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LIVER DISEASE AND PROTEIN NEEDS

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INTRODUCTION

The liver plays a central role in the metabolism of nutrients. Protein deficiency, often associated with liver disease, may be caused by decreased intake, decreased absorption, abnormalities in metabolism, or increased requirements for protein. Deficiencies of other nutrients in patients with liver disease, such as of carbohydrates and vitamins, also adversely affect the metabolism of protein. Abnormalities in the metabolism of protein in liver disease play a role in the pathogenesis of many of the clinical complications of liver disease.

DIETARY INTAKE AND NUTRITIONAL STATUS IN PATIENTS WITH LIVER DISEASE

Poor dietary intake is probably the principal cause of protein deficiency in liver disease. Decreased dietary intake is caused by symptoms of anorexia and nausea in association with liver disease, often compounded by minimal appeal of the special diets, restricted in salt and protein, that are prescribed for the patient. In alcoholic patients, additional causes of inadequate food intake are epigastric discomfort caused by gastritis, the high caloric value of alcohol, limited finances, a disorganized family and social life, and therefore a disrupted meal schedule.

Dietary histories are generally unreliable, especially in alcoholic patients. Nevertheless, a history of grossly substandard diets was obtained in 73% of 124 alcoholic patients with cirrhosis and hepatic failure (132), and in 64% of 172 alcoholic patients with various types of liver disease (79). In another study (113) poor dietary intake, defined as less than one meal per day for a period of 10 days before admission to the hospital, was found in 68% of 56 alcoholic patients with hepatomegaly caused by fatty infiltration of the liver.

The dietary intake of the alcoholic patient consists mostly of carbohydrate with inadequate amounts of protein and vitamins (132). During drinking sprees food intake is negligible, being often no more than a bowl of soup and a pretzel. In a study by Patek et al (133) a mean daily caloric intake in alcoholic patients with cirrhosis was in the range of 3200–3500, with alcohol contributing 51–58% of the calories, while protein intake was 50 g/day and contributed only 6% of the calories. Alcoholics without cirrhosis had a 13% higher intake of calories and protein than found in those with cirrhosis. In another group of alcoholics with cirrhosis the mean intake of protein was 56 g/day (136). In a study in France, alcoholics with cirrhosis had a mean caloric consumption of 3435 with alcohol contributing 31% of the calories; protein intake however was 81 g/day (only slightly lower than

that of 89 g/day of a control population) and contributed 13% of the calories. Total protein intake is only slightly influenced by the protein content of the alcoholic beverages ingested (134). Distilled spirits such as gin, rum, whiskey, and vodka contain no protein, while the protein content of one American beer (alcohol content, 3.6 w/v) and of one liter of wine (alcohol content, 9.9 w/v) are 1.1 g and 1.0 g, respectively (24).

A history of weight loss is obtained in most alcoholic patients with (132) and without cirrhosis (113), and almost invariably weight is gained following abstinence and reinstatement of a normal diet. A mean weight gain of 3.1 kg was found over a 3 week period in one study of 56 alcoholic patients after admission to the hospital (113). In a study by Leevy et al (2) circulating levels of water-soluble vitamins were frequently found deficient in alcoholic patients. Folic acid is the vitamin most commonly found deficient, low serum levels occuring in 30% of those with normal liver, 40% with fatty liver, and 47% with cirrhosis (79). Low serum levels of thiamine, riboflavin, nicotinic acid, and pyridoxine were found in 23% of patients with cirrhosis, whereas 20% had low levels of vitamin B_{12}, panthotenic acid, or biotin. Clinical stigmata of vitamin B deficiency were regularly associated with low serum vitamin levels. The serum albumin is usually normal in chronic alcoholics with no or mild hepatomegaly due to fatty infiltration (115) but was found decreased in 87% of patients with symptomatic fatty liver (75) and in 90% of patients with cirrhosis and hepatic failure (132). Lean body mass estimated from measurement of total body potassium using ^{40}K and whole body counting was found to be decreased in alcoholic cirrhosis (142); by contrast, skeletal mass determined by measurements of total body calcium using total body neutron activation analysis, and by bone mineral content of the radius using the photon absorption technique, was not decreased. Consequently, patients with alcoholic cirrhosis have a decrease in the ratio of lean body mass to skeletal mass.

Nonalcoholic patients with cirrhosis have also been documented to have decreased dietary intake. In a study of 39 patients a history of decreased caloric intake and dietary intake was found in 44 and 20% of the patients, respectively (120). The protein intake in 10 patients (26%) was below 55 g/day, but in all cases it was above the minimum of 0.85 g/kg body weight. In addition 18% of the patients were underweight by a mean of 9.3 kg below ideal weight. The fat-soluble vitamins were the principal vitamins found deficient. The plasma levels of vitamins A and E were low in 42 and 38% of the patients, respectively. In contrast to the alcoholic patients, deficiencies in the water-soluble vitamins (thiamine, riboflavin, and nicotinic acid) were found only once, each in a different patient. However, leukocyte ascorbic acid levels and serum folate were decreased in 35 and 17% of patients, respectively, whereas serum vitamin B_{12} was decreased in three patients.

DIGESTION AND ABSORPTION

Patients with liver disease often have abnormalities of digestion and absorption of nutrients that may contribute to protein deficiency. The disturbances in digestion and absorption may be related principally to alcoholism or may be associated with cirrhosis alone.

Alcoholism

Alcoholics with minimal hepatomegaly owing to fatty infiltration or no demonstrable liver disease frequently have abnormalities in intestinal absorption and pancreatic dysfunction after heavy alcohol ingestion. The substances that have been shown to be malabsorbed are: D-xylose, thiamine, folic acid, and fat (111). An increase in the fecal excretion of nitrogen ($>$ 2.75 g/24 hr) was found in 52% of alcoholic patients in one study (141). Radiological studies of the small bowel are normal and jejunal biopsies reveal no abnormalities of the mucosa when examined by light microscopy; but ultrastructural changes have been described in the jejunal mucosa of patients fed ethanol with an adequate diet (149). Pancreatic function assessed by means of the secretin stimulation test in one study was abnormal in 44% of 32 patients tested. Frequent abnormalities of pancreatic secretion in these patients are decreased outputs of bicarbonate, amylase, lipase, and chymotrypsin, but normal or increased volume output and normal trypsin output (114). Steatorrhea correlates best with a low lipase output (114). The abnormalities of intestinal absorption and pancreatic function are reversible to normal in most patients after abstinence from alcohol and ingestion of an adequate diet.

