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AMINO ACID IMBALANCE AND HEPATIC ENCEPHALOPATHY[1]

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I. BACKGROUND

A. Introduction

Hepatic encephalopathy is one of the most frequent terminal episodes of primary liver disease. It was already known at the time of Hippocrates, and it was well described by Johanne Baptista Margagni, teacher at the Padua Medical School, in the 18th century.

Hepatic encephalopathy is a neuropsychiatric syndrome seen in the presence of hepatic functional impairment secondary to acute liver failure or chronic parenchymal liver disease with or without spontaneous or surgically induced portalsystemic shunting of portal blood. The neuropsychiatric symptoms of liver failure are varied and range from subtle alterations, including mild personality change and disturbance in sleep rhythm, to confusion, drowsiness, stupor, and finally deep coma. In acute liver failure the progression of events may be extremely rapid, taking place in only a few hours. In chronic encephalopathy, in contrast, consciousness may be slowly altered over months, and may occasionally present as progressive and irreversible neurological symptomatology with dementia, parkinsonism, or other extrapyramidal signs suggesting involvement of the cerebellum or basal ganglia (170). Most often in the chronic situation, however, episodes of mental impairment are episodic, the result of defined stresses, and recovery to the premorbid state is the rule.

Other symptoms of hepatic encephalopathy are not specific for liver disease. Asterixis (flapping tremor of the extremities) is probably the most characteristic neurological sign of hepatic encephalopathy, is more common

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in uremia, and is also seen in patients with respiratory insufficiency, hypokalemia, congestive heart failure, sedative overdose, and even during hypoglycemia (35). Similar tremors can also be elicited in the feet, tightly closed eyelids, pursed lips, or in the protruded tongue. Conn has suggested that asterixis may be associated with a lesion of crossing corticospinal tracts, as it has been observed on the contralateral side of a developing cerebral thrombosis (36). Other neurological abnormalities, when present, are usually transient and are presumed to be a result of a metabolic disturbance rather than of permanent structural damage. Cerebral function may be assessed by simple tests for contructional apraxia such as the ability to draw a five pointed star, by psychometric methods such as the number connection test of Reitan (50, 173), and by electroencephalogram.

In the absence of preexisting liver disease, hepatic encephalopathy associated with acute fulminant hepatic failure is usually seen in the setting of acute viral hepatitis (125, 179), but is observed also with drug-induced liver damage [e.g. paracetamol, halothane (30, 114) or tetracycline (193)] and with the acute fatty degeneration seen in pregnancy (178). Against the background of chronic liver disease, a variety of clinical events, such as gastrointestinal bleeding, infections, and over-diuresis, may provoke hepatic encephalopathy.

B. Pathology

There are no striking findings in the brain pathology of patients dying in hepatic coma. A fairly consistent finding is hyperplasia of protoplasmic type II astrocytes, most prominent in the cortex but also found in other parts of the brain (215). The pseudopods of these cells, which likely are the cells that synthesize glutamine from ammonia, may contribute to the control of the passage of materials across the blood–brain barrier. In the cerebral edema that complicates acute fulminant hepatic failure, which does not respond to steroids, mannitol, urea, or other usual therapeutic measures (222, 229), it is possible that edema of the pseudopods disrupts the tight junctions normally present in brain capillaries and opens up abnormal passages through which toxic materials may enter the brain.

The meaning of these astroglial changes is not clear. It is increasingly thought that the astroglia are the cells that govern the extracellular environment of the brain; as such, their dysfunction may contribute to disruption in neurological function. As discussed elsewhere, glutamine is probably a glial metabolite (10, 12, 228). Elevation of cerebrospinal fluid glutamine is one of the most reliable biochemical correlates of hepatic encephalopathy (83, 98), and thus an anatomical-biochemical correlation may be implicit. Whether the astrocytic protoplasmic hyperplasia observed is an attempt on the part of the glia to maintain neuronal homeostasis or is a pathological

response to toxins and even contributes to the mechanism of hepatic encephalopathy is not known. In hepatocerebral degeneration, irreversible neuronal damage and degeneration as well as demyelination may occur.

C. Clinical Laboratory Investigation

In clinical laboratory investigation, there are no "hepatic function tests" or plasma electrolyte disturbances invariably associated with hepatic encephalopathy. Blood ammonia is elevated in the majority of patients with this syndrome; however, its level does not reflect well the degree of coma, and numerous patients with clinical encephalopathy may have normal blood ammonia levels. While arterial ammonia concentration is better correlated with clinical states, 10% of patients with hepatic coma have normal arterial ammonia levels (205); cerebrospinal fluid ammonia and glutamine are more often elevated than peripheral blood ammonia. Clotting factor deficiencies reflect only the severity of hepatic dysfunction, and are not related to the degree of encephalopathy. Electrolyte and acid-base disturbances are common; respiratory alkalosis, which is secondary to hyperventilation and may depress cerebral blood flow, and hypokalemic metabolic alkalosis may occur. Hypoglycemia generally presages a fatal outcome as it is usually encountered in greatly advanced hepatic failure. It is probably due to impaired gluconeogenesis and glycogen synthesis, but it has been reported in noncomatose patients with severe viral hepatitis (56). Renal failure may ensue, and urea and creatinine will rise, but again are not necessarily part of hepatic encephalopathy. Pathological findings in the cerebrospinal fluid (CSF) are not characteristic. CSF is clear and colorless, except when hyperbilirubinemia is present. No characteristic pattern of cells, glucose, protein and electrolytes has been discerned, while CSF glutamine is usually elevated (83, 98).

D. Pathogenesis of Hepatic Encephalopathy

Current knowledge about the pathogenesis of hepatic encephalopathy derives from studies in patients with this disorder and in animals with experimentally induced hepatic coma. Various theories of hepatic encephalopathy have been entertained for the past several decades. Any theory about the etiology and therapy of hepatic coma must accommodate several clinical observations: (*a*) Decreased hepatic function and shunting of blood around the liver are usually present in hepatic encephalopathy. (*b*) The substance(s) involved in the etiology of hepatic encephalopathy arise in the gut. (*c*) Those substances appear to be of nitrogenous origin, as coma is commonly provoked by increased dietary protein or gastrointestinal bleeding. (*d*) Gut bacteria play a role, since altering gut bacterial flora ameliorates hepatic encephalopathy. (*e*) While ammonia may not be the sole etiological agent,

serum ammonia generally reflects the presence of hepatic coma although the correlation is not exact.

The four prevalent theories of hepatic encephalopathy are those relating to: Ammonia, short-chain fatty acids, the synergistic hypothesis, and the neurotransmitter amino acid hypotheses.

1.AMMONIA Hyperammonemia has been implicated as a cause of hepatic coma. While patients with hepatic disease are especially sensitive to the cerebral toxic effects of ammonia (163) this does not necessarily mean that spontaneous encephalopathy is the result of ammonia intoxication. Hyperammonemia is common in patients with hepatic encephalopathy (135) and in laboratory animals with portacaval shunt. The concept that hyperammonemia is closely related to portal system encephalopathy is widely held and provides a rationale for treatment by a variety of agents designed to reduce intestinal ammonia production. Blood ammonia determinations are still the most widely performed biochemical tests for hepatic encephalopathy. The mechanism initially proposed by which ammonia exerts its toxic effect was that ammonia blocked energy production by depletion of the cerebral Krebs cycle of 2-oxoglutarate, utilized in the synthesis of glutamine (13). This hypothesis is no longer tenable, as the depletion of 2-oxoglutarate has not been observed in experimental hepatic coma (14, 195), and brain glutamine is derived from the synthesis of ammonia with a rapidly-turning-over pool of glutamic acid rather than directly from 2-oxoglutarate (12). Other possible toxic effects of ammonia that have been proposed include the accumulation of gamma-amino butyric acid (GABA), an inhibitory neurotransmitter (86). However, GABA measured in the brains of animals with acute ammonia intoxication and experimental hepatic coma is normal (192), although recent studies have again suggested that it may play a role in both chemical and experimental hepatic coma (59, 190).

