Pharmacology Biochemistry & Behavior, Vol. 13, Suppl. 1, pp. 17–30. Printed in the U.S.A.

Alcohol, Liver Injury and Protein Metabolism[1,2]

C. S. LIEBER

Alcohol Research and Treatment Center
Bronx Veterans Administration Medical Center and
The Mount Sinai School of Medicine (CUNY), New York, NY

LIEBER, C. S. *Alcohol, liver injury and protein metabolism.* PHARMAC. BIOCHEM. BEHAV. 13: Suppl. 1, 17–30, 1980.—Two mechanisms play a major role in mediating the effects of ethanol on intermediary metabolism which includes protein metabolism and liver injury. Ethanol is oxidized in the liver to 2 products (hydrogen and acetaldehyde) to which many of the effects of ethanol can be attributed. The hydrogen generation alters the redox state, and although this effect is attenuated after chronic ethanol consumption, it still may be sufficient to explain alterations in lipid and carbohydrate metabolism, possibly increased collagen deposition, and, under special circumstances, depression of the synthesis of protein whereas secretion of proteins is affected by acetaldehyde. It is noteworthy that after chronic consumption of ethanol, acetaldehyde is increased, in part, because of its decreased oxidation in the mitochondria, and, partly because of induction of an alternate pathway of ethanol metabolism, namely the microsomal ethanol oxidizing system. Increased acetaldehyde is associated with impaired function of microtubles, protein retention and swelling of the hepatocyte. Acetaldehyde also exerts toxicity with regards to other key cellular sites; it interferes with oxidations in the mitochondria, and it may promote peroxidation of cellular membranes. Hepatocellular injury is followed by extracellular collagen deposition, but what eventually attracts an increased number of myofibroblasts and promotes fibrosis is not known. Alcoholic hepatitis, when present, can obviously trigger fibrosis, but the cirrhotic process occurs even in the absence of florid alcoholic hepatitis. Cirrhosis can develop despite an associated adequate diet. However, decreased intake of dietary protein or decreased absorption of amino acids derived from dietary protein may contribute to the depletion of amino acids in the liver which, in turn, may potentiate the toxicity of alcohol. Selective deficiencies, such as those of methionine or vitamin A, may also play a contributory role.

Liver injury Protein metabolism Acetaldehyde Hydrogen Alcohol

ROLE OF DIETARY PROTEIN IN THE PATHOGENESIS OF LIVER INJURY

Until recently, the interrelationship between alcohol abuse, proteins and liver injury appeared relatively simple: protein deficiency was recognized as a complication of alcohol abuse and protein malnutrition was considered the sole culprit of liver injury in the alcoholic.

This exclusively nutritional theory was plausible since alcoholics do commonly suffer from malnutrition [66]. Chronic alcohol consumption does indeed interfere with normal food digestion and absorption because of its well known effects on gut and pancreas. Furthermore, alcohol, high in caloric value, displaces other foods in the diet. Each gram of ethanol provides 7.1 calories. Twelve ounces of an 86-proof beverage contains about 1200 calories or approximately one-half of the recommended daily dietary allowance for calories. But, unlike regular food, alcoholic beverages contain few, if any, vitamins, minerals, protein, or other nutrients and, therefore, the alcoholic's intake of these nutrients may readily become insufficient. Economic factors may also reduce consumption of nutrient-rich food, particularly those containing proteins.

The concept that alcoholic liver injury was exclusively of nutritional origin was also based on experimental studies. In rats, it was shown that alcohol given in the drinking water was not capable of producing liver damage unless associated with a diet deficient in essential nutrients, and it was concluded that "there is no evidence of a specific toxic effect of pure ethyl alcohol upon liver cells than there is for one due to sugar" [9]. However, rats display a natural aversion for ethanol and under the experimental conditions used, intake is relatively small and blood ethanol levels negligible. When the alcohol aversion was counteracted by incorporation of ethanol in a totally liquid diet, and the ethanol consumption was thereby increased to 36% of total calories, fatty liver resulted despite otherwise nutritious diets [81, 87, 91]. The question was then raised whether lesions more severe than steatosis, particularly cirrhosis, can be produced by alcohol in the absence of dietary deficiencies. Studies in rodents had

[1]Delivered, in part, as a Plenary Lecture at the Biennial International Symposium on Alcoholism, Cardiff, Wales, June 9, 1980.