Both a direct toxic effect of ethanol and malnutrition have been considered as causes of the absorption and pancreatic abnormalities. The acute administration of large doses of ethanol (0.8 g per kg of body weight, or more) in man has been shown to inhibit intestinal absorption of thiamine and folic acid in a few patients, and of D-xylose in all patients studied (111). The chronic administration of ethanol in man together with an adequate dietary intake has resulted in a decrease in vitamin B_{12} absorption in all of the patients studied (84), a decrease in folate absorption in only a few (42), but no changes in D-xylose absorption (111).

In support of the role of malnutrition as a factor in malabsorption and pancreatic dysfunction is the demonstration of the recovery to normal of D-xylose and folic acid absorption, the disappearance of steatorrhea (111), and the return to normal of exocrine pancreatic function (114) in patients after institution of a normal diet despite continuation of ethanol feeding in doses averaging 250 g per day (equivalent to 24 ounces of 86 proof whiskey per day). Exocrine pancreatic function in malnourished alcoholics ingesting

250 g of ethanol per day was shown to be dependent on the protein content of the diet. It remained abnormal as long as the patients were maintained on a low protein (25 g) 1800 calorie diet, but returned promptly to normal after institution of a normal protein (100 g) 2600 calorie diet. Furthermore readministration of the low protein diet resulted in decreased outputs of amylase and chymotrypsin (114).

Most likely, both alcohol and nutritional factors in combination are the cause of the malabsorption and pancreatic dysfunction. This is suggested by studies showing that, although neither the administration of ethanol nor the feeding of a folate-deficient diet alone resulted in malabsorption of folic acid and D-xylose, the combination of both did produce malabsorption of these substances (42). Also, chronic administration of ethanol decreased the pancreatic content of enzymes in rats fed a low protein diet, while increasing them in rats fed a high protein and high lipid diet (155).

Effects of Ethanol on Amino Acid Absorption

Ethanol has been shown to inhibit small intestinal transport of amino acids. In man the direct addition of ethanol to intestinal perfusates in a concentration of 2% was found to inhibit the intestinal uptake of L-methionine (57). In the rat intragastric administration of ethanol in a dose of 2.5 g/kg body weight together with the amino acid resulted in 50% inhibition of the absorption of L-phenylalanine, but did not modify the absorption of D-phenylalanine, an amino acid that is not actively absorbed (56). In everted sacs of small intestine 3% ethanol inhibited the transport of L-phenylalanine, L-leucine, glycine, L-alanine, L-methionine, and L-valine (16). L-alanine flux across the rabbit jejunal mucosa in Ussing Chambers was partially depressed by 3% ethanol and completely suppressed by 5.4% ethanol (72). Ethanol at these concentrations also decreased transport of sodium and 3-o-methylglucose, and decreased electrical potential difference and short circuit current. The effect of ethanol in inhibiting transport was found when added to the mucosal or to both the mucosal and serosal sides, but not when added to the serosal side alone, indicating that its effect was on active transport and not on permeability nor due to damage of the cellular membrane. The inhibitory effect of ethanol on amino acid transport is probably due to its inhibitory effect on the activity of intestinal basolateral membrane Na^+-K^+ ATPase (52). Besides its effect on inhibition of active transport, ethanol also causes an increase in intestinal permeability demonstrated by increases in serosal to mucosal flux of sodium, 3-o-methylglucose, and L-alanine (72). The increase in permeability is more marked at higher concentrations of ethanol, which are also known to result in erosions of the gastric and intestinal mucosa (8).

Cirrhosis

Steatorrhea is the most common manifestation of malabsorption in patients with cirrhosis. It occurs in about 50% of patients with cirrhosis whether or not they are alcoholic, and in the latter patients it persists even after several weeks of abstinence from alcohol. The steatorrhea is usually mild, not exceeding 10 g/day; however in 10% of cases it exceeds 30 g/day (85). The most common cause of steatorrhea in cirrhosis is decreased synthesis and biliary excretion of bile salts, resulting in decreased intestinal bile salt concentration for the formation of micelles (4). D-xylose malabsorption has been found in some (7, 36) but not in other (29, 176) studies of cirrhotic patients. The digestion and absorption of protein in patients with cirrhosis appear to be normal. Stool nitrogen excretion was not increased above normal at protein intakes as high as 100 g/day in one study (38). An increased gastrointestinal loss of albumin has been found in some patients with cirrhosis. In one study, there were positive correlations between protein loss into the intestine and the severity of the liver disease, the depression of the serum albumin, and the elevation of portal pressure (54). The increased losses of albumin into the gastrointestinal tract are probably the result of increased lymph flow and lymph pressure with resulting decreased drainage of lymph from the intestine caused by postsinusoidal obstruction to portal blood flow. Radiology of the small intestine has demonstrated thickening of the mucosal folds (7), which is more common in patients with hypoalbuminemia and probably is secondary to edema. Histological examination of the intestine in one study revealed edema, inflammation, and fibrosis of the villi, and dilation of the crypts of Lieberkühn (2); however, most recent studies have revealed little or no change in jejunal histology (101, 173).

Neomycin used in the treatment of hepatic encephalopathy, because it alters the intestinal flora that produce ammonia, may be a cause of malabsorption and steatorrhea. Changes produced by neomycin that may be responsible for the malabsorption and steatorrhea are: direct toxicity to the mucosal cell of the intestine, inhibition of intraluminal hydrolysis of long chain triglycerides, and precipitation of bile salts and fatty acids (178).

Intestinal Bacterial Overgrowth

An increase in small intestinal flora and an alteration in its composition have been found in patients with cirrhosis. Martini et al (102) found an increased total coliform count in the duodenum, jejunum, and ileum in one half of the patients studied; in addition, *Streptococcus fecalis* was present in the jejunum of 25% patients with cirrhosis but in none of the normal

subjects examined. Examination of the feces revealed a higher incidence of atypical coliform bacteria such as *Escherichia freundii* in patients with cirrhosis. Lal et al (74) reported an increase in urea-splitting bacteria, predominately *Klebsiella* and *Proteus* strains, in the small and large bowel of 88% of patients with cirrhosis. Contamination of the small bowel with these organisms was found in 5% of the patients. Some of the *Klebsiella* organisms were resistant to the action of neomycin. Intestinal bacterial overgrowth can have a deleterious effect on nitrogen metabolism (62). It can contribute to catabolism of ingested protein, increased loss of endogenous protein, and diminished absorption of protein. In addition it can alter the composition of amino acids and nitrogen breakdown products that are available for absorption (177). Rats with blind loops and bacterial overgrowth have decreased gain in body weight, steatorrhea, increased urinary excretion of bacterial degradation products from ingested protein, and increase in fecal nitrogen excretion (117, 127). In man bacterial overgrowth due to a blind loop was associated with decreased plasma concentration of essential amino acids, decreased synthesis of albumin and fibrinogen with an increased synthesis of urea (65); these data suggest that intestinal bacteria deaminate large quantities of protein with the formation of ammonia which is then available for incorporation into urea.