The second major hypothesis is the disruption in energy production. In studies carried out under carefully controlled experimental circumstances using the recently developed ultrarapid freeze-blow technique, slight decreases in phosphocreatine were found in ammonia-intoxicated animals (90). In a more recent study, after 60 minutes of massive ammonia intoxication in animals with portacaval shunts, ATP content was decreased in all regions of the brain, but the reduction in total high-energy phosphate was most marked in the brain stem. In other experiments the authors concluded that the cerebral dysfunction in chronic relapsing ammonia intoxication is not primarily due to energy failure (94).

In vivo toxicity of ammonia may be disassociated from venous ammonia levels by monoamine oxidase inhibitors in man (47) and by inhibitors of glutamine synthesis in experimental animals (223). Arterial ammonia con-

centrations, while correlating better with encephalopathy than venous ammonia, may still be normal with grade 4 hepatic encephalopathy (205). More recent physiological evidence localized the site of ammonia toxicity to the cortex and perhaps amygdyla, but the reticular activating system and the arousal response are not generally affected by ammonia and thus cannot explain all the symptoms of hepatic encephalopathy (95).

Acute ammonia intoxication does not reproduce all the cerebral changes seen in hepatic encephalopathy such as increased serotonin and tryptophan levels (7, 44, 63, 116), alterations in brain phenylalanine, tyrosine, and their derivative amines (63, 64), and alterations in metabolites of dopamine and serotonin (115). Furthermore, ammonia given to experimental animals in amounts far in excess of levels found in man failed to change cerebral serotonin, and this again suggests that ammonia intoxication does not completely mimic human hepatic encephalopathy (194, 218). Recent experiments from this laboratory have altered this view however.

According to the "glutamine exchange" hypothesis, prolonged infusion of ammonia in normal animals should reproduce, to some extent, the brain amino acid changes seen in experimental animals with impaired hepatic function. Preliminary experiments carried out in this laboratory show that while changes in serotonin and norepinephrine may be achieved by prolonged ammonia infusion, they are not of the same magnitude as seen in hepatic coma (105).

2. SHORT-CHAIN FATTY ACIDS Butyrate, valerate, and octanoate are increased in the blood and CSF of patients with hepatic encephalopathy (29, 208, 217). A close relationship has been found in at least one instance between slow-wave electrical activity on the EEG and the concentration of fatty acid in the spinal fluid (211). Muto (145) reported elevation in short-chain fatty acids, particularly C4 to C6, in hepatic coma resulting from nonfulminant disease. Precedent exists in another form of coma: that found with the Jamaican vomiting sickness, which is induced by hypoglycin A and results in isovaleric-acidemia (209).

The correlation of blood and CSF concentrations of short-chain fatty acids and grades of coma is not good (27). Furthermore, while the liver is presumably active in the catabolism of such fatty acids absorbed from the gut, it is not clear how such excesses of fatty acids might be linked to ingestion of protein, over-diuresis, sepsis, or some of the other stimuli known to provoke hepatic encephalopathy. Indeed, short-chain fatty acids of this chain length have been administered to patients with known cirrhosis without provoking encephalopathy (138). It is therefore unlikely that short-chain fatty acids alone are responsible for hepatic encephalopathy.

3. THE SYNERGISTIC HYPOTHESIS Synergism in coma production has been demonstrated experimentally with combined administration of ammonia, fatty acids, and mercaptans (137, 234). Zieve argues that by acting synergistically, smaller amounts of each of these three toxins might be necessary to produce hepatic encephalopathy than if given alone (234). Normal animals have been rendered comatose by ammonium salts supplemented with short-chain fatty acids. However, the concentrations of ammonia achieved in these experiments range from 590 to 1470 μmol/l (1000–2500 μg/dl), values almost never seen in patients except, occasionally, in Reye's syndrome. Thus the results obtained in support of this hypothesis are somewhat controversial.

4. THE NEUROTRANSMITTER AMINO ACID HYPOTHESIS The neuropsychiatric symptoms associated with hepatic encephalopathy in both acute and chronic circumstances suggest major disturbances in neurotransmission. The past several decades have seen the discovery of many candidates for neurotransmitter function in the central nervous system, including amines, such as acetylcholine, dopamine, norepinephrine (noradrenaline), serotonin, histamine; excitatory amino acids, such as glutamate and aspartate; inhibitory amino acids, including glycine, serine, and GABA; the prostaglandins; substance P; and more recently the peptide neurotransmitters (8). Progress has been made in the physiology of many central neurotransmitters, particularly the neuropharmacology of the adrenergic neurotransmitters, norepinephrine and dopamine. The structural requirements of the peripheral adrenergic system, the sympathetic nervous system, appeared to be a phenolic ring and a short carbon side chain with a hydroxyl group on the B position in the chain (69, 117).

The concept of "false neurochemical transmitters" evolved from evidence that the peripheral adrenergic neurone would take up, protect from destruction, and release in response to electrical stimulation compounds presumably less active sympathomimetically than the putative transmitter norepinephrine; these then could accumulate under toxic or pharmacologic conditions or in response to accumulation of exogenous precursors (42, 144).

The initial evidence for this hypothesis came from the laboratory. Octopamine is a known false neurochemical transmitter with a sympathomimetic action less than 1/50 that of norepinephrine in the periphery; it greatly increases in the brains and the hearts (representative of the peripheral sympathetic nervous system) of rats in acute hepatic coma (63, 66, 70). Norepinephrine, the putative neurotransmitter, decreases at least by approximately 50% in the brains of comatose animals, and the degree of decrease is proportional to level of coma (49, 53, 213). Serotonin can

compete with norepinephrine for storage in adrenergic neurons and is inhibitory (8); it has been implicated in normal regulation of consciousness through brainstem mechanisms (141) and is elevated in acute hepatic coma in rats (43).

II. PERIPHERAL AMINO ACID METABOLISM

The importance of the liver in amino acid metabolism was first noted in 1924, when Bollman, Mann & Magath (17) showed that the hepatectomized dog was unable to form urea from amino acids. The earliest studies of amino acid metabolism in hepatic failure utilized paper chromatographic techniques. In analysis of urine (220), no single pattern of abnormalities in amino acid excretion during hepatic failure was documented, presumably because of the relatively crude techniques available and because of deranged renal function in some patients. Excesses, but not deficiences, of many plasma amino acids were detected with increased plasma tyrosine and methionine being easily detected (100, 231). Several recent studies with more sophisticated analysis have revealed already a considerable amount of information about plasma amino acid levels in patients and animals with hepatic encephalopathy. In chronic liver disease complicated by acute decompensation a pattern was reported consisting of increased plasma phenylalanine, tyrosine, free but not necessarily total tryptophan, methionine, histidine, glutamate and aspartate, together with decreased or low normal levels of the branched-chain amino acids, leucine, isoleucine, and valine (4, 16, 20, 26, 73, 99, 101, 134, 177, 182, 206, 231). These findings assumed increased significance as it became clear that certain neutral amino acids served as precursors for CNS amine neurotransmitters. This group of large neutral amino acids includes tryptophan, tyrosine, phenylalanine, methionine, and histidine; they and the branched-chain amino acids compete for entry across the blood-brain barrier using a common carrier (151). Subsequent investigations have revealed that in addition to the classical amino acid pattern, another distinct amino acid pattern exists (182). While patients with chronic liver disease with superimposed acute insults—i.e. gastrointestinal bleeding, infection, alcoholic hepatitis—manifest the "usual" pattern of increased plasma phenylalanine, tyrosine and methionine, free tryptophan and decreased branched-chain amino acids that appears to be associated with hepatic encephalopathy (67, 72, 148), other investigators claim that it is associated with severe liver disease but not specifically with encephalopathy (140, 226). Others agree that it indicates the presence but not necessarily the grade of encephalopathy, as initially proposed (185). In acute fulminant hepatitis, a second pattern is present: severe hyperaminoacidemia of all amino acids. Only the branched-chain

amino acids leucine, isoleucine, and valine remain normal; plasma levels may even be slightly decreased (171, 182).