[2]Some of the original studies summarized here were supported, in part, by the Medical Research Service of the Veterans Administration and grants from NIAAA and NIAMDD.

been unsatisfactory because even when given with a liquid diet, the intake of alcohol in rodents does not reach the level of average consumption in the alcoholic, namely 50% of total calories [116]. Such a level of consumption was achieved in the baboon, again through incorporation of ethanol in totally liquid diets [83]. The calories (exclusive of alcohol) were provided by protein (36% of total nonalcohol calories), fat (42%) and carbohydrate (22%). This diet was also liberally supplemented with minerals and vitamins. All of the nutrients were calculated to exceed the normal requirements of the baboon [109], with choline being given at twice the level recommended by Foy *et al.* [38]. The fat and carbohydrate composition of the baboon diet was calculated to mimic an optimal clinical situation in which an alcoholic may be trying to achieve a high protein diet with available natural foods while drinking. In fact, even if the alcoholic tried hard it would be difficult for him to consume a diet richer in protein than the one administered to the baboons (36% of nonalcohol calories). Nevertheless, the baboons developed not only fatty liver, but after 2–5 years, one-third also had progression of liver damage to cirrhosis [123]. It was, therefore, concluded that in addition to dietary factors, alcohol itself plays a key etiological role in the development of liver injury.

If one can extrapolate from these baboon data to the human situation, it would seem that over the long haul heavy drinkers, even if they make an extra effort to maintain a nutritionally adequate high protein diet, will not necessarily succeed in preventing the development of cirrhosis. Many questions, however, still remain unresolved, particularly the extent to which malnutrition promotes the toxic effect of alcohol. In primates, although some fibrosis was produced with protein deficiency alone, no cirrhosis ever developed, except possibly in one animal [115]. For cirrhosis to develop in primates, dietary conditions were required which are not normally achievable with natural foods (such as an association of low protein and a high cholesterol content) [115,151]. In some patients subjected to intestinal bypass for obesity, lesions similar to those present in some alcoholics, namely hepatitis and fibrosis, have occurred [119] and it has been concluded that in both situations, a similar pathogenesis, namely malnutrition, may pertain. Such reasoning "by analogy", however, is hazardous, since pathogenesis of the liver lesion after bypass is still unsettled. Even if malnutrition were shown to be the culprit in the latter situation, extrapolation to the alcoholic is not necessarily warranted. Furthermore, the same lesion has been described after massive small bowel resection despite maintenance of adequate parenteral nutrition [18]. Thus whether an "across the board" nutritional deficiency may potentiate the alcohol induced liver damage in the primate has not been as yet determined. Selective deficiencies however could play a contributory role. For instance, it had been known for a long time that methionine deficiency may potentiate and methionine supplementation may partially alleviate alcohol induced fatty liver [9,78]. Originally, this was attributed to choline sparing properties of methionine, but, as discussed subsequently, a more likely mechanism is the depletion of glutathione. Similarly, judicious correction of vitamin A deficiency may affect the potentiation of alcohol induced liver damage by lack of vitamin A [73].

At present, the optimal diet for the alcoholic is not established. Obviously, specific nutritional deficiencies, such as lack of thiamine and folate, should be avoided and, when present, corrected. Concerning proteins, however, the situation is more complex. In rats, carbon tetrachloride induced cirrhosis can be prevented by a low protein diet [10]. The situation in alcoholic liver injury has not been clarified. Early studies relating beneficial effects of high protein diets [114] were uncontrolled. Subsequently, the risk of dietary induced encephalopathy in cirrhotic patients has become more apparent. Therefore, in the absence of experimental data to the contrary, high protein diets do not seem indicated at the present time. Until this issue is resolved it may be prudent to settle for an intake of proteins that does not exceed the recommended amount [110], or the individual protein tolerance of the cirrhotic, whichever is lower.

The demonstration of the hepatotoxicity of ethanol raises the question of the mechanisms of this action. The large caloric load that ethanol represents in the alcoholic has been alluded to before. Furthermore, only 2 to 10 percent of the ethanol absorbed is eliminated through the kidneys and lungs. The rest must be oxidized in the body, principally in the liver, which contains the bulk of the body's enzymes capable of ethanol oxidation. This relative organ specificity probably explains why ethanol oxidation produces striking metabolic imbalances in the liver. These effects are aggravated by the lack of feedback mechanism to adjust the rate of ethanol oxidation to the metabolic state of the hepatocyte, and the inability of ethanol, unlike other major sources of calories, to be stored or metabolized to a significant degree in peripheral tissues. When ethanol is present, it becomes the preferred fuel for the liver. By displacing up to 90% of all other substrates normally utilized by the liver [96], ethanol literally takes over the intermediary metabolism of the liver. The main pathway for ethanol metabolism in the liver proceeds via alcohol dehydrogenase (Fig. 1). Ethanol loses hydrogen and is oxidized to acetaldehyde and, as discussed subsequently, each of these two products is directly responsible for a variety of metabolic alterations, including abnormalities of protein metabolism and the development of liver injury.