PROTEIN REQUIREMENTS AND PROTEIN METABOLISM

Nitrogen Balance

The protein requirements of most patients with liver disease for maintenance of nitrogen balance are not different from those of normal individuals. In one study most of the patients with cirrhosis were in nitrogen equilibrium or in positive balance at protein intakes of 35–50 g/day (38, 39), which is in the range of the minimal requirement for normal adults. In patients with decompensated cirrhosis, manifested by the presence of ascites, an intake up to 75 g of protein a day may be necessary to maintain nitrogen balance, indicating an increased requirement of protein. With improvement in the clinical condition of these patients, and maintenance on the same protein intake, there was a decrease in the urinary nitrogen excretion, suggesting a defect in utilization of dietary protein in the presence of active disease and a more efficient utilization with improvement. Catabolism of endogenous protein does not account for the increased urinary excretion of nitrogen, since administration of a diet low in protein (4.3–13.0 g per day) but adequate in carboyhdrate reduced urinary nitrogen excretion to minimal levels ($<$ 4.6 g/ day) (38). Ingestion of 400 g of glucose a day in this study provided sufficient calories to prevent utilization of significant quantities of

nitrogen. Also in another study the normal ability to conserve nitrogen was demonstrated in cirrhotics placed on an even lower protein (2 g) diet for 8–10 days. On the other hand, an excessive degree of positive nitrogen balance was observed in these patients upon institution of a high protein diet (120 g/day) after a period of 8–10 days on the low protein diet. This excessive degree of nitrogen balance was associated with a subnormal urinary excretion of urea and no gain in body weight, suggesting that it was caused by a decrease in the synthesis of urea (151). Although estimates of total body nitrogen balance do not seem to be altered significantly in cirrhosis, there are profound alterations in the distribution of nitrogen between the liver and other organs, and in intermediary nitrogen metabolism. An increased demand for protein after liver injury drains nitrogen from other organs, leading to deficiencies in those other organs. Increases in plasma glucagon and an increase in the glucagon to insulin ratio results in increased gluconeogenesis with release of amino acids from muscle (168). The altered distribution of amino acids may contribute to the muscle wasting commonly observed in patients with cirrhosis (39).

Chronic ethanol feeding results in increased urinary excretion of nitrogen in rats (140) and in man (90). The increased excretion of nitrogen in man was associated with a negative nitrogen balance and weight loss. Possible causes for these findings are effects of ethanol in decreasing protein synthesis or in enhancing protein catabolism. Recent studies in rats show that chronic ethanol feeding decreases whole body protein synthesis and that this is due to reduced efficiency in recycling nitrogen for protein synthesis. An accompanying decrease in protein catabolism was not enough to maintain growth of the animals at levels similar to controls (17). Chronic ethanol feeding increases urea synthesis in liver slices of rats (58), while ethanol in concentrations of 50 and 100 mM decreases urea synthesis in isolated rat hepatocytes (123). Relatively low concentrations of ethanol and acetaldehyde inhibit protein synthesis by isolated muscle and liver mitochondria (150), and acetaldehyde depresses microsomal protein synthesis in the heart (157).

Amino Acids

Changes in the plasma concentrations of amino acids and increases in the urinary excretion of some amino acids are found in acute and chronic liver disease, but are most prominent during massive hepatic necrosis and in association with hepatic encephalopathy. Experimentally, in the dog, more than 85% of the liver must be removed before disturbances in amino acid patterns become apparent (97). The normal breakdown of tissue proteins results in a release of amino acids into the blood stream. These amino acids are continuously deaminated to serve as sources of energy. The catabolism

of many of the amino acids occurs in the liver, whereas essential branched chain amino acids such as valine, leucine, and isoleucine, and many of the nonessential amino acids, are preferentially taken up by extrahepatic tissues (118). In chronic liver disease there is a tendency for an increase in plasma concentrations of the amino acids normally removed by the liver, and a fall in the amino acids principally taken up by extrahepatic tissues. Therefore, the most common amino acid pattern observed in chronic liver disease consists of rises in the aromatic amino acids, tyrosine and phenylalanine, and glutamic acid, methionine, and sometimes cystine, and a fall in the branched chain amino acids, valine, leucine, and isoleucine (55, 126, 180, 186, 190). Increases in plasma tryptophan have been found only in cirrhotic patients with encephalopathy (50).

Increases in the circulating levels of both insulin and glucagon (100, 162) found in cirrhosis may be partly responsible for the changes in plasma amino acids observed. Elevated levels of insulin, by stimulating increased peripheral uptake of branched chain amino acids (138), contributes to their low levels in the circulation (162). On the other hand, elevated levels of glucagon stimulate gluconeogenesis and the release of amino acids from muscle, with a resulting accumulation of the aromatic amino acids which the diseased liver fails to metabolize. The principal cause of the high insulin levels found in cirrhosis appears to be insulin hypersecretion in association with insulin resistance. This is suggested by findings of inappropriately high plasma insulin levels in response to glucose administered either orally or intravenously (9, 20), increased insulin response even when glucose tolerance is normal (110, 158), and a diminished response of glucose to injected insulin (21). Decreased degradation of insulin does not appear to be a factor because rates of disappearance of injected insulin were found to be normal (21). Elevated levels of free fatty acids (9), fasting growth hormone (22), and glucagon (100, 162) as well as hepatic damage may be causes of insulin resistance. Glucagon levels are particularly high after portacaval shunting (162). Hyperammonemia may be a stimulus for increased secretion of glucagon, since plasma levels of glucagon correlated with elevated levels of blood ammonia (152), and administration of ammonia salts to normal dogs resulted in hypergluconemia (171).