Tryptophan is thought to be toxic in liver disease (2). Plasma total tryptophan is either slightly elevated (43) or reduced (155) in hepatic encephalopathy while free tryptophan is markedly increased (43). Absolute levels of plasma tryptophan are probably not as important as the ratio of protein-bound to free plasma tryptophan (61), the influence of plasma nonesterified fatty acids (161), and the distribution of branched-chain amino acids (107). Possibly because of these variables, brain tryptophan in animals with liver disease (107, 7) as well as CSF tryptophan and 5-hydroxyindoleascetic acid (5-HIAA) in both animals (63, 199) and patients with hepatic encephalopathy (115) are greatly increased, despite the relatively small elevations in the plasma concentration of total tryptophan.

Brain tryptophan is increased both because of the decreased plasma concentrations of the competing branched-chain amino acids (43, 60, 107) and because of increased plasma free tryptophan. Serotonin, an inhibitory neurotransmitter amine (89), is greatly increased in the experimental animal with acute hepatic coma (43, 63) and is thought to play a role in hepatic encephalopathy (43, 44, 45, 46, 107, 111, 115, 116, 120, 155, 158). Indeed, some investigators have proposed that the ratio of free tryptophan to competing neutral amino acids may be the most accurate plasma amino acid ratio describing hepatic encephalopathy, both spontaneously and in response to various forms of therapy such as lactulose and infusions of branched-chain amino acids (26, 185, 186). That tryptophan is an essential component of pathogenesis in hepatic encephalopathy is supported by experiments in which a coma–like state is induced by infusion into normal dogs of phenylalanine and tryptophan in combination but not alone (78).

Methionine had previously been used as a lipotropic factor in liver disease, but subsequently it was shown to be toxic (27). Intolerance to methionine has been correlated with the concentration of ammonia in portal blood collaterals (227). Intracarotid arterial injections of large amounts of methionine into normal dogs failed to produce coma (78). Oral tolerance of methionine may be increased by oral antibiotics (162), suggesting that the toxic factor is not methionine but a metabolic product. Other investigators have suggested that methionine may reduce brain ATP by the formation of S-adenosylmethionine (88). However, normal levels of brain ATP have been found in rats in hepatic coma with elevated blood and brain methionine (14). Exogenously administered methionine is probably of less importance to the brain since the brain synthesizes its own methionine when deprived of other sources (156).

Phenylalanine is the amino acid that has the highest concentration in the brain as well as in the blood of animals in hepatic coma. One factor is the

decreased conversion of phenylalanine to tyrosine by hepatic phenylalanine-4-hydroxylase, whose activity is decreased early in hepatic impairment.

A constant finding in both patients and experimental animals is the decreased level of the branched-chain amino acids (BCAA) valine, leucine, and isoleucine, both on a relative basis, as in acute fulminant hepatitis, in which they remain normal despite tremendous increases in other amino acids, and on an absolute basis, as in seen in chronic liver disease with acute exacerbation. They have a unique function in that they are not only incorporated into protein but can be catabolized by brain, muscle, and kidney for energy and be subsequently degraded to acetyl-coenzyme A. The low plasma concentration of BCAA is presumably the result of decreased release from muscle (where they are utilized for energy) as well as of increased oxidation by fat (201, 202). Presumably part of the function of the BCAA is to substitute for energy sources such as glucose and ketone bodies, possibly lacking in animals with severe hepatic parenchymal damage. In addition to the liver, the kidney is the one organ that may catabolize the branched-chain amino acids into glucose; it may be using the BCAA as a primary source of oxidizable substrate in an effort to maintain blood glucose when hepatic gluconeogenesis is deficient. In addition to regulation of effects of other amino acids across the muscle cell membrane (152), recent evidence suggestions that infusions of BCAA will increase not only muscle but hepatic protein synthesis (76, 77, 188).

A. Effects of Hormones

Alterations in the levels of insulin, glucagon, and presumably other hormones influence the plasma amino acid imbalance in cirrhotic patients. In advanced liver cirrhosis a remarkable increase in glucagon levels has been demonstrated (132), particularly when portalsystemic shunting is present (194). In spite of increased insulin levels (32), the insulin/glucagon molar ratio is decreased (131). This ratio may be a marker of a catabolic state characterized by enhanced gluconeogenesis from different sources, mainly amino acids (214). Insulin promotes the catabolism of the BCAA by both muscle and fat, and it has been suggested that the decreased concentrations of BCAA within the circulation are secondary to oxidation of these amino acids by muscle under the influence of insulin (142, 201), although increased oxidation by fat certainly plays a role (85, 202).

In animals, following end-to-side portacaval shunt, both insulin and glucagon increased modestly, with a normal insulin/glucagon ratio. As the animal enters hepatic encephalopathy, there is a dramatic rise in the plasma concentration of glucagon while insulin either remains stable or decreases (142, 202). In a recent study, Marchesini et al (130, 131) found that hyperglucagonemia in cirrhotics closely correlates with encephalopathy grade

since the plasma concentration of glucagon progressively increases with the deterioration of the mental state. Infusion of glucagon in conjunction with epinephrine and steroids brings about a state of sustained catabolism and gluconogenesis; with this, aromatic amino acids are presumably released in increased amounts, accumulating within the circulation owing to decreased hepatic catabolism.

B. Effects of Catabolism

In animals and patients with normal liver function, anabolism and catabolism exert relatively minor influences on plasma and brain amino acid levels. However, in animals with liver disease the effects of positive nitrogen balance and intracellular deposition of amino acids are to decrease plasma and brain aromatic amino acids as well as brain amine neurotransmitter derivatives as rats with end-to-side portacaval shunts go into more markedly positive nitrogen balance (183).

C. Effects of Acute Hepatic Necrosis

Patients during the course of fulminant hepatitis with coma have been shown to have grossly abnormal plasma amino acid profiles, characterized by substantially increased levels of most amino acids, but normal or slightly reduced concentrations of the branched-chain amino acids valine, leucine, and isoleucine (171, 182).

The origin of the raised plasma amino acids in fulminant hepatic failure is not certain, although on the basis of a close relationship between plasma tyrosine and serum glutamic oxaloacetic transaminase levels it has been suspected that hepatic necrosis per se is responsible for high plasma amino acid levels (182). Thus the degree of aromatic amino acid elevations, particularly of tyrosine, may be useful in predicting the extent of hepatic necrosis, and possibly outcome, although others have denied this (197).

The concentrations of phenylalanine, tyrosine, and methionine may be as high as 700–800% of normal, and in a series of 15 patients no patient with a tyrosine level higher than 60 μg/ml (or 600% of normal) survived acute fulminant hepatitis regardless of therapy (182). High plasma tyrosine and phenylalanine levels have been found in association with elevated brain concentrations of these amino acids in patients dying from fulminant hepatic failure (171). Conversion of excess tyrosine and phenylalanine to tyramine, octopamine, phenylethylamine and B-hydroxyphenylethanolamine, respectively, act as weak or false neurotransmitters present in excess. Other laboratories have confirmed the hyper-tyrosinemia seen in decompensated cirrhosis and encephalopathy (54, 55). Increased plasma tyrosine and its amine derivative tyramine have also been documented in hepatic encephalopathy (53, 54, 55). Tyramine is, of course, a precursor of octopa-

mine (β–hydroxytyramine), and at present tyramine is not regarded as unique in the amino–acid–neurotransmitter hypothesis of hepatic encephalopathy but rather merely as a representative of the class of phenylethylamines and phenylethanolamines.