EFFECTS OF EXCESSIVE HEPATIC NADH GENERATION

As shown in Fig. 1, the oxidation of ethanol results in the transfer of hydrogen to NAD. At blood levels commonly achieved by drinking, hydrogen generation exceeds the capacity of the liver for hydrogen disposition. The resulting altered redox state (characterized by an enhanced NADH-NAD ratio), in turn, produces a change in the flux of those substrates that are dependent on the NADH-NAD couple for reduction. It was therefore proposed that the altered NADH/NAD ratio is responsible for a number of metabolic abnormalities associated with alcohol abuse [77].

ALTERATIONS IN INTERMEDIARY METABOLISM, LIPIDS AND CARBOHYDRATE

The enhanced NADH/NAD ratio is reflected by an increased lactate/pyruvate ratio that results in hyperlactacidemia [90,92] because of both decreased utilization and enhanced production of lactate by the liver. The hyperlactacidemia contributes to acidosis and also reduces the capacity of the kidney to excrete uric acid, leading to secondary hyperuricemia [90]. Alcohol induced ketosis may also promote the hyperuricemia. The latter may be related to the common clinical observation that excessive consumption of alcoholic beverages frequently aggravates or precipitates gouty attacks. Furthermore, the altered redox state may also

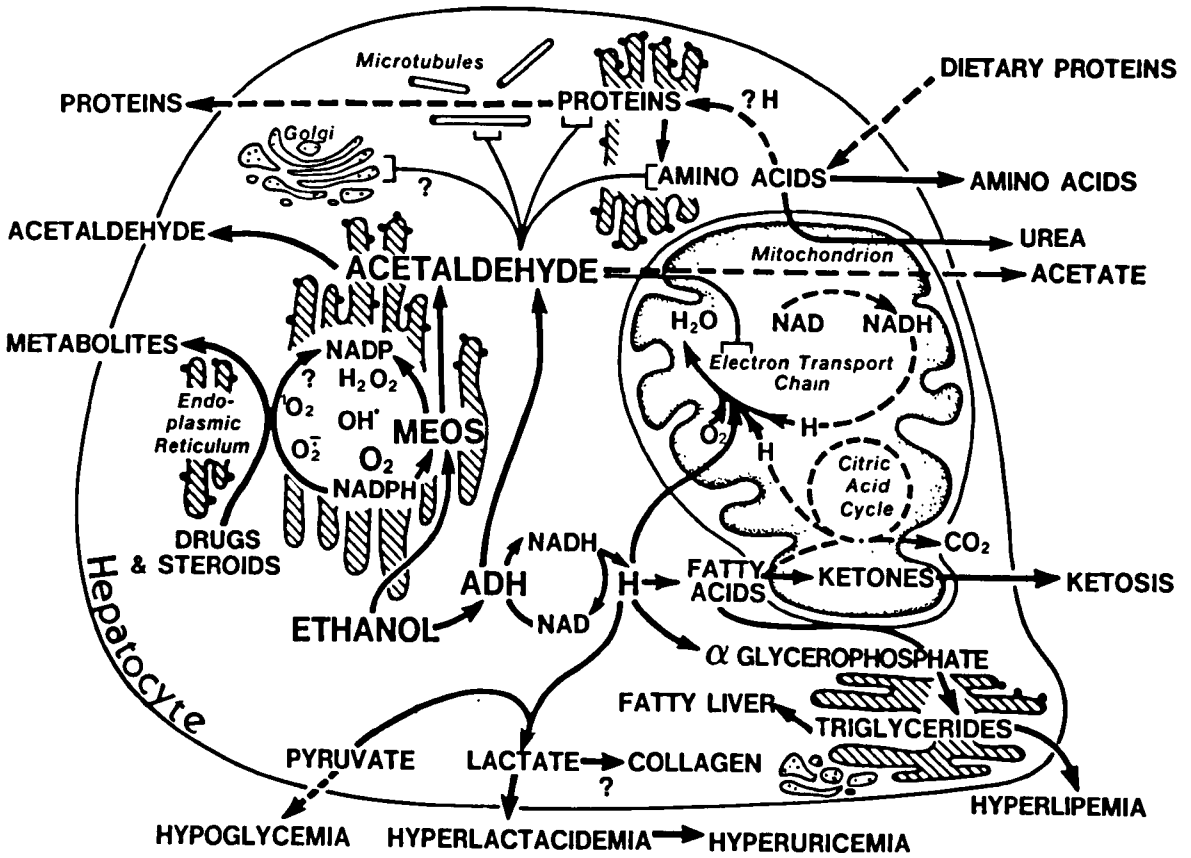

FIG. 1. Oxidation of ethanol in the hepatocyte and link of the two metabolites (acetaldehyde and H^+) to disturbances in intermediary metabolism, including abnormalities of amino acid and protein metabolism. NAD denotes nicotinamide adenine dinucleotide, NADH reduced NAD; NADP nicotinamide adenine dinucleotide phosphate, NADPH reduced NADP, MEOS the microsomal ethanol oxidizing system, and ADH alcohol dehydrogenase. The broken lines indicate pathways that are depressed by ethanol. The symbol -[denotes interference or binding by the metabolite. (From Lieber [76])

contribute to the hypoglycemia through impairment of gluconeogenesis from amino acids [40]. The increased NADH/NAD ratio also raises the concentration of α-glycerophosphate [111] that favors hepatic triglyceride accumulation by trapping fatty acids [59]. Some reducing equivalents can be transferred into the mitochondria by various "shuttle" mechanisms. However, the activity of the citric acid cycle is depressed, as discussed elsewhere [84]. The mitochondria will therefore use the hydrogen equivalents originating from ethanol, rather than from oxidation through the citric cycle of two carbon fragments derived from fatty acids. Thus, fatty acids that normally serve as the main energy source for the liver are supplanted by ethanol. Decreased fatty acid oxidation by ethanol has been demonstrated in liver slices [85], perfused livers [93], isolated hepatocytes [112], human liver biopsy tissue [36] and *in vivo* [11]. This results in the deposition in the liver of dietary fat, when available, or fatty acids derived from endogenous synthesis in the absence of dietary fat [86, 88, 89, 101], and can be considered a major cause for the development of alcoholic fatty liver, the first stage of alcoholic liver injury.