The increase in the plasma levels of aromatic amino acids in liver disease as mentioned previously is due to a combination of increased release of amino acids from muscle and a decrease in their metabolism. In a recent study, Heberer et al (44) showed a decrease in the elimination of intravenously administered L-phenylalanine, associated with a decreased formation of tyrosine in patients with cirrhosis and acute hepatitis, but not in those with alcoholic hepatitis. The activity of hepatic phenylalanine hydroxylase was decreased in patients with cirrhosis and acute alcoholic

hepatitis. The total phenylalanine hydroxylase activity in patients with cirrhosis was estimated to be 20% of the normal value, suggesting that reduced enzyme activity was the principal cause of the decreased metabolism of this amino acid. In another study (59) the elimination of L-phenylalanine from the plasma was normal in patients with cirrhosis, but the total body clearance was decreased, owing to a decrease in the volume of the central compartment of distribution. This compartment corresponds to highly perfused organs, such as the liver, that rapidly equilibrate with plasma. The elimination of tyrosine, the metabolic product of phenylalanine, had been shown to be decreased in cirrhosis (83). A decrease in the elimination of orally administered methionine has also been demonstrated in cirrhosis (51); the simultaneous decrease in urine sulfate excretion suggested that the retarded elimination of methionine is due to a block in the transsulfuration pathway. The increases in plasma free tryptophan may be the result of decreased binding of tryptophan to plasma albumin due to a decreased concentration of albumin and a raised concentration of free fatty acids that compete for binding to albumin (109).

Ethanol feeding for two or four weeks to alcoholic volunteers resulted in increases in plasma α-amino-n-butyric acid and the branched chain amino acid isoleucine (160). Studies in rats and baboons demonstrated that the increased concentration of plasma α-amino-n-butyric acid was caused by an increased hepatic production and release into the circulation of this amino acid following chronic ethanol ingestion (161). It is postulated that the increased production of α-amino-n-butyric acid may be due to increased catabolism of threonine, serine, and methionine to α-ketobutyrate, which is then transaminated to α-amino-n-butyric acid. In another study, chronic ethanol feeding in rats resulted in increases in plasma and muscle, but not in the hepatic concentration of α-amino-n-butyric acid (169), while the concentration of leucine was increased in plasma, muscle, and liver. Most of these amino acid changes were prevented by the addition of pyruvate, dihydroacetone, and riboflavin to the diet. Alanine concentration in plasma and liver, but not in muscle, is decreased by chronic ethanol feeding. The decrease in alanine is observed despite the fact that ethanol inhibits its conversion to glucose by gluconeogenesis; rather the alanine is converted to lactate via pyruvate (71). Ethanol (80–100 mM) inhibits basal and insulin-stimulated uptake of the nonmetabolizable amino acid, α-amino-isobutyric acid, by rat hepatocytes in culture (143). The inhibition of insulin-stimulated transport was greater than that of basal transport, suggesting an effect on the "A" system of amino acid transport in the liver (18). The "A" system of transport, which is Na^+-dependent and requires energy, serves mainly for short-chain amino acids such as alanine, glycine, and

serine. The effect of ethanol was not inhibited by pyrazole, suggesting that it is a direct physical effect of ethanol on the cell membrane. A number of recent studies have demonstrated that ethanol in vitro increases membrane fluidity; by contrast, chronic ethanol administration increases resistance to the fluidizing effect of ethanol (175). These changes appear to be caused by alterations in the composition of membrane phospholipids (182).

Urea Synthesis

The normal liver disposes of the nitrogen in amino acids by transamination with formation of glutamic acid. In addition, ammonia produced in various tissues can be removed by amination of glutamic acid with the formation of glutamine. The nitrogen is then released in the liver as ammonia and enters the urea cycle with the eventual formation of urea (Figure 1). In patients with liver disease there is a decrease in the synthesis of urea with the resultant accumulation of ammonia. Maximal rates of urea synthesis have been shown to be decreased in patients with cirrhosis to values ranging from 10–90% of normal. In a study of 34 cirrhotic patients, mean maximal rate of urea synthesis was 27 mg of urea N/hr/kg of body weight, as compared with a rate of 65 mg of urea N/hr/kg of body weight in normal subjects (152). The depressed maximum rate of urea synthesis correlated strongly with elevated fasting venous ammonia, impaired ammonia tolerance, elevations in plasma glutamine and glycine, and a prior history of hepatic encephalopathy (1). The increase in plasma glutamine is most likely the result of a reaction of excess ammonia with α-ketoglutarate; however, the mechanism for the hyperglycinemia is unknown. Maximum rate of urea synthesis showed no correlation with serum levels of albumin, bilirubin, and glutamic oxaloacetic transaminase, or with prothrombin time or hemoglobin concentration (1, 152). A decrease in the ability to synthesize urea could be detected earlier in the natural history of cirrhosis than hyperammonemia, hyperaminoacidemia, and hepatic encephalopathy. A further decrease in the maximal rate of urea synthesis occurs after venous shunts for the therapy of bleeding esophageal varixes, and this decrease is more marked after total portacaval shunts, which divert blood flow from the liver, than after selective splenorenal shunts, which only decompress the varixes (40). The greater decrease in maximal rate of urea synthesis after portacaval shunt is associated with a higher incidence of hepatic encephalopathy. Decreases in urea synthesis have also been demonstrated in perfused livers of rats made cirrhotic with a diet deficient in choline and protein (66) or with the administration of carbon tetrachloride (135).

Factors that may be responsible for the decreased urea synthesis in liver disease are: decreases in the enzymes or substrates of the Krebs-Henseleit urea cycle, a reduced hepatic blood flow, and a smaller functional hepatic

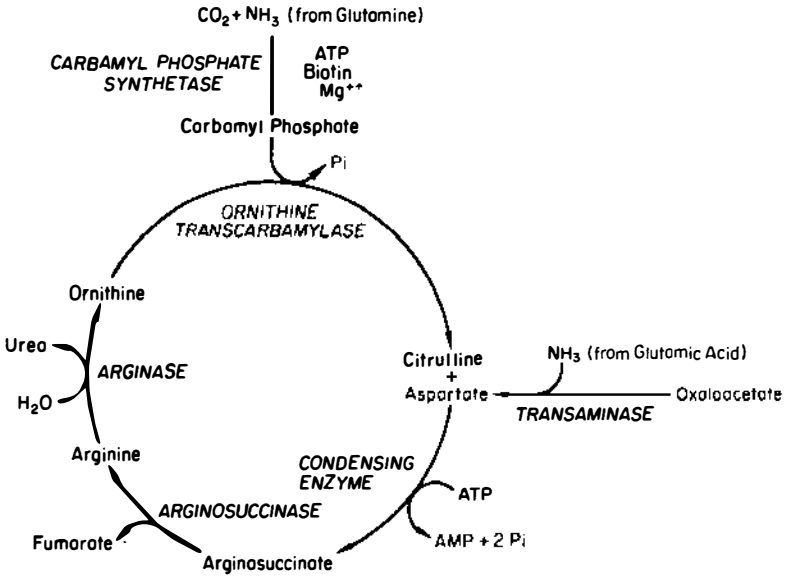

Figure 1 Reactions involved in the utilization of ammonia and formation of urea. Reproduced from (111a).

