

After three days of treatment 56% of the CC group showed improvement, 26% did not change and 19% worsened. For the AH group the equivalent figures were 25%, 63%, and 13% (Table 2).

Pretreatment and post-treatment (after approximately 3 days on FO80) mean amino acid values for

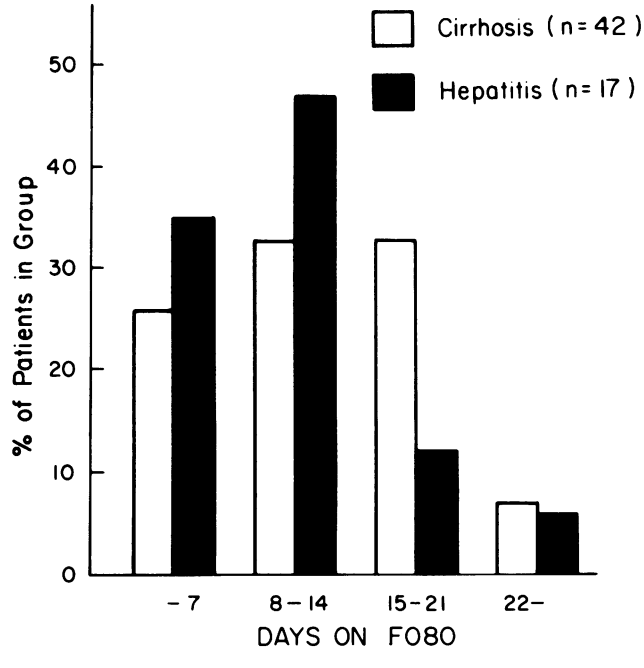

FIG. 2. Duration of therapy with FO80. The number of days of treatment for both patients with cirrhosis and hepatitis are given. Most of the patients were treated for 2 to 3 weeks, particularly those patients with cirhosis who required prolonged therapy generally following a surgical catastrophe.

TABLE 2. *Initial Improvement in Encephalopathy in Response to Treatment with FO80 (First 3 Days of Treatment with FO80)*

	Per cent of Patients Improving	
	Cirrhosis	Hepatitis
Within 24 hours	29%	25%
Within 24–48 hours	35%	42%
Within 48–72 hours	23%	8%
Total within 72 hours	56%	25%

the CC and AH groups are shown in Table 3. Pretreatment blood chemistry, ammonia and liver function tests appear in Table 4.

In the hepatorenal group, three out of four patients improved by day 3 of treatment; one patient worsened. Despite the improvement, there was a 75% mortality in this small group, not unexpected.

Improvement in Electroencephalogram (EEG)

Only 43% of the CC group and 50% of the AH group had a pretreatment EEG. In the CC Group, 14% had mild, 18% moderate, and 7% severe abnormalities of EEG. In the hepatitis group, 10% had normal EEG, and 40% moderate to severe EEG changes. EEG improvement, judged by increasing frequency, disappearance of theta frequency and triphasic waves, and by increase in amplitude, occurred in 59% of the patients with chronic cirrhosis and in 70% of patients with AH. The improvement in EEG usually was slower than the clinical improvement and patients reversing to Grade 0-I encephalopathy still showed EEG signs of metabolic encephalopathy.

Plasma Amino Acid Pattern in Chronic Cirrhosis and Acute Hepatitis

Table 3 and Figure 3 show the amino acid patterns for the CC and AH groups compared with normal. It can be seen that levels of methionine phenylalanine, tyrosine, aspartate, and glutamate were markedly increased for the CC group, while the levels of the branched-chain amino acids valine, leucine and isoleucine were depressed.

The AH group had even more marked increases in methionine, phenylalanine, tyrosine, and glutamate. Increases in lysine, glycine, and ornithine were also present. The branched-chain amino acids valine, leucine, and isoleucine were present at approximately normal levels.

After 3 days of treatment the levels of some of the amino acids in the CC group were altered to a more normal level, noticeably aspartate, methionine, tyrosine, serine, alanine, histidine, arginine, and also two of the branched-chain amino acids valine and leucine. Isoleucine increased above the normal level (Fig. 4).

In the AH group 3 days of treatment with FO80 resulted in reduction in levels of methionine, phenylalanine, tyrosine, aspartate, and asparginase occurred while threonine, taurine and glycine increased (Fig. 5).

TABLE 3. *Plasma Amino Acid Levels Before and After 3 Days of Treatment with Hepatic Failure Fluid in Chronic Cirrhosis and in Acute Hepatitis (nmol/ml)*