Normal BCAA concentration in acute hepatic necrosis is, perhaps, related to two opposing influences: hepatic necrosis releasing branched-chains into the circulation versus increased fat uptake or branched–chain metabolism by muscle for energy.

III. BRAIN AMINO ACID METABOLISM

A. Blood-Brain Barrier

The capillary circulation in the brain is unique in that capillaries do not have pores but tight junctions. The permeability properties of these vessels resemble those of a cell membrane, and these properties have led to the use of the term blood-brain barrier. The blood-brain barrier is altered in a subtle way in chronic liver disease (104) and completely disrupted in experimental models of the late stages of acute hepatic coma (123), but not after 18 hr in a properly supported anhepatic rate (91a).

Blood-brain transport of various substances has been investigated by determining the brain uptake index (BUI) (151). James and his collaborators (104) found that the BUI of tryptophan, phenylalanine, tyrosine, and leucine was significantly increased after portacaval anastomosis (PCA) in rats. No increase was observed in the BUI of tyramine or for inulin (mol wt 5000–5500), which are normally excluded from the brain. The BUI of glucose, for which a hexose-specific transport system has been demonstrated (159), was also unchanged after PCA. In contrast, the BUI of arginine, which is transported by a separate system specific for basic amino acids, was significantly decreased in rats after PCA (104). A significant positive correlation was found between plasma free tryptophan and brain tryptophan, brain tryptophan, and the BUI of tryptophan, strongly suggesting that experimentally obtained BUI values are a valid index of the physiological activity of the blood-brain amino acid transport system. These data suggest that not only brain tryptophan but also brain methionine, tyrosine, phenylalanine, and histidine may be influenced by the activity of the blood-brain transport system.

These results also strongly support the postulated role of the neutral amino acids in the pathogenesis of hepatic encephalopathy. This increase in blood-brain barrier transport activity has been independently confirmed [(91, 93, 235) and D.B.A. Silk, personal communication].

Another phenomenon that appears to be independent of the competition phenomenon is a direct relationship between CSF and, presumably, brain

aromatic amino acids and the plasma concentration, suggesting that in acute fulminant hepatitis, when branched-chain amino acids may be normal, the extremely elevated plasma aromatic amino acids will result in flooding of the brain with, for example, phenylalanine and tyrosine. Whether these CSF data are in keeping with a selective active transport phenomenon or whether this is the result of partial breakdown of the blood-brain barrier is not yet clear, although evidence at present favors the former alternative (91a).

B. Glutamine, Ammonia

According to the hypothesis suggested by James et al (109), peripheral hyperammonemia increases the influx of ammonia into the brain where it is detoxified in astrocytes by its reaction with glutamic acid to yield glutamine, a neutral amino acid whose efflux from the brain is mediated by the large neutral amino acid carrier system (108). Glutamine has a low affinity for the neutral amino acid transport system of the blood-brain barrier, whereas it readily exchanges with tryptophan, tyrosine, and methionine in the plasma to enhance the concentration of these amino acids in the brain. There is an excellent correlation between the concentration of glutamine in the brain and tryptophan in the cerebrospinal fluid (199). According to this hypothesis, hyperammonemia contributes indirectly to the brain accumulation of neutral amino acids. Cangiano et al (24) showed that the presence of physiologically high concentration of ammonia significantly increased the uptake of neutral amino acids by isolated brain capillaries. These studies support the concept that hyperammonemia is able to modify the neutral amino acid L-system by increasing the glutamine intracellular levels and then stimulating the exchange of outside neutral amino acids for inside glutamine. The increased level of these precursors alters the balance of neurotransmitter substances in the brain and causes encephalopathy.

C. Significance of the Unified Neurotransmitter Amino Acid Hypothesis

If the above hypothesis is correct, hepatic encephalopathy is the result of altered plasma amino acid profile, changes in the blood-brain barrier, hyperammonemia, and altered neutral amino acid blood-brain barrier transport in turn at least partially related to hyperammonemia and increased exchange for brain glutamine. No single factor, neither ammonia nor plasma amino acid molar ratios, could be expected to have a clear one-to-one correlation with encephalopathy. The various therapeutic approaches, including decreasing ammonia and altering plasma amino acid ratios, would all work via neurotransmitter alterations.

The ultimate proof of this hypothesis in man would be the demonstration that in patients with hepatic coma, decreasing peripheral blood ammonia

would result in alterations (decreases) in CSF concentrations of neutral amino acids despite no changes in plasma amino acid concentrations. This apparently has been observed by measuring serial CSF amino acid concentrations in patients in hepatic coma treated with lactulose (L. Capocaccia, personal communication). In those patients, blood ammonia decreased while plasma amino acid concentrations remained unchanged. Nonetheless, CSF aromatic amino acids decreased markedly.

Hyperammonemic Models

If hyperaminonemia increases brain glutamine and this is at least partially responsible for increased exchange, infusing large quantities of ammonia into normal rats should at least partialy mimic amino acid changes seen in animals with portocaval anastomosis. Ammonia infusions, carefully done to avoid seizures, increase brain aromatic amino acids in parallel with rises in brain glutamine (84). Blocking synthesis of brain glutamine appears to prevent at least partially this rise in brain aromatic amino acids (110).

IV. BRAIN TRANSMITTERS

A. Catecholamines

In practice the term "catecholamine" usually implies dihydroxyphenylethylamine (dopamine) and its metabolic products, norepinephrine and epinephrine. At the present time it is well accepted that norepinephrine acts as a neurotransmitter in the central nervous system. Both in the peripheral adrenergic system and the brain, other variables such as exogenous intake, and particularly the liver and its metabolism of amino acids and amines, can control the entry and the concentration of neurotransmitter material into the neurons. The portal circulation absorbs amino acids and amines derived from gut bacterial action. They are generally inactivated by the liver, and their plasma concentrations are kept within a narrow range despite a wide range of intake by hepatic enzymatic function. The blood-brain barrier contributes to the control of neurotransmitter material by selectively allowing precursor amino acids to penetrate and excluding most circulating amines. Although tyrosine is normally the principal precursor for catecholamines, phenylalanine by hydroxylation to tyrosine similarly serves as a precursor for catecholamine synthesis. In liver failure, the peripheral and the central nervous system can accept and synthesize other precursor amino acids or unphysiologic amounts of those precursors, leading to altered neurotransmitter synthesis and derangements in neurotransmission. The depletion of a normal neurotransmitter (norepinephrine) in the periphery (49, 133) might result in a state of high cardiac output and low vascular resistance often seen in hepatic failure (133, 148). Other laboratories have

confirmed depletion of brain norpinephrine in animals in acute hepatic coma (53).