mass. The activities of all five enzymes that catalyze the steps of the urea cycle—i.e. of carbamyl phosphate synthetase, ornithine carbamyltransferase, arginosuccinate synthetase, arginosuccinate lyase, and arginase—are decreased in cirrhosis (68, 94). The most likely limiting enzymes in the urea cycle on the basis of tissue enzyme levels are carbamyl phosphate synthetase and arginosuccinate synthetase (68, 159). The decreases in the activities of these enzymes in cirrhosis were of the order of 60% for carbamyl phosphate synthetase and 37% for arginosuccinate synthetase (154). Patients with chronic active hepatitis had decreases in all the five enzymes similar to those found in cirrhosis (154), while patients with alcoholic hepatitis only had decreases in the activities of carbamyl phosphate synthetase and arginase, but not in the other three enzymes (95). No changes in enzyme activity were demonstrated in patients with fatty liver. However, substrate availability rather than enzyme activity may be rate-limiting for the urea cycle under physiologic conditions. A direct relationship exists between dietary protein intake and urea synthesis (156). Feeding of a low protein diet results in a greater decrease in urea synthesis by the perfused rat liver than the associated decrease in the activity of ornithine carbamyltransferase, while the addition of ornithine and acetylglutamate to the perfusate enhances the rate of urea synthesis (154). Similarly, urea synthesis in the perfused liver which had been made cirrhotic by carbon tetrachloride was found to exceed the

decrease in the activity of the arginine synthetase system (66). Alterations in portal blood flow due to portal hypertension and the presence of spontaneous venous collaterals or surgically constructed portal-systemic shunts also contribute to decreases in urea synthesis and the disposition of ammonia (40, 185). The diversion of portal blood flow causes hepatocellular atrophy and a decrease in the enzyme activities of the urea cycle in experimental animals (99); furthermore, the shunting of portal blood to the systemic circulation not only reduces the ammonia immediately available for extraction by the liver, but also reduces the concentrations of amino acids necessary for maximal activity of the urea cycle. Finally, a direct correlation between decreased liver mass produced by partial hepatectomy and urea synthesis was demonstrated in rats (12).

The administration of corticosteroids to patients with cirrhosis and chronic active hepatitis results in an increase in urea synthesis (64). In patients with chronic active hepatitis, corticosteroid-induced histological and laboratory remission of the disease is associated with a return to normal of the activities of the urea cycle enzymes (93). By contrast, corticosteroid administration did not improve depressed activities of the enzymes of the urea cycle in patients with alcoholic hepatitis.

HEPATIC ENCEPHALOPATHY

The abnormalities of nitrogen metabolism, amino acid metabolism, and urea formation found in chronic liver disease are important in the pathogenesis of hepatic encephalopathy.

Nitrogenous Breakdown Products

Nitrogenous breakdown products produced by the action of bacteria in the large intestine have ready access to the systemic circulation and brain in patients with a diseased liver and portal-systemic collaterals. Of these nitrogenous products ammonia is the best studied and is the principal potential substance in the pathogenesis of hepatic encephalopathy. Elevations of ammonia are common in patients with encephalopathy, and decreases in levels after treatment often correlate with improvement of the encephalopathy, whereas administration of ammonia or substances that give rise to it often precipitates encephalopathy; however, the correlation is far from perfect, and in fact about 10% of patients with encephalopathy have normal arterial levels (174). Of course, it is likely that blood ammonia does not reflect brain ammonia concentration. Mercaptans, which are derived from bacterial metabolism of methionine, can produce coma in animals and have synergistic properties when administered with ammonia and fatty acids (188). Changes in the blood concentration of methanethiol (methyl mercap-

tan) were found to correlate with changes in the severity of encephalopathy in man (106).

Amino Acid Imbalance

A number of recent studies have suggested that changes in plasma and, hence, brain amino acids may be important in the pathogenesis of hepatic encephalopathy by their production of changes in central neurotransmitters (28, 31, 187). Fischer et al found a correlation between the degree of hepatic encephalopathy and the molar ratio of the branched chain amino acids, valine, leucine, and isoleucine, to the aromatic amino acids, phenylalanine and tyrosine, in dogs (33) and man (34). The normal value for the ratio is approximately 3.0, and in severe hepatic encephalopathy the value drops to 1.0. In other studies, an excellent correlation was found between elevation of plasma free tyrptophan, or the ratio between free tryptophan to branched chain amino acids, and the grade and evolution of hepatic encephalopathy (15, 98). These amino acids compete for entry across the blood-brain barrier (128); hence, a decrease in the plasma concentrations of branched chain amino acids would result in an increased entry and brain concentration of the aromatic amino acids, which are the precursors of central neurotransmitters. Indeed an increased transport of the neutral amino acids tryptophan, phenylalanine, tyrosine, and leucine from plasma into brain was demonstrated in rats after portacaval anastomosis (60). Elevated ammonia levels may contribute to the entry of these amino acids into the brain due to the increased formation of glutamine in the brain. The increased efflux of glutamine from the brain stimulates influx of other neutral amino acids by a mechanism of exchange using the neutral amino acid carrier system (61). Decreases in the brain concentration of the normal neurotransmitter norepinephrine (23) and increases in the false neurotransmitters (β-hydroxylated phenylethylamines and octopamine) were demonstrated in experimental hepatic encephalopathy (32). In patients, elevations of serum phenylethanolamine (14) and serum octopamine (96) were found to correlate with the degree of hepatic encephalopathy. The hypothesis has been challenged, however, by the observation that the intraventricular infusion of octopamine, resulting in very high brain concentrations of octopamine and a reduction in brain norepinephrine and dopamine, failed to result in hepatic encephalopathy in rats (189). Of course, dopamine may not act alone and may not be the principal false neurotransmitter responsible for hepatic encephalopathy. Also in one study a significant decrease in the molar ratio of valine, leucine, and isoleucine to phenylalanine and tyrosine was found in severe liver disease irrespective of the presence or absence of encephalopathy (121).