	Chronic Cirrhosis					
	Before Treatment			After Treatment		
	N	Mean	S.D.	N	Mean	S.D.
TAU	18	37.50	28.88	38	61.48	51.31
ASP	16	31.44	77.40	31	13.19	11.73
THR	20	92.25	48.31	38	173.54	117.84
SER	20	48.15	32.52	38	87.36	77.11
ASA	7	88.57	64.18	15	281.82	257.16
GLU	17	143.47	134.66	37	204.72	163.78
PRO	20	192.30	120.15	38	315.00	189.15
GLY	20	242.25	113.35	37	358.59	101.13
ALA	20	242.95	118.35	38	337.72	167.49
AAB	13	10.31	6.50	27	13.65	25.78
VAL	20	122.15	73.54	38	228.17	105.99
CYS	15	81.13	70.88	14	53.60	59.38
MET	20	67.45	41.89	37	47.16	89.58
CTH	16	14.19	9.09	31	20.71	17.30
ILE	20	36.85	17.95	37	80.25	41.64
LEU	20	71.45	34.05	38	123.65	55.56
TYR	20	70.35	35.86	37	54.46	42.19
PHE	20	90.50	43.26	38	84.37	71.09
ORN	18	61.67	59.01	37	94.79	49.26
LYS	20	160.40	69.12	38	212.19	96.62
HIS	20	54.00	21.08	38	72.20	37.21
ARG	20	54.25	32.71	38	83.38	53.21
	Acute Hepatitis					
	Before Treatment			After Treatment		
	N	Mean	S.D.	N	Mean	S.D.
TAU	5	55.00	26.60	13	75.31	70.29
ASP	4	21.75	5.62	11	41.55	10.70
THR	5	168.80	112.65	13	280.92	245.68
SER	5	112.60	64.65	13	153.38	112.22
ASN	3	274.00	110.01	6	239.83	225.76
GLU	5	196.80	178.10	12	193.34	183.50
PRO	5	247.60	162.94	12	327.83	207.06
GLY	5	370.20	235.20	11	425.36	444.72
ALA	5	389.20	263.37	11	303.82	177.36
AAB	4	24.25	15.11	11	19.45	19.93
VAL	5	157.40	86.32	13	186.23	81.47
CYS	4	127.75	84.93	5	104.60	60.71
MET	5	206.40	132.62	13	135.31	156.34
CTH	4	24.50	13.18	12	37.50	34.87
ILE	5	64.60	32.66	13	81.31	38.33
LEU	5	101.60	55.03	13	104.77	44.39
TYR	5	157.40	124.07	13	117.31	135.54
PHE	5	168.60	99.37	13	116.23	106.06
ORN	5	86.00	49.08	13	90.69	56.10
LYS	5	316.80	204.42	12	340.25	218.54
HIS	5	68.80	42.49	13	121.00	128.97
ARG	5	99.80	77.44	13	148.54	128.50

TABLE 4. *Pretreatment Blood Chemistry, Ammonia and Liver Function Test in a Group of Patients with Cirrhosis and a Group with Hepatitis*

	Cirrhosis			Hepatitis		
	N	Mean	S.D.	N	Mean	S.D.
GLUC	16	166.63	88.33	7	139.14	61.94
BUN	22	37.59	38.22	8	45.13	30.10
CREAT	19	1.71	2.51	8	2.21	1.94
ALB	13	2.88	0.37	8	2.90	0.46
BILI	19	12.07	15.07	8	24.75	13.05
SGOT	15	196.33	390.17	5	567.80	757.84
ALKP	15	8.13	6.02	4	7.07	1.78
PT	19	123.26	16.61	8	143.75	31.27
NH3	7	116.00	40.66	3	245.33	64.93

β-hydroxyphenylethylamines

Octopamine (OCT) and phenylethanolamine (PEA) levels were significantly elevated in both CC and AH patients during encephalopathy and coma.

In the CC group mean OCT levels which were 7.6 ± 1.9 ng/ml (normal 0.7 ± 0.04) during encephalopathy and decreased to 2.5 ± 2.3 ng/ml after 3 days treatment with FO80.

Phenylethanolamine levels which were 8 ± 3.8 ng/ml (normal 2.6 ± 0.14) during encephalopathy did not change significantly after 3 days treatment and were 7.7 ± 4.1 ng/ml.

In the AH group OCT levels were 4.8 ± 0.7 during encephalopathy and decreased to 3.1 ± 0.6 after 3 days of treatment with FO80. Insufficient data was available on PEA levels in AH.

Correlation Between Degree of Encephalopathy and Plasma Amino Acid Levels

In the group of patients with CC a statistically significant correlation was found between the grade of encephalopathy and plasma levels of α-amino-butyric acid ($p < 0.01$), cysteine ($p < 0.02$), citrulline ($p < 0.05$), tyrosine ($p < 0.02$), phenylalanine ($p < 0.01$), and the BCAA:AAA ratio between the branched-chain amino acids (valine, leucine, isoleucine) to the aromatic amino acids (tyrosine and phenylalanine) ($p < 0.02$).

Separating the group of CC into surviving and nonsurviving patients, the correlations between plasma amino acids and degree of encephalopathy differed. The surviving patients still showed statistically significant correlations with α-amino-butyric acid, citrulline, tyrosine, and phenylalanine, but the correlation with cysteine was lost, and two new significant correlations appeared with glutamate and asparginine. The nonsurvivors lost most of the significant correlations except for the one with cysteine and acquired a new correlation with asparginine.

In the AH group different correlations between plasma amino acids and degree of encephalopathy were found. Statistically significant correlations were found with aspartic acid ($p < 0.005$), asparginine ($p < 0.01$), glutamate ($p < 0.01$), α-amino-butyric acid ($p < 0.02$), valine ($p < 0.005$), isoleucine ($p < 0.01$), leucine ($p < 0.003$), methionine ($p < 0.001$), tyrosine ($p < 0.02$), lysine ($p < 0.02$), and BCAA:AAA ratio ($p < 0.002$). Again when dividing the group into surviving and nonsurviving patients, marked differences were apparant.