Once a fatty liver has developed, however, fat accumulation does not increase indefinitely, even though alcohol consumption may be continued [81]. If the ethanol induced redox change plays a major role in fat accumulation in the liver, one would expect that the redox alteration becomes attenuated at the time the fat accumulation stabilizes. This was, indeed, found to be the case: in rats fed alcohol chronically, the redox change produced by an acute dose of ethanol in the liver was attenuated after chronic ethanol administration [26]. Similarly, in baboons fed alcohol chronically, the redox changes induced by alcohol (as measured by inhibition of galactose metabolism) progressively decreased after chronic ethanol consumption [133]. In the latter experimental model, chronic alcohol consumption is associated with the progression of alcoholic liver injury beyond the fatty liver stage [123]. It is apparent, therefore, that although the alcohol induced redox change may play an important role in the initial development of the alcoholic fatty liver, progression beyond the fatty liver stage must be attributed, at least in part, to mechanisms other than the redox alteration, which will be discussed subsequently.

ALTERATIONS IN PROTEIN SYNTHESIS

We now realize that in addition to changes in carbohydrate and lipids, the abnormal redox state may also affect protein metabolism.

Effects in vitro

Acute changes. There is a widely held belief that acute ethanol administration inhibits the synthesis of both constituent and export proteins of the liver. This opinion is supported mainly by experimental evidence that has been obtained after addition of ethanol to various *in vitro* preparations, such as perfused livers [128], liver slices [118], isolated hepatocytes [57], microsomes [126], mitochondria [130] and ribosomes [69]. Some of these effects may be explained by the fact that various *in vitro* preparations become deficient in substrates for the hydrogen-translocation shuttles during preparation [19], particularly if obtained from fasted animals [97]. Substrates may be diverted away from protein synthesis either because of "leakiness" of the *in vitro* preparations, or because, as suggested by Perin and Sessa [117], the excess of reducing equivalents generated by ethanol in the cytosol may promote utilization of pyruvate and amino acids for the operation of the shuttles needed to transport the reducing equivalents into the mitochondria. These interpretations are consistent with the aggravation of the inhibitory effects of ethanol by fasting [128], the prevention with massive supplementation of amino acids [58,128], pyruvate [117] or other substrates for the hydrogen-translocation shuttles, especially those of the malic-aspartic acid shuttle [7,117].