Therapy of Hepatic Encephalopathy

Present therapy of hepatic encephalopathy results in the development of a negative nitrogen balance because it is largely based on decreasing the production of ammonia and other nitrogenous breakdown products in the intestine by limiting protein intake. Additional measures that decrease ammonia production are the administration either of antibiotics such as neomycin, which decreases the flora of ammonia-producing organisms, or of lactulose, which traps nitrogen and increases its fecal loss (184). Efforts have been made recently to treat hepatic encephalopathy while maintaining an adequate nitrogen balance. This has been attempted by the administration of special mixtures of amino acids to normalize plasma amino acids or by the administraiton of keto analogs of amino acids to offset both hyperammonemia and protein deficiency. Parenteral administration of mixtures of amino acids, high in branched chain but low in aromatic amino acids, has resulted in normalization of plasma amino acids and improvement in hepatic encephalopathy in some patients (34). In rats studied after portacaval shunt, administration of the same mixture of amino acids, resulting in normalization of most of the plasma and brain amino acids, was shown to be associated with a positive nitrogen balance (144). Improvement of hepatic encephalopathy in man has also been reported after the intravenous infusion of the single branched chain amino acid valine (70) or leucine (27). Administration of the keto analogs of the essential amino acids, valine, leucine, isoleucine, methionine, and phenylalanine, resulted in an increase in the plasma concentrations of the amino acids corresponding to the infused analogs, and an increase to normal of the ratio of essential to nonessential plasma amino acids. There was a delayed but only slight decrease in blood ammonia. Clinical improvement as assessed by mental and psychological studies was obtained in 8 of the 11 patients studied (92). More recently the administration of ornithine salts of branched chain ketoacids by nasogastric tube was found to be more effective than that of branched chain amino acids in improving encephalopathy (47). In both cases there was an improvement in nitrogen balance equal to the nitrogen content of the medication. The administration of ornithine α-ketoglutarate was not effective in improving the encephalopathy. L-Dopa has been used in the therapy of hepatic encephalopathy with the idea that it may replenish the normal neurotransmitters dopamine and norepinephrine and displace the false neurotransmitters that accumulate. Although a few patients have awakened from hepatic coma after the administration of L-Dopa, no efficacy was demonstrated in the one controlled trial that has been done (116). In chronic hepatic encephalopathy a change from animal-protein to a vege-

table-protein diet has been found to result in improvement of the encephalopathy in association with decreased arterial ammonia levels (41). The exact mechanism whereby vegetable-protein is better tolerated than animal-protein is unknown. However, vegetable-protein contains smaller amounts of ammonia, methionine, and aromatic amino acids and also results in alterations of small intestinal and colonic bacteria flora. The beneficial effects of a vegetable-protein and lactulose on hepatic encephalopathy were additive.

PROTEIN SYNTHESIS

Proteins synthesized by the liver are frequently decreased in patients with liver disease. This is manifested clinically by decreases in circulating proteins such as albumin, clotting factors, ceruloplasmin, transferrin, and retinol-binding protein.

Albumin

Albumin is normally the most abundant of the serum proteins synthesized in the liver. The synthesis of albumin is normal in most cases of viral hepatitis and only decreased in severe cases (105). In patients with cirrhosis, hypoalbuminemia, and ascites, albumin synthesis although depressed in some cases is more often normal or elevated (147). The exchangeable pool of albumin is often normal, or greater than normal, owing to expansion of the plasma volume and leakage of albumin into the ascitic fluid, whereas the catabolic rate of albumin is frequently decreased (10, 43, 147). A very important factor affecting albumin synthesis in liver disease is nutrition. Protein deficient diets can result in decreases in albumin synthesis and albumin levels of more than 50%, and this effect is readily reversed by the administration of either protein or amino acids (69, 165, 183). Both an adequate supply of total amino acids and an availability of individual amino acids are important for albumin synthesis. Tryptophan, which is the least abundant amino acid in the hepatic intracellular amino acid pool, is often rate-limiting in albumin synthesis (146, 164). Animals fed tryptophan-deficient amino acid diets have disaggregation of polysomes and depressed hepatic protein synthesis, which reverts to normal following tryptophan administration (146, 164). Acute exposure of the liver to ethanol either by the oral route or in the perfused liver inhibits albumin synthesis, which, however, can be prevented by the simultaneous addition of a mixture of amino acids (148). Chronic ethanol administration, on the other hand, was reported to increase the synthesis of albumin but inhibit its export into the circulation (5). The decrease in the export of albumin coincided with decreases in polymerized tubulin and the number of visible microtubules

(137). These decreases were also demonstrated in vitro by incubation of hepatocytes with either ethanol (50 mM) or acetaldehyde (mean concentration of 234 μM). Acetaldehyde may be responsible for the observed effects since pyrazole, an inhibitor of alcohol dehydrogenase, blocked the effect of ethanol on microtubules in isolated hepatocytes (104). In addition disulfiram, an inhibitor of aldehyde dehydrogenase, exaggerated the in vivo effect of ethanol on either microtubules or polymerized tubulin (6), and acetaldehyde has been shown to compete with colchicine binding to liver tubulin (37). In another study, however, no change in albumin synthesis was found after chronic administration of ethanol to rabbits (145). Also, ethanol in vitro in a concentration of 50 mM had no effect on the polymerization of purified bovine neurotubulin, while acetaldehyde produced only slight inhibition at a concentration of 1 mM, which is much higher than micromolar concentrations found after ethanol ingestion (63). Furthermore, ethanol in concentrations of 50 and 100 mM did not inhibit the release of prelabelled total export proteins and albumin from isolated hepatocytes (122).

Increases in the gastrointestinal loss of albumin, mentioned in a previous section, can also contribute to hypoalbuminemia in some patients with cirrhosis (32).

Clotting Factors

The clotting factors most likely to be decreased in parenchymal liver disease are factors II, VII, IX, and X. Fibrinogen and factor V are reduced only in severe liver disease. In one study, 85% of patients with liver disease had at least one abnormal clotting test and 15% had abnormal bleeding (25). Decreases in clotting factors can be caused by decreased synthesis or increased utilization. Decreased synthesis is the principal cause of decreases in clotting factors in liver disease. Vitamin K controls the synthesis of prothrombin (factor II) and also of factors VII, IX, and X. Deficiency of this vitamin, owing to decreased intake or to decreased absorption, is a cause for the decrease of the above clotting factors, with resultant prolongations of the one-stage prothrombin time (affected by factors II, V, VII, and X) and of the partial thromboplastin time (affected by factors II, V, VIII, IX, and X). Vitamin K deficiency is readily corrected by the parenteral administration of aqueous preparations of vitamin K. The administration of 15 mg of Aquamephyton® will result in a return of vitamin K-dependent clotting factors (assessed by the determination of the prothrombin time) to normal within 48 hr of its administration in patients with vitamin K deficiency. However, patients with severe acute liver disease or advanced chronic liver disease there often is a parenchymal defect in the synthesis of clotting factors not correctable with vitamin K. In these cases, measurements of the prothrombin time after vitamin K repletion are useful clini-

cally in the assessment of the severity and prognosis of the liver disease. Clotting factors may also be decreased because of increased utilization due to disseminated intravascular coagulation and excessive fibrinolysis, which occur on occasion in various types of liver disease (139).