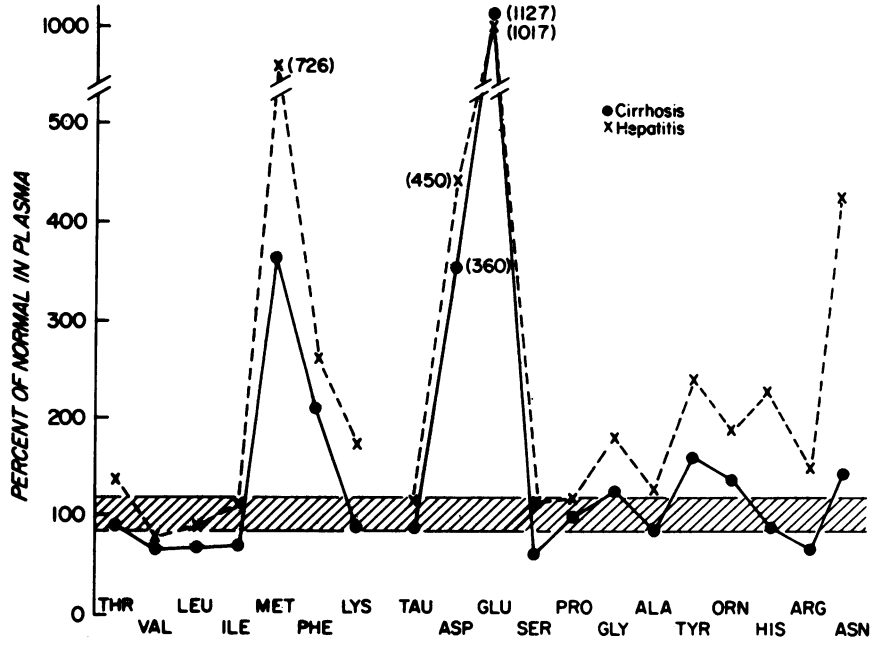

FIG. 3. Amino acid patterns in patients in the cirrhosis and hepatitis groups. Note that as in patients previously described, the patients with cirrhosis tend to have elevations of methionine, phenylalanine, tyrosine, glutamate, and aspartate. Branched-chain amino acids are reduced. The patients with acute alcoholic hepatitis demonstrate a diffuse hyperaminoacidemia with methionine and phenylalanine being the most elevated, in addition with aspartate and glutamate.

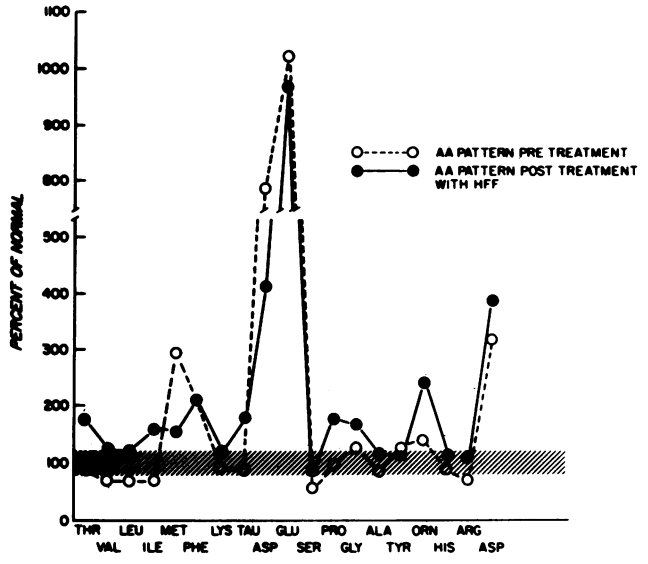

FIG. 4. Amino acid pattern in patients with cirrhosis before and after therapy. Note that following therapy with FO80, there is a decrease in methionine, as well as tyrosine and an increase in the branched-chain amino acids valine, leucine, and isoleucine. In addition, arginine, previously reduced slightly but not significantly returns to normal, and ornithine becomes elevated.

The survivors maintained significant correlations with aspartic acid, glutamate, and valine, and acquired new correlations with taurine and phenylalanine. The nonsurviving patients maintained significant correlations with α-amino-butyric acid, valine, leucine, methionine, tyrosine, lysine, and the BCAA:AAA ratio and did not acquire new correlations.

Correlation Between the Branched Chain Amino Acids (Valine, Leucine and Isoleucine) to Aromatic Amino Acids (Phenylalanine and Tyrosine) Ratio (BCAA:AAA) and Treatment with FO80

The BCAA:AAA ratio has been useful in determining neurologic status and degree of encephalopathy in chronic cirrhosis. Although arbitrary, this ratio takes into account two major presumed factors in hepatic encephalopathy, namely the increases in phenylalanine and tyrosine leading to alterations in central neurotransmitters and the changes of the branched-chain amino acids that are important in the competition for the common transport system across the blood–brain barrier and in decreasing muscle catabolism and efflux of aromatic amino acids from muscle.

In the CC group the mean pretreatment BCAA:AAA ratio was 1.61 ± 0.11 and increased to 5.01 ± 0.93 on day 3 of treatment with FO80 ($p < 0.001$). When the CC group was divided into survivors and nonsurvivors, those surviving this episode of encephalopathy or coma improved their ratio from 1.81 ± 0.17 to 6.80 ± 1.82 ($p < 0.02$), while the nonsurviving CC patients improved from 1.45 ± 0.14 to only 3.58 ± 0.62 ($p < 0.01$).

In the AH group the mean pretreatment BCAA:AAA ratio was 1.35 ± 0.31 and did not change significantly after 3 days of treatment with FO80 (1.51 ± 0.35).

Blood Chemistry, Liver Function Tests, Blood Ammonia, and Correlations with the Degree of Encephalopathy

Pretreatment blood chemistry levels, liver function tests, and ammonia levels in CC and AH are shown in Table 4.

Statistically significant correlations were found to exist in the surviving patients of the CC group between the degree of encephalopathy and SGOT levels ($p < 0.05$) and prothrombin time ($p < 0.02$).