B. Indoles

Serotonin (5-HT) is found in many cells that are not neurons, such as platelets, mast cells, and the enterochromaffin cells of the intestinal mucosa. Only about 1–2% of the serotonin in the whole body is found in the brain (41), and as a 5-HT cannot cross the blood-brain barrier it is clear that brain cells must synthesize their own. Within the central nervous system the first important step is the uptake of the amino acid tryptophan, the primary substrate for the synthesis. An active uptake facilitates the entry of tryptophan into cells, and this entry site can be competed for by certain other amino acids, such as phenylalanine or any of the other neutral amino acids. The next step is hydroxylation of tryptophan to form 5-hydroxytryptophan (5-HTP). This step in the synthesis can be specifically blocked by parachlorophenylalanine, an inhibitor of tryptophan hydroxylase. In the rat, a single intraperitoneal injection of 10 mg/kg of this inhibitor lowers the brain serotonin content (28). Once synthesized, 5-HTP is almost immediately decarboxylated to yield serotonin. The only effective route of continued metabolism for serotonin is deamination by monoamine oxidase (MAO). The products of this reaction can be further oxidized to 5-hydroxyindoleacetic acid.

Disturbances in serotonin metabolism in liver disease have been widely documented. Serotonin, which may replace norepinephrine or dopamine in the adrenergic system, is markedly increased in both chronic encephalopathy and acute hepatic failure (43, 44, 45, 115, 116). Increased 5-HIAA has long been known to be present in the CSF and brain of animals and CSF of patients with hepatic failure (115, 199). In both dogs and primates, when specific nutritional therapy for hepatic encephalopathy is undertaken 5-HIAA promptly returns to normal as the animal recovers (199).

C. "False," Weak, or Inactive Neurochemical Transmitters

In the peripheral sympathetic nervous system, where norepinephrine is the normal transmitter, it has been amply shown that other β-hydroxylated phenylethylamines can replace norepinephrine and act as false or relatively inactive neurochemical transmitters (31, 69). Similar phenolic amines have been shown capable of being taken up, retained, and released in the central nervous system as well as in the peripheral sympathetic nervous system (69).

The role of the false neurochemical transmitters (FNT) may explain the production of some of the symptoms of hepatic failure as follows: Amines are produced in the gut by the action of bacterial amino acid decarboxy-

lases. They and their amino acid precursors, such as phenylalanine and tyrosine, are absorbed into the portal circulation. In normal circumstances these are largely cleared from the portal blood by the liver. When hepatic function is impaired and blood is shunted around the liver, either spontaneously or surgically, these substances flood the peripheral and central nervous system, releasing and replacing the normal endogenous neurotransmitters.

Such events might explain not only the CNS malfunction but also the cardiovascular changes—e.g. the high cardiac output and low peripheral resistance state—that may be associated with hepatic failure (133, 148). The cardiovascular changes associated with the high output state are therefore viewed as secondary. Normally, vascular beds with high resting peripheral vascular resistance are dependent on the presence of norepinephrine in the nerve terminals to maintain normal vascular tone. When norepinephrine is depleted, such normally high vascular resistance areas as skin, muscle, and the splanchnic bed vasodilate, leading to uneconomical shunting of blood through these areas. "High-preference areas" such as the brain, heart, and kidney normally have a relatively low resting peripheral resistance and cannot markedly vasodilate; their metabolic requirements must be satisfied by an increase in cardiac output. Shunting of blood away from the kidney can then cause internal reflex changes, which are associated with the hepatorenal syndrome. The finding of an accumulation of octopamine, a well known FNT, in the brain and blood of experimental animals and in the blood and urine of patients with hepatic encephalopathy has been confirmed with an excellent correlation between octopamine and the grade of encephalopathy (66, 70, 121, 129, 148, 184, 187). Phenylethanolamine, another amine capable of acting as a false neurochemical transmitter, increases as well (187). Similar results have been seen with tyramine, an octopamine precursor, (53, 54, 55). In a recent experiment in which CSF has been measured simultaneously with blood, CSF levels of these inactive or "false" neurochemical transmitters increase even before the elevation of blood octopamine and phenylethanolamine, reaching maximal levels with grade 3–4 coma and decreasing rapidly when appropriate therapy is undertaken. (199).

D. Other Neurotransmitters

The many candidates for neurotransmitter function in the central nervous system include amines such as acetylcholine, norepinephrine, dopamine, serotonin, and histamine. Many other synaptic transmitters are present in the brain and serve various functions, some stimulatory, some inhibitory. GABA, an inhibitory neurotransmitter, is normal in the brains of rats with liver disease or following ammonia administration (14, 172), but more

recent work has suggested elevations in plasma GABA levels in experimental animals and man in hepatic coma (59, 190). Brain levels have thus far not been studied. The excitatory neurotransmitters glutamate and aspartate are significantly decreased in the supratentorial part of the brain obtained from rats in acute hepatic coma (94); other inhibitory amino acid neurotransmitter candidates include taurine, serine, and glycine.

Thus a final pathophysiological mechanism would explain (*a*) the alterations in plasma and brain aromatic amino acids; (*b*) the depletion of dopamine and norepinephrine, and increased serotonin and β-hydroxyphenolethanolamines; (*c*) a widespread derangement in neurotransmission; and (*d*) the relationship between *a, b,* and *c.*

V. FACTORS PRECIPITATING HEPATIC COMA

A. Introduction

Many factors may cause encephalopathy and coma in patients with preexisting liver disease, including infection, over-diuresis, gastrointestinal hemorrhage, sedatives, tranquilizers, electrolyte imbalance, surgical procedures, acute alcoholism, abdominal paracentesis, and also occasionally a large protein meal or severe constipation. The most frequent factors in 100 episodes of hepatic coma were, in order of occurrence: azotemia, either spontaneous or diuretic induced; the use of sedatives, tranquilizers, or analgesics; gastrointestinal hemorrhage; hypokalemia; alkalosis; and protein intoxication (62). The syndrome may appear spontaneously, without a precipitating factor, usually in a deeply jaundiced patient with ascites and in the terminal states.

1. DIURETIC THERAPY The commonest precipitating factor of coma is a brisk response to a potent diuretic that causes hypokalemia and alkalosis. The kidney is an important source of ammonia, and hypokalemia increases ammonia production in the renal vein (5, 81), although whether this increased output represents increased production or a pH-dependent diversion of ammonia from the urine into the circulation is uncertain (164). With the development of metabolic alkalosis, freely diffusible ammonia rises rapidly, favoring the diffusion of ammonia into the brain, whereas a lower pH will convert it to the NH_4^+ form (25). The same may be true for amines. The passage of amines through the blood-brain barrier if it occurs at all (it does occur, for example, with phenylethylamine) should also be facilitated by alkalosis, since they are also weak bases. The mechanism by which diuretics may cause coma need not depend on the toxicity of ammonia. Apart from the liver, the kidney is the only organ capable of manufacturing glucose from amino acids. With a failing liver, the kidney may augment

production in an attempt to maintain peripheral energy supply, thus resulting in decreased BCAA facilitating the entry of the toxic aromatic amino acids into the brain (72, 107). In addition, one major source of renal venous ammonia may be the deamination of branched-chain and other amino acids, which may increase with hypokalemia and alkalosis (164).

Over-diuresis may also decrease intravascular volume, decreasing hepatic perfusion, and thus further compromise hepatic function.

2. OVERDOSE OF SEDATIVES The second most common precipitant is sedative overdose, and half of the episodes of coma are attributed to the use of chlordiazepoxide, a sedative-tranquilizer often prescribed for alcoholic patients, who frequently have liver disease. Clearance of chlordiazepoxide and diazepam, also frequently used in sedating alcholics, is reduced in advanced liver disease (143). Chlorpromazine resulted in EEG changes typical of portal systemic encephalopathy in cirrhotic patients (191). Morphine and other opiate derivatives such as methadone, meperidine, and codeine are metabolized in part by the liver (18, 103, 119), and the excretion of some barbiturates, especially the short-acting, is dependent on the liver (23). Factors besides clearance by the liver contribute to clearance of barbiturates, including protein binding and transport by serum albumin, which is decreased in most patients with liver disease. If the patient is uncontrollable and some sedation is necessary, half the usual dose of barbitone or oxazepan is given. An antihistaminic such as phenergan may be useful. It is not clear whether the coma produced by oversedation is similar to spontaneous coma or merely represents oversedation.