VITAMIN DEFICIENCIES AND PROTEIN METABOLISM

Vitamin deficiencies that are found commonly in patients with liver disease contribute to abnormalities of protein metabolism and cell replication. Deficient dietary intake is the principal cause of the vitamin deficiencies. In addition malabsorption, decreased storage, defects in their metabolism to their active forms, and increased requirements of some of the vitamins after liver injury also contribute significantly to vitamin deficiencies in liver disease.

Thiamine

Red blood cell transketolase activity is dependent on thiamine pyrophosphate and, therefore, subject to the ability of the liver to phosphorylate thiamine. Administration of thiamine to thiamine-deficient alcoholic patients with cirrhosis and peripheral neuropathy resulted in an increase in blood thiamine levels, but no significant change in red blood cell transketolase activity and no effect on peripheral neuropathy (30). In vitro addition of thiamine pyrophosphate to red cell hemolysates resulted in increases in enzyme activity of the hemolysates from thiamine deficient patients with liver disease, but not in those with cirrhosis. These studies suggest that alcoholic patients without liver disease have a defect in the conversion of thiamine to thiamine pyrophosphate (the active form of the vitamin). Patients with liver disease in addition may have poor utilization of the active form of the vitamin. The Wernicke-Korsakoff syndrome probably occurs only in individuals who have a genetically determined abnormal transketolase enzyme with a low affinity for its coenzyme thiamine pyrophosphate (11).

Folic Acid

The metabolic conversion of absorbed folic acid (pteroylglutamic acid) to 5-methyltetrahydrofolic acid, which is the principal circulating form of folate, occurs in the liver. 5-methyltetrahydrofolic acid is also the folate coenzyme that serves as a donor of methyl units for the conversion of deoxyuridylate to methyl deoxyuridylate (thymidylate), which is necessary for the synthesis of DNA. Folate is stored in the liver predominantly as reduced polyglutamate forms (163). Ethanol has been shown to suppress

the hematological response of anemic, folate-deficient patients to folic acid (172). Also, the acute administration of ethanol results in a fall in serum folate levels in alcoholic patients and normal subjects, suggesting that ethanol interferes with the formation or release of 5-methyltetrahydrofolic acid (131). Recent studies in the rat show that ethanol inhibits folate excretion into the bile by shunting pteroylglutamic acid, returning via the enterohepatic circulation to the liver for reduction and methylation, into a hepatic pentaglutamate storage pool. The resulting decrease in folate in the enterohepatic cycle probably explains the fall in serum folate after the acute administration of ethanol (49). In one study, the induction of moderate alcoholic hepatitis with alcohol despite a normal diet was associated with a low serum folate level and decreased in vitro hepatic DNA synthesis, which could be corrected to normal by the administration of extra folate despite continuation of alcohol intake (77, 80).

Vitamin B_6

The biologically active form of vitamin B_6 compounds is the coenzyme pyridoxal-5'-phosphate (PLP). The liver is the principal site of PLP formation (88). Decreases in plasma PLP have been demonstrated in alcoholic patients with (73, 119) and without liver dysfunction (89). The decreases in PLP are not due to a decrease in its formation but rather the result of an acceleration of its degradation. Acetaldehyde acts by displacing PLP from its binding protein, thereby making it susceptible to hydrolysis by membrane-bound alkaline phosphatase (89). In patients with cirrhosis an increased clearance of PLP after its intravenous administration and an increased excretion of 4-pyridoxic acid after the oral administration of either pyridoxine or PLP (119) also suggests that increased degradation of PLP is the principal cause of low plasma PLP in these patients. However, the mechanism for the increased degradation of PLP in cirrhosis remains unknown. The aminotransferases require PLP as a cofactor. Pyridoxine deficiency in rats results in a greater decrease in the liver and serum glutamate pyruvic transaminase (SGPT) than in the liver and serum glutamate oxalacetic transaminase (SGOT) (87). Also, lower levels of GPT were found in patients with alcoholic liver disease as compared with normal individuals and those with viral hepatitis (103), suggesting that low levels of SGPT in alcoholic hepatitis are due to decreased hepatic concentration available for release into the circulation.

Vitamin A

Vitamin A is transported in the plasma by a retinol binding-prealbumin complex. In patients with acute and chronic liver disease, the complex is decreased in association with decreases in plasma vitamin A. These studies

suggest that the decrease in plasma vitamin A in patients with liver disease may be attributable in part to a decrease in its release from the liver because of decreased synthesis of the retinol-binding protein and prealbumin necessary for its transport (166). This is supported by the finding of an increased percentage of unbound retinyl esters to total vitamin A in the fasting serum of cirrhotics with abnormal dark adaptation (153). In a few cirrhotic patients with low serum vitamin A and low zinc concentrations, and impaired dark adaptation with poor or no response to vitamin A administration, the administration of zinc sulfate resulted in improved or normal dark adaptation (124). Decreased plasma retinol-binding protein (167) or decreased retinal alcohol dehydrogenase activity (53), both of which have been demonstrated in zinc-deficient rats, may account for the association between zinc deficiency and impaired dark adaptation.

Vitamin D

Vitamin D is metabolized to 25-hydroxyvitamin D in the liver, and this metabolite is converted in the kidney to 1, 25-hydroxyvitamin D, which is the most active form of vitamin D. An increased incidence of osteoporosis has been described in chronic liver disease in association with low serum 25-hydroxyvitamin D levels (19, 46, 179). Therapy of patients with large doses of vitamin D corrects serum 25-hydrovitamin D levels to normal, but fails to prevent the progression of the osteoporosis, suggesting that factors other than the hepatic conversion of vitamin D to 25-hydroxyvitamin D plays a role in the development of bone disease (86). Ethanol administration in chickens decreased renal 25-hydroxyvitamin D-1-α-hydroxylase, which catalyzes the formation of 1,25-hydroxyvitamin D, while it increased the activity of 25-hydroxyvitamin D-24-hydroxylase, which catalyzes the formation of 24,25-hydroxyvitamin D, probably an inactive metabolite (67).