In the nonsurviving cirrhotic patients in the degree of encephalopathy was significantly related to glucose levels ($p < 0.05$), BUN ($p < 0.02$), creatinine ($p < 0.05$), bilirubin ($p < 0.05$), and alkaline phosphatase ($p < 0.01$).

In the hepatitis group, surviving patients had significant correlations between encephalopathy and glucose ($p < 0.001$), BUN ($p < 0.002$), and creatinine ($p < 0.02$), somewhat similar to the correlations in dying cirrhotics.

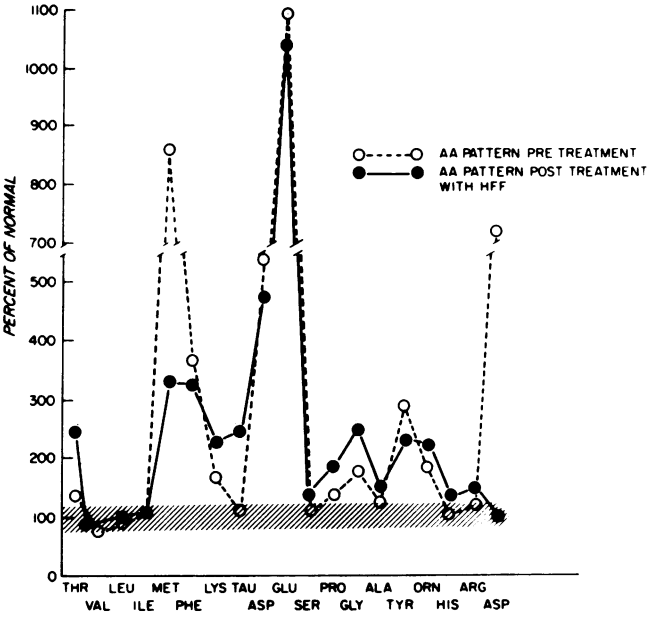

FIG. 5. Amino acid pattern before and after therapy in patients with hepatitis. The marked elevations in plasma methionine and phenylalanine are restored toward, but not to, normal. The same may be said of tyrosine. Aspartate and glutamate do not change significantly. Branched-chain amino acids, previously normal, stayed within normal range.

TABLE 5. *Nitrogen Intake as FO80, Urinary Nitrogen and Nitrogen Balance in Patients with Cirrhosis or Hepatitis Treated with Infusions of FO80*

	Cirrhosis	Hepatitis
FO80 nitrogen	9.61 ± 4.3	7.38 ± 3.0
Urinary nitrogen	10.22 ± 5.17	7.93 ± 3.1
Nitrogen balance	−0.61 ± 2.83	−0.55 ± 0.12

In the nonsurviving hepatitis patients encephalopathy was related significantly to creatinine ($p < 0.005$), bilirubin ($p < 0.005$), ammonia ($p < 0.001$), and prothrombin time ($p < 0.001$).

Interestingly, in none of the groups except for the group of dying hepatitis patients was ammonia well correlated with the degree of encephalopathy. Furthermore, after 3 days of treatment with FO80 only minor changes in ammonia levels occurred. In the CC group mean ammonia levels were 160 ± 14 μg% pretreatment and 173 ± 22 μg% after 3 days of treatment. In the AH group ammonia levels decreased from a pretreatment value of 267 ± 64 μg% to 206 ± 9 μg% (p = NS).

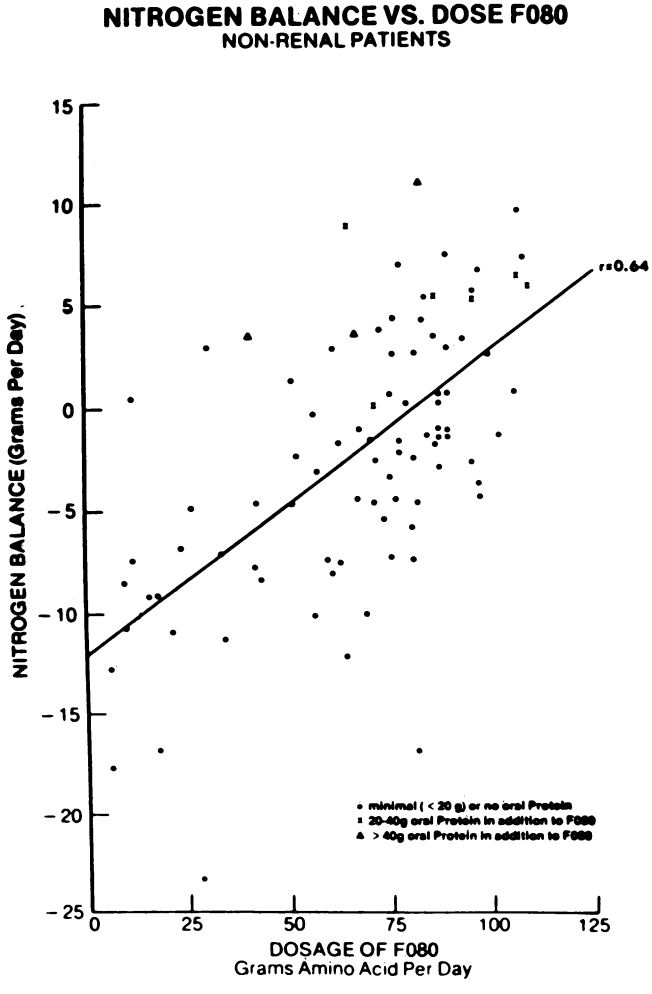

FIG. 6. Nitrogen balance *vs.* dose of FO80 as given as grams of amino acids per day. Note that the line crosses the nitrogen equilibrium point at about 75 g of amino acids administered per day. In a few patients taking significant oral intake, greater than 40 g of oral protein, in addition to FO80, (triangles) nitrogen balance deviates to significantly more positive as would be expected.