3. GASTROINTESTINAL HEMORRHAGE Gastrointestinal hemorrhage, usually from esophageal varices, is another common precipitant, and rapid detection is essential in the treatment of hepatic encephalopathy. Gastrointestinal bleeding increases the protein load of the gut. Blood is particularly poorly tolerated because it contains large amounts of aromatic amino acids. In addition, the hypotension associated with gastrointestinal hemorrhage decreases perfusion of the already compromised liver.

Peripheral intravenous Pitressin (antidiuretic hormone) infusion appears to be the preferred treatment as few advantages have been demonstrated with Pitressin infused into the superior mesenteric artery. The Sengstaken-Blakemore tube may be used as a temporary measure and only postpones the decision definitely to arrest a variceal bleeding. Other temporary measures include the embolization of either endogenous clot or gelfoam into varices. Sclerotherapy, recently gaining popularity (188, 210), may serve until the patient can tolerate a definitive portal hypertension-relieving procedure. Surgery, poorly tolerated in such patients, may be necessary. Emer-

gency shunts tend to have a high mortality, and trials of end-to-side portacaval shunts have revealed only equivocal evidence of life extension, although the mode of death is altered from gastrointestinal bleeding to hepatic failure (102, 157, 175), perhaps secondary to further impairment of hepatic function due to deprivation of the liver of substrate-rich portal blood (7, 16).

4. INFECTION Especially with bacteremia and including spontaneous bacterial peritonitis in patients with ascites, infection may be the precipitating factor (38). Presumably patients with severe liver disease are less resistant to infection than normal patients. In addition, alcohol has an adverse affect on leukocyte migration (22) and serum bacteriocidal activity (12), depresses white cell mobilization, and impairs phagocytosis (in mice) (124). An active serum inhibitor has recently been invoked (48, 112). Infection and fever increase metabolic demands, resulting in increased lysis of lean body mass to utilize the BCAA, which decrease even further with infection (75, 221). This protein breakdown in turn results in excessive plasma concentration of aromatic amino acids and toxic metabolites. Thus therapy of infection in patients with hepatic decompensation should include proper nutritional support.

IV. THERAPY OF HEPATIC COMA

A. Effects of Established Treatment In Chronic Liver Disease

1. GENERAL SUPPORT

a. Identify and treat the precipitating factor—e.g. hemorrhage, infection, alcoholism, electrolyte imbalance, sedation, large protein meal.

b. Carefully adjust fluid and electrolyte balance, particularly avoiding overhydration and hyponatremia. The administration of "salt-poor" 25% albumin to patients with low concentration of serum albumin maintains intravascular volume and urinary output. The measurement of urinary sodium in these patients gives an excellent guide to the requirement for administered sodium.

c. Adequate oxygenation may be measured by arterial blood gases.

d. Vitamins, especially of the B group, and calories are essential. Parenteral nutrition may be necessary in patients whose oral intake is interrupted for more than a day or two. These patients have notoriously poor oral intake, and even during unrestricted oral intake many patients with alcholic liver disease are so anorectic that they eat only a thousand calories daily only with difficulty.

e. Finally, other causes of coma may exist in hepatic failure, particularly since trivial trauma may give rise to subdural hematomas or intracranial hemorrage in patients with deficient clotting mechanisms. Meningitis should be ruled out by lumbar puncture.

2. DIET Protein restriction in patients capable of oral intake is a cornerstone of therapy and usually includes restriction to 40 g protein/24 hr. Oral intake of fewer than 20 g protein/24 hr is not compatible with prolonged survival. Following portacaval shunt, survival in experimental animals has been related to various diets. Those resulting in the shortest survival were rich in the aromatic amino acids, phenylalanine, tyrosine, and tryptophan (33). Milk and cheese are apparently better tolerated than diets containing equal amounts of protein given as meat (57). Perhaps the reason for this is that casein contains more BCAA and fewer aromatic amino acids than meat. Protein derived from vegetables may be tolerated better than animal protein (87). Vegetable protein is less ammoniagenic and contains smaller amounts of methionine and aromatic amino acids. A defined amino acid diet with these properties for patients with chronic encephalopathy is discussed in the section below on BCAA diet.

3. BOWEL STERILIZATION Bowel sterilization aims to alter bowel flora by the suppression of urease producing organisms, thereby decreasing the production and absorption of ammonia. This is accomplished by means of socalled nonabsorbable antibiotics, generally aminoglycosides, such as neomycin, kanamycin, or paramomycin. Approximately 1% of oral or rectal neomycin is absorbed, with some risk of chronic nephrotoxic or ototoxic effects (11, 118). In the acute case 6–8 g are given daily in divided doses, an amount subsequently reduced to a maintenance dose of 2–4 g/day. In addition to preventing absorption of ammonia, these antibiotics may also cause selective malabsorption of the aromatic amino acids (52, 71). A similar mechanism has been suggested in germ-free animals treated with oral antibiotics(146).

4. LACTULOSE Lactulose is a nonabsorbable synthetic disaccharide (β-1, 4-galactoside fructose). When given by mouth the bulk reaches the caecum, where it is hydrolyzed by bacterial action to lactic acid and small amounts of acetic acid, producting acidic diarrhea when amounts of 60–160 g/24 hr are utilized. The mechanism of action of lactulose in hepatic encephalopathy is still uncertain (37). It may be mediated by reduction in colonic pH with resulting decreased ammonia absorption, or it may decrease the absorption of aromatic amino acids, and decrease brain octopamine (80). An alternative explanation is that by decreasing the time in which a stool is in

contact with bacteria lactulose decreases ammonia production (3). Since the original observation (15), lactulose has been shown to be effective in three controlled studies (39, 51, 198). An additional study has demonstrated that it may be as effective as neomycin and that the additive effects of lactulose and neomycin may be superior to those of neomycin or lactulose alone (39). Although lactulose is more expensive than neomycin, it is of potential benefit in patients with renal impairment in whom neomycin may be toxic, or in those who fail to respond to neomycin. Other studies have suggested that patients treated with lactulose, despite no changes in plasma amino acid pattern, resulted in decreases in CSF aromatic amino acids, presumably by decreasing exchange for glutamine (L. Capoccia, personal communication).

5. OTHER FORMS OF THERAPY

Colon bypass Colon bypass may indeed ameliorate chronic portal systemic encephalopathy (136, 174). The operative morbidity and mortality, however, have made this a rare procedure. Ileostomy appears superior to ileorectal anastomosis as colonization of the small intestine above the ileorectal anastomosis decreases the effectiveness of the procedure with time.

Miscellaneous forms of therapy Recently, bromocriptine, a specific dopamine receptor agonist, in a dose of up to 15 mg daily, has been shown to be effective in patients with chronic encephalopathy (139). Acetohydroxamic acid has not been totally evaluated. In a few patients it has lowered venous blood ammonia but yielded little clinical improvement (204). Colonic lavage with a double-lumen tube has been utilized (230). It is not clear whether this potentially hazardous technique has anything to offer compared with the usual cleansing enemas, and fluid overload is a hazard. Lactobacillus colonization has not been successful (127). The rationale of feeding pure cultures of non-urease-producing lactobacilli is to alter the bowel flora so as to decrease production of ammonia.

B. Effects of Established Treatments In Acute Liver Disease

The management of encephalopathy due to acute hepatic failure is less well understood and much less rewarding than that for portal-systemic encephalopathy. To survive, the patient needs aggressive therapy, which is best administered in an intensive care unit.