HEPATIC REGENERATION

An increased requirement for protein and vitamins for use in tissue regeneration occurs after liver injury. In addition, alcohol increases nutritional requirements and has adverse effects on hepatic regeneration. Partial hepatectomy has been the principal model for the study of mechanisms of hepatic regeneration and the factors that influence it.

Liver mass is restored with extraordinary rapidity following partial hepatectomy in experimental animals and in man. In the rat following partial hepatectomy, DNA synthesis increases after a lag of 12 hr, reaching a maximum at 20 hr followed 6–8 hr later by mitosis (13). An increase in protein synthesis is detectable at about 12 hr and reaches a peak at 36 hr. The increase in protein synthesis is accompanied by an increase in free

amino acid pools, which in the case of some amino acids such as lysine appears to be due to a decrease in their catabolism (48). The residual lobe after partial hepatectomy in rats doubles in size in two days and the liver is almost back to normal weight in seven days (13). Administration of a protein-free diet results in a delay in DNA synthesis and protein accumulation in partially hepatectomized rats, which is readily eliminated by the administration of casein hydrolysate, but not by glucose or incomplete amino acid mixtures (108). The early increase in hepatic protein after partial hepatectomy is more the result of a cessation of protein breakdown than due to an increase in protein synthesis, and the decrease in degradation is less in animals protein-depleted prior to hepatectomy (3). The administration of ammonia in the form of ammonium acetate inhibited the incorporation of ^{3}H-thymidine into hepatic DNA after partial hepatectomy (26). In carbon tetrachloride-induced hepatic injury there are decreases in liver folate during the first 24 hr owing to release of folate in the serum, followed by further decreases during maximum regeneration 48 hr after the administration of carbon tetrachloride. This is accompanied by a reduction in DNA synthesis, which is corrected by folate administration (78). In rats with thiocetamide-induced cirrhosis the incorporation of ^{3}H-thymidine into hepatic DNA was only slightly delayed as compared to controls, and the final level of incorporation was similar (170).

Acute and chronic ethanol administration were found to depress ^{3}H-thymidine incorporation into DNA, mitotic activity, and protein synthesis of the regenerating rat liver after partial hepatectomy (35, 181). Yet despite these effects, ethanol had no effect on total DNA and the restoration of liver mass (35, 129), suggesting that ethanol does not influence the overall ability of the liver to regenerate. Total hepatic protein was increased in ethanol-fed animals before and after hepatectomy as compared with controls (129). Increases in enzymes of short half-life such as ornithine decarboxylase and tyrosine aminotransferase (137), which occur in the regenerating liver after partial hepatectomy, were more pronounced in animals ingesting ethanol. The increase in these enzymes appeared to be caused by a decrease in their degradation. Accumulation of these enzymes and other proteins as a result of decreased degradation may contribute to the observed increase in total hepatic protein caused by ethanol in normal and regenerating liver.

In man a 65–90% hepatectomy for tumor or abscess results in a fall in serum albumin and clotting factors during the first week following operation to dangerously low levels (107, 130). The development of both of these deficiencies needs to be anticipated and prevented by the administration of parenteral albumin and plasma during the immediate post-operative period (107). Both liver function and mass return to normal in six weeks to six months following partial hepatic resection (107, 130).

The treatment of alcoholic liver disease has consisted of abstinence from alcohol, bed rest, and intake of a diet of normal or high protein content, provided there is no evidence of encephalopathy. Fatty liver and alcoholic hepatitis produced by chronic administration of ethanol while on a normal diet is associated with low serum levels of folate, thiamine, riboflavin, vitamin B_6, and nicotinic acid (81). About 20% of the patients with alcoholic hepatitis have negligible in vitro hepatic DNA synthesis, which is restored to normal by administration of vitamins found deficient (76, 78). In a recent study, administration of 70–80 g of parenteral amino acids a day of either 7% Aminosyn® or 8.5% Travasol® for four weeks in patients with alcoholic hepatitis resulted in greater clinical and laboratory improvement and less mortality than found in a control group (125). The administration of prednisolone was shown to increase survival in a subgroup of severely ill patients manifested by encephalopathy in one study (45), and this effect was associated with an increased caloric intake, suggesting that this was a factor in the decreased mortality. However, in another study by the same group prednisolone was again demonstrated to increase survivial in patients with alcoholic hepatitis and encephalopathy as compared to those receiving oral or intravenous nutrient supplements of at least 1600 calories a day, leading to the conclusion that the effect of prednisolone was not related to total caloric intake (82). The administration of prednisolone results in a more prompt decrease in serum bilirubin and prothrombin time, and increases serum albumin (91). The effect of corticosteroids in decreasing mortality in severely ill patients with alcoholic hepatitis was confirmed by another group of investigators (91); however, it was found of no benefit in other studies in which all patients with alcoholic hepatitis were grouped together regardless of the severity of the disease (112).

SUMMARY

Protein deficiency is often associated with liver disease. The principal cause of protein deficiency is decreased dietary intake. Deficiencies in digestion and absorption that are common in alcoholics contribute to protein deficiency in alcoholic liver disease. The protein requirements in most patients with compensated chronic liver disease are not different from normal, but increase during episodes of hepatocellular deterioration. An increased demand for protein after liver injury drains nitrogen from other organs such as muscle. Aromatic amino acids released from muscle in increased amounts accumulate in the circulation of patients with chronic liver disease because of their decreased hepatic metabolism. By contrast branched chain amino acids decrease in the circulation because of their preferential uptake by extrahepatic tissues. Decreases in urea synthesis in liver disease result

in the accumulation of ammonia. The causes of the decrease in urea synthesis include decreases in the enzymes and substrates of the urea cycle, alterations in portal blood flow, and a decrease in total hepatic mass. The resulting increase in ammonia in association with an increased accumulation and entry of aromatic amino acids into the brain are important factors in the pathogenesis of hepatic encephalopathy. Circulating proteins synthetized by the liver, such as albumin and clotting factors, are frequently decreased in chronic liver disease. Vitamin deficiencies that are common in liver disease contribute to abnormalities of protein metabolsim. Hepatic regeneration following hepatic resection or injury is adversely affected by protein and vitamin deficiencies and by alcohol ingestion.

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