Nitrogen Intake, Nitrogen Balance, and Caloric Intake

During their treatment period patients with CC received a mean of 9.6 ± 4.3 grams nitrogen and 1970 ± 57 calories per day. Patients with AH received a mean 7.4 ± 3.0 grams nitrogen and 1451 ± 106 calories per day (Table 5). Nitrogen balance was achieved when 75 to 80 g of amino acids were infused/24 hours with a statistically significant linear correlation between nitrogen balance and daily intake of FO80 (Fig. 6).

When dividing the two groups into survivors and nonsurvivors, no significant difference could be found in nitrogen intake, urinary nitrogen, or nitrogen balances between survivors and nonsurvivors within the two groups.

Correlation Between the BCAA:AAA Ratio and the Amounts of Nitrogen Infused as FO80 and Nitrogen Balance

A significant correlation was found to exist between the BCAA:AAA ratio and the amount of nitrogen infused as FO80 and the nitrogen balance in both the CC ($p < 0.001$) and the AH ($p < 0.01$) groups.

Complications

Complications related to parenteral nutrition were quite common although not severe and never lethal. Twenty-seven of the cirrhotic patients (64%) and 11 of the hepatitis patients (65%) experienced blood sugar levels above 200 mg% necessitating the use of more insulin at some stages of their treatment with FO80. However, none of the patients developed hyperglycemic, hyperosmolar nonketotic coma, and in no case was the treatment discontinued because of glucose intolerance.

Ninety per cent of CC patients and 94% of the hepatitis group showed some electrolyte imbalance (electrolytes out of normal range for 48 hours [24]), mainly hyponatremia.

Five patients (12%) in the cirrhosis group suffered an episode of catheter sepsis during treatment with FO80. This is considerably higher as compared with the 2 to 3% infection rate in the authors' General Hospital population receiving parenteral nutrition.

Survival and Causes of Death

The survival rate in the cirrhosis and in the hepatitis group was similar, 45% and 47% respectively. The survival rate in the hepatorenal group was only 25%.

The main causes of death were liver failure and sepsis. Most patients died of a combination of two or more of the factors and causes mentioned in Table 6, mainly the combination of liver failure, renal failure and sepsis.

TABLE 6. *Causes of Death in 63 Patients with Cirrhosis, Hepatitis, and Hepatorenal Syndrome*

	Cirrhosis No. pts. (%)	Hepatitis No. pts. (%)
Hepatic failure	10 (22)	6 (33)
Sepsis	9 (19)	3 (17)
Respiratory failure	6 (13)	3 (17)
Renal failure	6 (13)	2 (11)
Cardiovascular failure	6 (13)	2 (11)
Gastrointestinal bleeding	5 (11)	1 (5.5)
Hepatorenal syndrome	4 (9)	—
Irreversible coma	—	1 (5.5)

Treating Hepatic Encephalopathy with FO80 Alone

For 24 encephalopathic patients FO80 was the only treatment modality for their encephalopathy. When the results in this group are compared with the group receiving conventional treatment modalities for hepatic encephalopathy as well, such as oral neomycin and/or lactulose, response to treatment in both groups was similar with the group getting FO80 alone, showing a 66% improvement in encephalopathy and a 50% survival rate compared with a 46% survival rate in patients receiving FO80 and other treatment modalities. Even in retrospect there was no selection in the group receiving FO80 alone.

Discussion

This report concerns an unselected group of patients with severe liver disease in a large urban hospital. A large percentage of patients with chronic cirrhosis who entered the hospital did so because of variceal or other types of gastrointestinal bleeding. Severe alcoholic hepatitis made up a good portion of the patients in the alcoholic group. This was rather a sick group of patients. The long duration of hospitalization before consultation for nutritional support, the comparatively long duration of coma before FO80 therapy despite, in most cases, standard treatment for hepatic coma, and the liver chemistries clearly point to a severely ill group of patients in whom nutritional support was required. The pretreatment liver chemistries show a mean bilirubin in the chronic cirrhosis hepatitis group of 13 mg/100 ml and in the acute hepatitis group of 25 mg/100 ml. Albumin was 2.8 g/100 ml in the cirrhosis group and 2.9 in the hepatitis group despite albumin supplementation. Despite this there was a significant salvage in each of the groups. One feature of the study is that the patients remained on the service of the primary physician and most of the decisions concerning major treatment modalities continued to be made by the patient's primary physician with a nutritional support team serving only in an advisory capacity. In this study nutritional support using relatively large amounts of protein was achieved despite hepatic coma, and in a significant number of patients, nitrogen balance was achieved. Despite this, improvement in encephalopathy was seen in the majority of patients suggesting that this specially designed solution was well tolerated in these protein intolerant patients.

It could be argued that in this group of patients, wake-up was a function of improvement in the liver disease and had absolutely nothing to do with FO80 therapy. This is unlikely for several reasons.

1. Most of the patients had been in coma for five days, despite standard therapy, and did not appear to be improving.
2. Many of the patients had been in the hospital for approximately 14 days and improvement in liver disease, should it occur spontaneously, is less likely after this period of time.
3. Even after improvement with nutritional support, most of the patients remained in the hospital for between 2 to 6 weeks.

Although it is impossible to state with certainty that nutritional intervention was associated with wake-up from hepatic encephalopathy, these factors make a chance association less likely but do not rule it out.