Prevention of the inhibition of protein synthesis was also achieved by reducing the shift of cytosolic redox state with an acceptor of reducing equivalents (methylene blue) [7]. In regard to all these experiments, it must be pointed out that some degree of anoxia is almost unavoidable in *in vitro* preparations, and anoxia has been shown to enhance the toxic effects of ethanol on protein synthesis [57]. In addition, the redox changes produced by ethanol *in vitro* are much greater than *in vivo* [7]: the addition of ethanol *in vitro* increased the lactate/pyruvate ratio 13-fold, whereas the administration of ethanol *in vivo* increased this ratio only 2 times [26]. Moreover, the change of the ratio *in vitro* was essentially due to a marked decrease in the concentration of pyruvate, while a more moderate decrease in pyruvate with a corresponding increase in lactate is observed *in vivo*. These abnormalities in the lactate/pyruvate ratio have been also found in perfused livers [128] in association with ethanol-induced inhibition of protein synthesis. Clearly, the *in vitro* observations referred to above do not pertain to the conditions prevailing *in vivo* in the well-nourished rat, in which similar ethanol concentrations do not inhibit protein synthesis (vide infra). Actually, it would be of great interest to identify *in vivo* conditions that mimic the alterations of ethanol metabolism occurring in isolated liver preparations and that induce inhibitory effects of ethanol on protein synthesis. Indeed, under such conditions, the toxicity of ethanol should be greatly enhanced.

Effects of chronic ethanol consumption. Whatever their mechanism, the acute effects of ethanol on hepatic protein synthesis in naive rats may not pertain to the conditions prevailing in alcoholics or in animals in which metabolic adaptations have developed in response to prolonged alcohol exposure. Indeed, ethanol failed to inhibit amino acid incorporation into cell protein when hepatocytes were obtained from rats fed alcohol-containing diets for several weeks [7]. These hepatocytes also displayed small changes of the lactate/pyruvate ratio in the presence of ethanol. The decreased effect of ethanol in these hepatocytes compared to those in naive pair-fed controls could be due to attenuation of the redox change following chronic alcohol consumption, as reported *in vivo* in rats [26], and in baboons [132].

In summary, in naive animals ethanol inhibits protein synthesis *in vitro*, in part because of the redox shift and/or pyruvate depletion. The latter changes, however, are much more striking than those observed *in vivo*. After chronic ethanol feeding, when the redox change becomes attenuated, the *in vitro* alterations in protein synthesis also tend to disappear.

Effect in vivo

Acute changes. In vivo, the acute effects of ethanol on amino acid incorporation into protein have been less consistent than those described *in vitro*. No changes in the synthesis of total liver protein were found after administration of ethanol to naive rats [134]. Decreased hepatic production of export proteins (such as albumin and transferrin) has been demonstrated after acute administration of ethanol (3 g/kg) to the rat *in vivo* [58] but, as discussed subsequently, ethanol affects the secretion of such proteins, and under these conditions, effects on synthesis and secretion may be difficult to dissociate. The same reservation also pertains to the inhibition of the hepatic production of serum lipoproteins reported after acute ethanol administration, especially when high ethanol concentrations are achieved [20]. However, no changes or even an increase in lipoprotein production were observed with more moderate doses [3, 28, 134]. Hypo- and hyper-lipemic effects of ethanol have been produced by varying the dose of alcohol administered [30]. Thus it is not clear whether the decreased production of plasma proteins is due solely to a defect in synthesis or to a concomitant impairment of secretion (vide infra).

Effects of chronic ethanol consumption. In vivo studies of protein synthesis after chronic ethanol administration have yielded conflicting results. Decreased incorporation of labeled amino acids into liver proteins was found by Banks *et al.* [1] and Morland [105] after several weeks of administration of a mixture of solid and liquid diets containing ethanol. Furthermore Morland [105] showed that livers isolated from alcohol-fed rats have a decreased ability to incorporate amino acids into protein after stimulation with dexamethasone, and reduced activity of enzymes (tryptophan oxygenase, tyrosine amino-transferase) involved in protein metabolism. It is noteworthy that in all these studies there was no fatty liver, hepatomegaly or significant weight gain after administration of the alcohol-containing diets, suggesting that the ethanol intake may have been relatively low and perhaps associated with some degree of undernutrition.