The most frequent cause of this condition is acute virus hepatitis of both A and B types (167), although non-A, non-B can also become fulminant (233). Other causes include sensitivity reactions to halothane, to isoniazid, and to monoamine oxidase inhibitors such as iproniazid. Acetaminophen

(paracetamol) has a high mortality from acute hepatic necrosis (30). Mushroom poisoning is commonly associated with acute liver disease in France (9).

Therapy in acute hepatic failure is a holding action, an attempt to support the patient until hepatic recovery and regeneration can take place. Numerous heroic measures have been devised to provide temporary biological support. These include exchange transfusion, plasmapheresis, crosscirculation, and perfusion with isolated porcine, bovine, or baboon livers (1, 19, 34, 122, 157, 169, 176, 180, 212). Perfusion using healthy livers has a theoretical advantage if the liver secretes a substance necessary for brain metabolism. Dramatic awakening from hepatic coma has been reported by several investigators, but increased survival has not resulted. In a recent prospective controlled trial, exchange transfusion did not provide any benefit (172). Two uncontrolled studies have recently reported the use of polyacrylonitrile-membrane hemodialysis (to remove middle molecules) and have reached opposite conclusions. Nusinovici et al (150) concluded that this procedure transiently improved consciousness but did not alter survival; Silk et al (196) reported that survival was improved.

Although corticosteroids are apparently valuable in the treatment of chronic active hepatitis (203), their benefits in fulminant hepatitis have never been adequately documented. The initial report (113) has never been confirmed either in uncontrolled studies (40, 179) or in controlled trials (191). Thus, because of the questionable benefit and the possibility of serious complications, steroid therapy is no longer advocated in the treatment of hepatic encephalopathy.

Hyperbaric oxygenation has also been employed without convincing effect (82).

Conservative but intensive care remains the most reasonable course of action. It includes the furnishing of clotting factors when necessary and at least some minimal means of nutritional support, such as hypertonic dextrose, because patients with fulminant hepatic failure often have impaired hepatic mobilization of glucose, and prolonged hypoglycemia may lead to irreversible deterioration of cerebral function. The provision of nutrition in the form of amino acids and/or protein to patients with hepatitis should theoretically foster hepatic recovery and enable regeneration and more rapid recovery to take place. In a recent study, patients with alcoholic hepatitis who were supplemented with a commercially available amino acid diet had increased survival as compared with patients treated in standard fashion, without emphasis on nutritional support (147). These patients were only moderately ill and hepatic encephalopathy was not a major problem. In sicker patients, where encephalopathy limits oral protein intake, a branched chain enriched amino acid mixture is better tolerated (97). It is

tempting to extend these conclusions to sicker patients, but one must do so with caution, as the liver that is destroyed by fulminant hepatitis may be unable to respond with improved regeneration. Thus it is imperative to measure serum glucose frequently and to treat hypoglycemia promptly and aggressively with intravenous dextrose solutions. Also necessary are meticulous attention to fluid and electrolyte balance, prevention and treatment of infection, and the prevention of further gastrointestinal bleeding. Decreasing intestinal flora by administering neomycin or acidifying the stool by lactulose, which may be given orally or by enema, ameliorate the symptomatology of hepatic coma. This may be secondary to decreased production or absorption of ammonia. It has been previously noted that intestinal sterilization in animals with chronic end-to-side portacaval shunts decreases brain FNT amines to almost normal levels (71). Because most FNT amines are similar to ammonia in their pH dependency of absorption, rendering the bowel lumen more acidic certainly decreases the absorption of tyramine, octopamine, and similar substances.

C. Experimental Treatments

1. L-DOPA IN HEPATIC COMA L-dopa is the precursor of dopamine and norepinephrine in the normal metabolic synthesis of the catecholamines. Since the first report by Parkes et al (160) on the use of L-dopa in hepatic coma, numerous investigators have confirmed the apparent awakening of patients with acute or chronic hepatic encephalopathy with L-dopa therapy (68, 70, 126, 166, 189). The modes of administration have included oral (nasogastric tube), rectal, and intravenous routes. While most studies have been uncontrolled, in crossover studies L-dopa apparently increases cerebral oxygen consumption and decreases response time (126). However, another randomized prospective trial has failed to confirm the efficacy of L-dopa.

Various explanations have been offered for the effect of L–dopa in hepatic encephalopathy. The mode of action may be secondary to a number of mechanisms including replenishment of such normal transmitters as serotonin (149), release of β-hydroxyphenylethanolamines (21, 106), or the absorption of methyl groups, thus preventing the synthesis of potentially toxic methylated amines (232).

Up to 30% of patients may not absorb L-dopa even when antacid is added to aid absorption in the duodenum (181). Recent evidence suggests that coma may pass from a reversible to an irreversible stage; thus if initiated early in the course of hepatic coma, L-dopa therapy may be of some benefit (68). Side effects exist; gastrointestinal tolerance decreases with time, and the incidence of gastritis, nausea, and anorexia increases.

2. AMINO ACID INFUSION Although most investigators accept the concept that plasma aminograms are variously deranged in patients with chronic liver disease and hepatic encephalopathy, controversy surrounds the interpretation of such data and their relation to the pathogenesis of hepatic encephalopathy.

Part of the problem lies in the nature of our understanding of the blood-brain barrier and its alteration in hepatic encephalopathy (104, 109). Our laboratory had proposed that the ratio (Val + Leu + Ileu)/ (Phe + Tyr) could accurately predict the concentrations of aromatic amino acids in the brain (67, 72). It has long been clear, however, that this is not so (199) and that alterations in the blood-brain barrier (104, 235), particularly via exchange for glutamine (109), may be more important in determining intracerebral neutral amino acid monoamine precursor concentrations. Thus whether this ratio is (148) or is not (140, 226) an accurate prediction of amino acid concentrations within the brain seems unimportant, as it is now known that many other factors influence such concentrations. Furthermore, there may be other differences between medical and surgical patients as well (74). Thus the controversy may be unimportant. Suffice it to say that in most patients, plasma amino acid patterns will show elevated levels of phenylalanine, tyrosine, methionine, free but not necessarily total tryptophan (which may be decreased), and decreased concentrations of the BCAA.

Based in part on these findings that deranged amino acid concentrations are pathogenically related to hepatic coma, corrective amino acid solutions have been proposed (67, 72). The theoretical purpose of this form of therapy is to provide comparatively large amounts of BCAA (*a*) to decrease muscle catabolism, (*b*) to decrease the release of aromatic amino acids from muscle and promote their utilization via skeletal muscle and hepatic protein synthesis (76, 77), and (*c*) to compete with the aromatic amino acids for blood-brain transport at the blood-brain barrier. Infusion of a branched-chain enriched amino acid solutions, will "normalize" the plasma amino acid pattern, increasing BCAA and reducing plasma aromatic amino acids. Following the initial report of improvement in hepatic encephalopathy in patients with chronic liver disease given large amounts of amino acids in the form of special amino acid formulation (F080, a BCAA-enriched solution) (72) many additional studies, including several randomized prospective trials, have been carried out. BCAA enriched mixtures were originally proposed based on abnormal plasma amino acid patterns which were similar in dogs and man (4, 67, 72, 73). In initial animal experiments a randomized trial of a commercially available amino acid mixture vs a branched-chain enriched amino acid mixture demonstrated the superiority of the BCAA-enriched mixture (67). Neurologic normality and prolonged

survival were seen when the branched-chain enriched amino acid mixture was given. In contrast, dogs with commercially available amino acid mixtures died regularly in hepatic encephalopathy (67). When animals maintained in neurological normality on prolonged infusions of BCAA were switched to freamine ® they died within 10 days in hepatic coma (67).