If the controversial aspects of these studies are eliminated, such as etiology of hepatic encephalopathy, the results can be said to show the following:

1. In a group of patients with severe liver disease, protein intolerance, and hepatic encephalopathy, significant amounts (60–120 g/day) of intravenous amino acids (normally contraindicated) were tolerated with either no deterioration or improvement in hepatic encephalopathy.
2. Nitrogen balance was achieved when between 75 and 80 g of amino acids/24 hours were administered with hypertonic dextrose.
3. Improvement in encephalopathy occurred simultaneously with the administration of a branched-chain enriched amino acid solution with hypertonic dextrose. This improvement was evident on neurologic examination by three different observers, Reitan trailmaking test, and objective improvement in EEGs.
4. It is possible that survival was improved in this group of patients from what one might ordinarily expect. Because the study was not prospectively randomized it is not possible to claim that survival was due to nutritional support.

What is the mechanism by which branched-chain enriched amino acid solutions and hypertonic dextrose

presumably operate in hepatic encephalopathy? We believe that the patient with chronic liver disease begins with "a sensitized brain" as proposed by Shenker,[25] with an abnormal plasma amino acid pattern with increased aromatic amino acids, mildly decreased branched-chain amino acids,[10,26,27] and a significant increase in plasma-free tryptophan.[26,28,29] Ammonia may or may not be elevated. This abnormal plasma amino acid pattern results in threshold disturbances in neurotransmission including increased aromatic amino acids within the brain, phenylalanine, tyrosine and tryptophan and their aminergic by-products, the β-hydroxyphenylethylamines, with decreased dopamine and norepinephrine, and increased indoleamines. Such threshold manifestations can be detected by careful neurologic examination in patients[30,31] and in disturbances in behavior[32,33] and/or circadian rhythm in experimental animals.[34]

When hepatic coma supervenes a variety of biochemical events increase the neurotransmission abnormalities in the central nervous system. Metabolic changes leading to increased lipolysis[4,28,35,36] and increased catabolism are perhaps mediated by increased glucagon. This in turn contributes to elevation of the already elevated aromatic amino acids,[1,26,28] but also a striking increase in the plasma free tryptophan, making it more available for penetration across the blood-brain barrier. When and if ammonia is increased, it is "detoxified" in the brain to glutamine which then is exchanged via the L-transport system in an effort to normalize brain glutamine.[7,37] As glutamine leaves the brain it is exchanged for neutral amino acids, thus increasing the penetration of the toxic aromatic amino acids and increasing their amine by-products. This results in greatly increased serotonin[20,28,29] and β-hydroxyphenylethanolamine, decreased dopamine, and decreased norepinephrine.

According to this hypothesis any therapy that decreased peripheral ammonia will, by decreasing brain glutamine, decrease the penetration of the toxic aromatic amino acids across the blood-brain barrier.[8]

According to this hypothesis, therefore, the effect of ammonia is indirect, mediated via changes in the central nervous system amino acids and their neurotransmitter by-products. It follows that if one prevents the formation of glutamine, the toxicity of ammonia is decreased. This has been demonstrated *in vivo* using methionine sulfoximine in mice[38] and *in vitro* using isolated brain capillary preparations.[39] In the latter situation, pretreatment of isolated brain capillaries, which prevents synthesis of glutamine, abolished the increased transport of amino acids secondary to incubation in high ammonia media.[39]

The purpose of the FO80 therapy is several-fold. The provision of hypertonic glucose and increased amounts of branched-chain amino acids presumably decrease catabolism and proteolysis by providing exogenous calories. In addition hypertonic dextrose will decrease plasma glucagon which the authors now feel is important in initiating lipolysis, increasing plasma nonesterified fatty acids and liberating tryptophan from binding sites on albumin, thus making it much more available for entry into the central nervous system. The branched-chain amino acids have recently been thought of as having a regulatory role in efflux of amino acids from peripheral muscle,[14,15] which the authors believe to be the peripheral source of aromatic amino acids in the catabolic situation. In recent experiments when increasing amounts of branched-chain amino acids are given, the addition of hypertonic dextrose as opposed to low-dose dextrose does not appear to increase glucose protein sparing significantly once an optimal amount of branched-chain amino acids is given.[40] Additionally, the provision of glucose calories and amino acids should increase protein synthesis thereby reducing levels of the plasma aromatic amino acids and making them less available for entry into the brain.

In addition to these peripheral effects of both the branched-chain amino acids in decreasing catabolism and promoting protein synthesis, increase in plasma concentrations of the branched-chain amino acids will also result in greater competition with the aromatic amino acids for entry across the blood-brain barrier by the common L-system carrier.[16]

In these studies, a direct effect of FO80 therapy on ammonia concentrations within the peripheral venous blood was not seen. Samples were drawn on ice and transported rapidly to the laboratory to minimize artifact. Nonetheless, statistically significant decreases in ammonia were not obtained. It becomes increasingly clear, however, that ammonia does not only arise from the gut but that a large amount of ammonia is generated by deamination of amino acids by muscle. The provision of adequate calories and amino acids for protein synthesis should decrease the efflux of ammonia from skeletal muscle.