The effects of chronic alcohol administration on protein synthesis were reassessed by Baraona *et al.* [5] using rats pair-fed the liquid diets described by Lieber and DeCarli [81]. Ethanol-fed rats developed hepatomegaly and fatty liver and the incorporation of leucine into protein was not decreased. Moreover, as also reported in other species [135], alcohol feeding increased the concentration of leucine (and other branched chain amino acids) in the plasma and even more so in the liver, which diluted the specific activity of the tracer in the amino acid precursor pool. After correction for the dilution of the amino acid pool, the rate of protein synthesis was found actually to be enhanced after chronic ethanol administration. Similarly, the ethanol-induced inhibition of albumin synthesis that had been observed in naive

animals was not reproduced when ethanol was administered to alcoholics [57]. It is possible, of course, that under special circumstances, such as relative hypoxia or starvation, the redox changes *in vivo* may approximate those found to be required *in vitro* to inhibit protein synthesis, but this has not as yet been established.

EFFECTS OF ACETALDEHYDE

All known pathways of ethanol oxidation in the liver result in production of acetaldehyde, and it is now generally accepted that more than 90% of the acetaldehyde formed from ethanol is in turn oxidized by the liver.

Chronic ethanol consumption results in a significant reduction in the capacity of rat liver mitochondria to oxidize acetaldehyde [50]. The decreased capacity of mitochondria of alcohol-fed animals to oxidize acetaldehyde, associated with unaltered or even enhanced rates of ethanol oxidation (and therefore acetaldehyde generation) [80,82], may result in an imbalance between production and disposition of acetaldehyde. Such a mechanism may cause the elevated blood acetaldehyde levels observed after chronic ethanol consumption in rats [65], baboons [120] and man [67]. Recent methodological advances have revealed that concentrations of "free" acetaldehyde in the blood are lower than the total levels hitherto reported; however, the levels were still higher in alcohol-fed animals than in controls [120]. Levels of acetaldehyde were also elevated in the liver [6]. The higher acetaldehyde concentrations that result may in turn enhance the functional disturbance of the mitochondria by reducing the activity of various shuttles involved in the disposition of reducing equivalents and by inhibiting oxidative phosphorylation [13]. The concentrations of acetaldehyde required to achieve the hepatic effects in mitochondria of normal animals were greater than those seen in the blood. However, mitochondria of rats fed ethanol chronically were found to have an increased susceptibility to the effects of acetaldehyde; under these conditions, concentrations of acetaldehyde that could occur in some hepatic compartments were found to depress mitochondrial functions, including lipid oxidation [100]. Since the rate of ethanol clearance in alcoholics is either equal to or faster than that of non-alcoholics, this 'induction' may be the source of greater production of the toxic metabolites (acetaldehyde) resulting in a 'vicious cycle': acetaldehyde causes mitochondrial dysfunction, which in turn promotes higher acetaldehyde levels; this circular process could result in elevation of acetaldehyde and further damage to the liver (Fig. 2). Indeed, in view of its reactivity, the increased acetaldehyde can exert a number of adverse effects with regard to protein metabolism and the integrity of the hepatocyte.

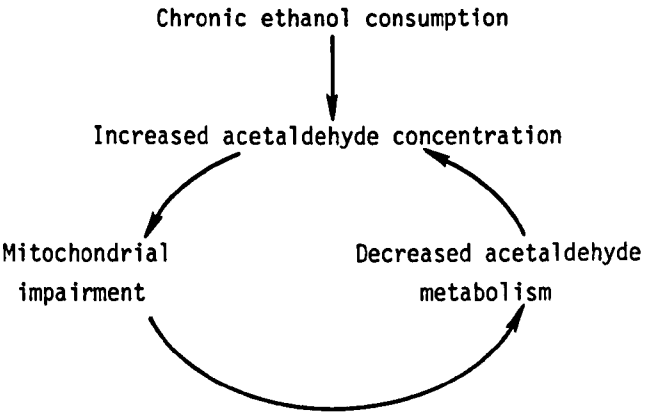

FIG. 2. Possible relation between ethanol consumption, altered acetaldehyde levels, and mitochondrial impairment. (From Hasumura *et al.* [49]).