Subsequent studies revealed that in patients with hepatic encephalopathy and/or those with impaired hepatic function who did not tolerate commercially available amino acid mixtures, infusion with BCAA enriched mixture resulted in improvement in encephalopathy and nitrogen balance (72). A host of studies from a variety of institutions have subsequently confirmed such efficacy on an anecdotal basis as well as in three randomized prospective multi-centered trials (186).[2] In five or six other trials in which the randomization is suspect, efficacy has also been claimed (50, 93, 96, 153, 154, 168).

The mechanism is of such an improvement in hepatic encephalopathy is the result of decreased plasma aromatic amino acid concentrations due to increased hepatic and skeletal protein synthesis. Competition of the BCAA at the blood-brain barrier is also important in normalizing brain monamine precursors.

Two studies have appeared in abstract form in which the efficacy of the BCAA in both acute and chronic patients was not demonstrated (165, 216). It should be noted, however, that the principal caloric source in both of these studies was intravenous fat. There is little evidence that fat is utilized in hepatic failure; it may, in fact, be injurious, as nonesterified fatty acids may compete with tryptophan for binding sides on albumin, thereby displacing bound tryptophan, increasing free tryptophan, and increasing central nervous system indoles. In confirmation of the lack of utilization of aromatic amino acids for protein synthesis, the plasma concentrations of aromatic amino acids were unchanged in the study in which acute patients were treated (165). These findings suggest that fat may be an inappropriate caloric source for such infusions and will not be efficacious; glucose in adequate caloric amounts is required.

Further investigations in (*a*) experimental animals with chronic indwelling cannulas in the lateral ventricles from which CSF may be collected and (*b*) animals in which brain and sagittal sinus blood amino acids have measured have revealed the following (199): (*a*) The changes in brain and/or CSF amino acids, amines, and transmitter metabolites precede those of the plasma. (*b*) In general it is impossible to predict brain and/or CSF concentrations from plasma concentrations of these various substances. (*c*) Hepatic coma occurs when phenylalanine, tyrosine, tryptophan, and

[2]A multi-centered trial in the United States is approaching its conclusion. A multicentered trial in Brazil has also been completed.

their CNS neurotransmitter metabolites, octopamine, phenylethanolamine, and 5-hydroxy indolacetic acid are at their highest levels. (*d*) Improvement and awakening from hepatic encephalopathy appears to occur simultaneously with or immediately following the normalization of these amino acids and neurotransmitter metabolites. With BCAA infusions, CSF and presumably brain concentrations of the aromatic amino acids and their amine derivatives decrease precipitously, as do the sulfur-containing amino acids in both dogs and monkeys (199, 200).

Subsequent studies appear to confirm that exchange for glutamine may be a mechanism for accumulation of CNS amino acids in patients with hepatic encephalopathy. Thus treatment of patients with hepatic encephalopathy with lactulose, which does not change plasma concentrations of amino acids, nonetheless was accompanied by precipitous decreases in aromatic amino acids within the cerebrospinal fluid. CSF ammonia and glutamine decreased as well.

There is a growing trend toward nutritional support and metabolic modification as a major therapeutic approach to encephalopathy. Theoretically, supplying BCAA enriched mixtures with hypertonic dextrose should have the following beneficial effects: (*a*) It should promote muscle protein synthesis, thereby lowering plasma aromatic amino acids. (*b*) In the presence of overall energy deficiency, BCAA may satisfy a greater percentage of peripheral energy requirements than the estimated 5–6% they provide under normal circumstances. (*c*) At the blood-brain barrier, the BCAA compete with the aromatic amino acids for transport by system-L. (*d*) By decreasing catabolism, decrease ammonia production, brain glutamine, and exchange of glutamine for aromatic amino acids across the blood-brain barrier (109). (*e*) BCAA may improve hepatic protein synthesis and regeneration (76, 77).

Up to 120 g protein equivalent in the form of amino acids have been administered to patients with protein intolerance and hepatic encephalopathy with beneficial effects, including the clearing of encephalopathy, positive nitrogen balance (when 80 g protein equivalent and 32–37 kcal/kg/day are administered), and in some cases apparent improvement in hepatic function. Prospective randomized trials have confirmed efficacy.

3. ALPHA-KETO ANALOGS Another possible approach is the administration of alpha-keto analogs of amino acids, either orally or intravenously. These keto acids are the derivates of the branched chain amino acids. Since abundant nitrogen is available as urea or glutamine, providing the keto-acid carbon-chain skeletons of amino acids facilitates transamination with available nitrogen to convert these keto acids into amino acids, which become available for protein synthesis. In addition, utilization of alpha-keto acids does not depend on the liver (219, 225). In a small series of patients with

chronic hepatic encephalopathy given alpha-keto acids intravenously, improved nutrition and clearing of hepatic encephalopathy were observed in some patients (128). The nitrogen donor in hepatic failure is glutamine, not urea as in renal failure. One beneficial effect of the alpha-keto analogs appears to be decreased muscle catabolism, as decreased plasma levels of tyrosine and glycine were observed in these patients despite the fact that tyrosine and glycine were not given (64, 128). The keto acids become more soluble when made into sodium or calcium salts, but it is questionable whether many patients with chronic hepatic insufficiency could tolerate amounts of calcium or sodium ions that would be contained in useful doses of water-soluble keto-acid formulations. More recent studies suggest that the ornithine salts of the alpha-keto acids are more efficacious than equimolar amounts of the calcium salts or the BCAA themselves (92). Watanabe has utilized a similar approach using ornithine and the BCAA (Hep-OU) (93, 224). Here again the decrease in blood ammonia may be more efficacious, since less glutamine is synthesized and less is available for exchange across the blood-brain barrier. In addition, glutamine itself appears to be the nitrogen donor.

4. BRANCHED-CHAIN AMINO ACID ENRICHED DIET Until recently there had been few attempts to explore diet therapy (other than protein restriction) of patients with liver disease. Protein restricted diet may increase catabolism and result in deterioration of liver function and mental state. Patients with hepatic encephalopathy are frequently treated with a 40 g protein diet for prolonged periods of time. This may result in negative nitrogen balance since earlier studies indicated that cirrhotics require a minimum of 35–50 g protein/day (153, 207), and negative nitrogen balance cannot be sustained indefinitely. The parenteral approach appears to be useful in an acute or an in-hospital setting where patients cannot take nourishment orally. A much larger problem exists in patients who, either following portacaval shunt or spontaneously, demonstrate severe protein intolerance and hepatic encephalopathy accompanied by incapability of accepting minimal intakes of protein, even when given nonabsorbable antibiotics and lactulose. Recent studies suggest that a nonprotein diet in a patient with hepatic impairment releases large amounts of aromatic amino acids, resulting in greater plasma amino acid imbalances. Many patients with hepatic impairment can tolerate 50–60 g of protein orally. For those who can not, we have proposed a branched-chain enriched amino acid diet: 35% BCAA (Hepatic Aid) with a composition similar to F080. This amino acid-glucose formulation consists of L-amino acids in similar proportion to the intravenous formulation F080 (67, 72). BCAA make up 35% of the amino acids and aromatic amino acids are decreased as compared with normal commercially available diets. The results, especially in patients with

portacaval shunt, have been interesting (58, 65, 79, 97) and include improvement in encephalopathy as well as liver function. A recent prospective randomized study appears to demonstrate the efficacy of such a BCAA diet as compared with conventional protein (97). Some problems remain, especially because the commercially available diets are wedded to high-dose glucose. In patients who are usually diabetic and hyperglucagonemic, such diets make difficult the control of blood glucose. Under these circumstances, we have used up to 30 g of BCAA alone on a daily basis, in divided doses.

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