Of all of the biochemical parameters that were measured in this study, only the plasma amino acids and the plasma amino acid ratio in most patients appeared to correlate with the clinical grade of encephalopathy. The correlation is not exact, but on the basis of recent work both from this and other laboratories and data which indicate exchange of amino acids for glutamine across the blood-brain barrier one would not expect a one-to-one correlation between a plasma amino acid ratio and the concentration of various amino acids within the central nervous system, as the CNS concentration of amino acids is dependent on the activity of the blood-brain barrier,[8] the concentration of ammonia,

plasma concentration of amino acids, and the concentration of nonesterified fatty acids displacing tryptophan from albumin, which in turn are probably related to release of glucagon and so forth. Thus, a simple ratio would be expected to give an indication, but not the total picture, of the status of amino acid transmitter precursors within the CNS. Experiments in chronic animal preparation in which chronic indwelling lateral CSF ventricular cannulas monitored the progress of hepatic encephalopathy compare such central nervous system findings with plasma amino acid ratios confirm this concept,[41,42] since in those experiments it was concluded that it was impossible to predict at a given time what the central nervous system concentrations of amino acids were from the plasma concentrations. In these studies, as stated earlier, there was little or no correlation between grade of encephalopathy and peripheral blood ammonia measured in a good clinical laboratory under conditions in which ammonias were drawn on ice and delays were avoided before measurement. It is not clear whether there would have been a better correlation had arterial blood or a research technique for ammonia been used.

The results herein reported appear to support the amino acid neurotransmitter hypothesis. Unexpected confirmation of this hypothesis related hormone whose control is partially dopaminergic in patients with liver disease. Hyperprolactinemia has been reported both in Reye's Syndrome[43] and in patients with cirrhosis and hepatic encephalopathy,[44] thereby confirming abnormalities in central dopaminergic transmission in liver disease.

It is difficult to say in these patients whether provision of appropriate parenteral nutrition altered the clinical course and resulted in increased survival. These are a very sick group of patients, particularly those patients with acute (alcoholic) hepatitis with a mean bilirubin of 25 in whom an extremely high mortality can be expected. In previous studies of nutritional support in patients with other acute organ injuries such as renal failure,[45] it has been suggested that the appropriate type of nutritional support may result in faster recovery from a given insult and demonstrated improved survival if proper nutrition is provided.

More recently, in a randomized prospective trial in a far less sick group of patients with alcoholic hepatitis without significant encephalopathy (bilirubin = 8 mg/100 ml), increased survival was seen in the group with aggressive nutritional support in this case being carried out with chemically-defined diets.[46] Thus, aggressive nutritional support does seem to be associated with increased survival in acute alcoholic hepatitis, as may have occurred in this study.

These results are encouraging. In a disease whose course is varied it is clear that the only study that will answer the question is a randomized prospective double-blind trial, currently being carried out, in which nutritional support with a branched-chain enriched amino acid solution is randomized against a known form of therapy such as neomycin.

References

1. Fischer JE, Funovics JM, Aguirre A, et al. The role of plasma amino acids in hepatic encephalopathy. Surgery 1975; 78:276–290.
2. Fischer JE, Rosen HM, Ebeid AM, et al. The effect of normalization of plasma amino acids on hepatic encephalopathy in man. Surgery 1976, 80:77–91.
3. Fischer JE, Baldessarini RJ. Pathogenesis and therapy of hepatic coma. In: Popper H, Schaffner F, eds. Progress in Liver Disease. New York: Grune and Stratton, 1976; 363–397.
4. Fischer JE. Current concepts of pathogenesis of hepatic encephalopathy. Presented at the Third International Gstaad Symposium. In: Preisig R, Bircher J, eds. The Liver. Berne, Switzerland: Editio Cantor Aulendorf, 1979; 374–385.
5. Fischer JE. Portasystemic encephalopathy. In: Wright R, Alberti KGMM, Karrân S, Millward-Salder GH, eds. Liver and Biliary Disease: Pathophysiology, Diagnosis, Management. Philadelphia: WB Saunders, 1979; 973–1001.
6. Fischer JE, Baldessarini RJ. False neurotransmitters and hepatic failure. Lancet 1971; ii:75–80.
7. James JH, Jeppsson B, Ziparo V, Fischer JE. Hyperammonemia, plasma amino acid imbalance, and blood-brain amino acid transport: a unified theory of portal-systemic encephalopathy. Lancet 1979; ii:772–775.
8. James JH, Escourrou J, Fischer JE. Blood-brain neutral amino acid transport activity is increased after portacaval anastomosis. Science 1978; 200:1395–1397.
9. Fischer JE, Yoshimura N, James JH, et al. Plasma amino acids in patients with hepatic encephalopathy: effects of amino acid infusions. Am J Surg 1973; 127:40–49.
10. Rosen HM, Yoshimura N, Hodgman JM, Fischer JE. Plasma amino acid patterns in hepatic encephalopathy of differing etiology. Gastroenterology 1977; 72:483–487.
11. Sapir DG, Walser M. Nitrogen sparing induced early in starvation by infusion of branched-chain keto-acids. Metabolism 1977; 26:301–308.
12. Freund H, Hoover HC Jr, Atamian S, Fischer JE. Infusion of the branched-chain amino acids in postoperative patients: anticatabolic properties. Ann Surg 1979; 190:18–23.
13. Rosen HM, Soeters PB, James JH, et al. Influences of exogenous intake and nitrogen balance on plasma and brain aromatic amino acid concentrations. Metabolism 1978; 27:393–404.
14. Buse MG, Reid M. Leucine, a possible regulator of protein turnover in muscle. J Clin Invest 1975; 56:1250–1261.
15. Odessey RK, Kairallah EA, Goldberg AL. Origin and possible significance of alanine production by skeletal muscle. J Biol Chem 1974; 249:7623–7629.
16. Oldendorf WK. Brain uptake or radiolabelled amino acid amines and hexoses after arterial injection. Am J Physiol 1971; 221:1629–1639.
17. Okada A, Ikeda Y, Itakura T, et al. Treatment of hepatic encephalopathy with a new parenteral amino acid mixture (abstr). JPEN 1978; 2:218.
18. Streibel JP, Lutz H. Free plasma amino acid in patients with liver cirrhosis during parenteral nutrition (abstr). JPEN 1978; 2:217.
19. Capocaccia L, Rossi-Fanelli F. Results of infusion of branched-chain amino acids to patients with hepatic coma. Presented at the Second International Symposium, Portal-Hypertension. Rome: June 1979 (in press).
20. Ferenci P, Funovics J, Wewalna F. Therapie der hepatischen