INTERACTION OF ACETALDEHYDE WITH AMINO ACIDS AND PROMOTION OF LIPID PEROXIDATION

Acetaldehyde has been shown to participate in and to favor the condensation reactions of biogenic amines [16,21]. Some of the condensation products may be hepatotoxic [108]. Another possible mode of toxicity of acetaldehyde is its interaction with amino acids. Indeed, aldehydes react quite readily with mercaptans. L-cysteine could complex with acetaldehyde to form a hemiacetal, which would then transform to L-2-methylthiazolidine-4-carboxylic acid. It has been suggested that such a complex may be a nontoxic detoxification product, since cysteine was reported to protect against death from acetaldehyde toxicity *in vivo* [144]. Cysteine, *in vitro*, afforded protection against the depression of CO_2 production from palmitate, octanoate, and α-ketoglutarate by acetaldehyde [12]. On the other hand, cysteine is one of the three amino acids which constitute glutathione. Binding of acetaldehyde with cysteine and/or glutathione may contribute to a depression of liver glutathione [140]. Glutathione offers one of the mechanisms for the scavenging of toxic free radicals; a severe reduction in glutathione favors peroxidation, and the damage may possibly be compounded by the increased generation of active radicals by the "induced" microsomes following chronic ethanol consumption (Fig. 3).

Indeed, there is a microsomal pathway which requires O_2 and NADPH and which is capable of generating lipid peroxides. Enhanced lipid peroxidation has been proposed as a mechanism for ethanol induced fatty liver [25], but its role is controversial [48]. Theoretically, increased activity of microsomal NADPH oxidase following ethanol consumption [79] could result in enhanced H_2O_2 production, thereby also favoring lipid peroxidation.

In any event, it was found that in naive rats, very large amounts of ethanol (6 g/kg) are required to produce lipid peroxidation, whereas a smaller dose (3 g/kg) had no effect. By contrast, after chronic ethanol administration to the rat, even the smaller dose of ethanol administered acutely induced liver peroxidation and this effect could be prevented, at least in part, by the administration of methionine, a percursor of glutathione [139]. The ethanol induced lipid peroxidation was even more striking in the baboon: administration of relatively small doses of ethanol (1–2 g/kg) produced after 5–6 hours, lipid peroxidation and GSH depletion. In the baboon chronically fed alcohol (50% of total calories for 1–4 years) fatty liver and, in some, cirrhosis develops and such animals exhibit evidence of enhanced hepatic lipid peroxidation and GSH depletion. These changes were observed following an overnight withdrawal from ethanol and were exacerbated by the readministration of ethanol.

It is tempting to speculate that the propensity of the ba-

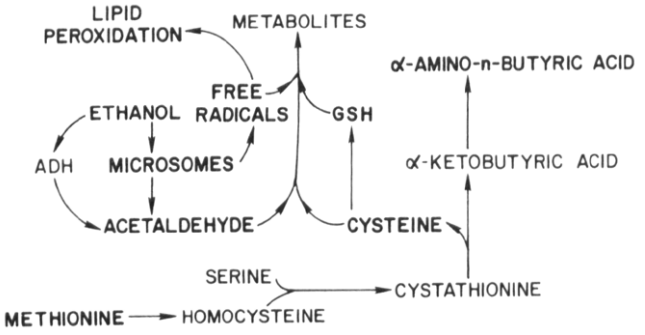

FIG. 3. Possible link between microsomal induction, enhanced acetaldehyde production, lipid peroxidation and alpha amino-n-butyric acid generation after chronic ethanol consumption.

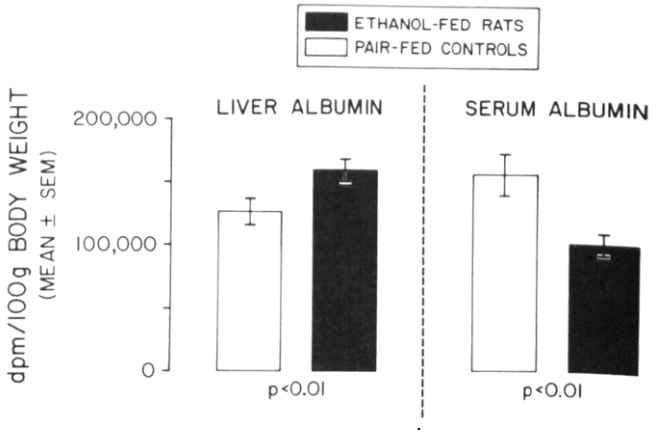

FIG. 4. Comparison between total liver and serum albumin labeling 30 minutes after ^{14}C-leucine injection. At this time, newly labeled albumin was being released into the plasma. (From Baraona and Lieber [2])

boon to develop more severe lesions than the rat after chronic ethanol consumption may in some way be related, at least in part, to the greater susceptibility to glutathione depletion and the initiation of lipid peroxidation.