enzephalopathie: beeinflussung des plasmaaminogrammes durch aminosaure infusion. Langenbecks Arch Chir Suppl Forum 1978; 183–189.
21. Adams RD, Foley JM. Neurological disorder associated with liver disease. In: Metabolic and toxic diseases of the nervous system. Res Publ Assoc Nerv Ment Dis 1953; 32:198–237.
22. Reitan RM. Validity of the trailmaking test as an indicator for organic brain damage. Percept Mot Skills 1958; 8:271–278.
23. Rossi-Fanelli F, Escourrou J, Smith AR, Fischer JE. Phenylethanolamine and octopamine in hepatic encephalopathy. In: Mosnaim AD, Wolf ME, eds. Noncatecholic phenylethylamines. New York: Marcel Dekker, 1980:231–244.
24. Edmunds LM Jr, Williams GM, Welsh CE. External fistulas arising from the gastrointestinal tract. Ann Surg 1960; 152:445–471.
25. Schenker S. Hepatic coma: current concepts of pathogenesis. Viewpoints Dig Dis 1970; 2:1–4.
26. Salerno F, Dioguardi FS, Abbiate R. Tryptophan and hepatic coma. Gastroenterology 1978; 75:769–770.
27. James JH, Freund H, Fischer JE. Amino acids in hepatic encephalopathy. Gastroenterology 1979; 77:421–422.
28. Ono J, Hutson DG, Dombro RS, et al. Tryptophan and hepatic coma. Gastroenterology 1978; 74:196–200.
29. Knell AJ, Davidson AR, Williams R, et al. Dopamine and serotonin metabolism in hepatic encephalopathy. Br Med J 1974; 1:549–551.
30. Gilberstadt S, Gilberstadt H, Buegel B, et al. Defective intellectual function in alcoholic cirrhotics and noncirrhotic alcoholics: relationship to severity of liver disease (abstr). Gastroenterology 1978; 74:1037.
31. Elsass P, Lund Y, Ranek L. Encephalopathy in patients with cirrhosis of the liver: a neuropsychological study. Scand J Gastroenterol 1978; 13:241–247.
32. Beubernard C, Saloman F, Grange D, et al. Experimental hepatic encephalopathy. Changes of the level of wakefulness in the rat with portacaval shunt. Biomedicine 1977; 27(4):169–171.
33. Warbritton JD, Geyer MA, Jeppsson B, Fischer JE. A behavioral model of early encephalopathy in the end-to-side portacaval shunted rat. Surg Forum 1979; 30:394–396.
34. Campbell A, Ziparo V, James JH, Fischer JE. Loss of day-night rhythm in rats following portacaval shunt. Surg Forum 1979; 30:388–390.
35. Ebeid AM, Soeters PB, Murray P, Fischer JE. Release of vasoactive intestinal peptide (VIP) by intraluminal osmotic stimuli. J Surg Res 1977; 23:25–30.
36. Soeters PB, Fischer JE. Insulin, glucagon, amino acid imbalance and hepatic encephalopathy. Lancet 1976; ii:880–882.
37. Cardelli-Cangiano P, Cangiano C, James JH, et al. *In vitro* isolated brain microvessel studies confirm blood-brain abnormalities in hepatic encephalopathy (abstr). Gastroenterology 1980; 78:1303.
38. Warren KS, Schenker S. Effect of an inhibitor of glutamine synthesis (methionine sulfoximine) on ammonia toxicity and metabolism. J Lab Clin Med 1964; 64:442–449.
39. Cangiano C, Cardelli-Cangiano P, James JH, Fischer JE. Ammonia stimulates amino acid uptake by isolated bovine brain microvessels (abstr). Gastroenterology 1980; 78:1302.
40. Freund H, Yoshimura N, Fischer JE. The effect of branched-chain amino acids and hypertonic glucose infusions on postinjury catabolism in the rat. Surgery 1980; 87:401–408.
41. Smith AR, Rossi-Fanelli F, Ziparo V, et al. Alterations in plasma and CSF amino acids, amines and metabolites in hepatic coma. Ann Surg 1978; 187:393–404.
42. Smith A, Rossi-Fanelli F, Freund H, et al. Sulfur-containing amino acids in experimental hepatic coma in the dog and monkey. Surgery 1979; 677–683.
43. Newman SL, Caplan DB, Camp VM, et al. Prolactin and the encephalopathy of Reye's syndrome. Lancet 1979; ii:1097–1100.
44. McLain CJ, Kromhout JP, VanThiel D. Serum prolactin concentrations in portal systemic encephalopathy (abstr). Gastroenterology 1979; 76:1292.
45. Abel RM, Beck CH Jr, Abbott WM, et al. Improved survival from acute renal failure following treatment with intravenous essential L-amino acids and glucose. N Engl J Med 1973; 288:695–699.
46. Nasrallah SM, Galambos JT. Amino acid therapy in alcoholic hepatitis (abstr). Gastroenterology 1980; 78:1315.