INTERACTION OF ACETALDEHYDE WITH PROTEIN SYNTHESIS

As with ethanol, addition of acetaldehyde to liver inhibits protein synthesis [117, 118, 143]. There is, however, dissociation between the inhibitory effect of ethanol on protein synthesis and the concentration of acetaldehyde. Addition of either aspartate with α-ketoglutarate or of methylene blue prevents the ethanol-induced inhibition, despite a marked increase in acetaldehyde accumulation [121]. Acetaldehyde oxidation can produce an excess of reducing equivalents and increases the lactate/pyruvate ratio [37]. The partial prevention of the acetaldehyde-induced inhibition by supplementation with pyruvate, malate, oxaloacetate, aspartate, etc. [117] suggests that this inhibition is mediated, at least in part, by changes in redox state due to oxidation of acetaldehyde in the cytosol (in addition to its prevailing oxidation in the mitochondria). Moreover, while the inhibition of protein synthesis produced by acetaldehyde in concentrations of 320–660 μM is irreversible [143], that produced by ethanol is reversible [117], suggesting that different mechanisms may be involved.

Thus, several consequences of ethanol metabolism can be incriminated in the pathogenesis of the ethanol-induced inhibition of protein synthesis *in vitro*, namely, a marked shift in cytosolic redox state, high acetaldehyde and low pyruvate concentrations. Metabolism of acetaldehyde may, in turn, exacerbate the redox change. However, neither of these changes have been demonstrated to occur *in vivo* to the extent required for inhibition of protein synthesis, at least not in well-fed subjects.

INTERACTION OF ACETALDEHYDE WITH PROTEIN SECRETION

Whereas total protein concentration remains unchanged after chronic ethanol consumption, the concentration of export proteins (such as albumin and transferrin) increases, suggesting an impairment of protein secretion [4,5]. Such a secretory defect was further documented, in rats fed alcohol-containing diets, by the observation of delayed appearance of newly labeled albumin and transferrin in the serum with a corresponding retention of these newly labeled proteins in the liver [4,5] (Fig. 4). Acute alcohol administration *in vivo* to naive rats was also associated with delayed appearance of newly labeled albumin in the serum, retention of labeled albumin in the liver and increased hepatic content of immunoreactive albumin [7]. After an acute ethanol dose, the degree of albumin retention was considerably smaller than that of rats chronically fed an alcohol-containing diet [5]. Some *in vitro* studies [142] also favor the possibility that the acute inhibitory effects of ethanol are exerted not only on protein synthesis but on secretion as well. Indeed, 10 mM ethanol (or 340–580 μM acetaldehyde) inhibited the release of prelabeled protein and glycoproteins from liver slices when synthesis was blocked with cycloheximide or puromycin. Other studies in isolated hepatocytes [107] did not reveal any inhibitory effects of ethanol on the release of prelabeled proteins. In the latter system, however, colchicine (a potent inhibitor of secretion) decreased protein release by only 27–34%, suggesting that this system may lack the sensitivity required to detect the much weaker antisecretory effect of ethanol.

The majority of plasma proteins (with the exception of albumin) are exported from the liver in the form of glycoproteins after glycosylation in the Golgi apparatus. However, a defect in glycosylation cannot account for the concomitant impairment in albumin secretion, a protein which does not require glycosylation. Therefore, ethanol must interfere with a secretory step which affects both glycoproteins and non-glycoproteins. Microtubules represent such a likely site. Indeed, drugs (such as colchicine and Vinka alkaloids) that alter microtubules have been reported to impair secretion of macromolecules (such as proteins and lipoproteins) from various organs, including the liver [72]. Reaven and Reaven, who had first questioned the relationship between protein secretion and polymerization of microtubules, now [125] also find a "tight coupling" between the reduction in hepatic

VLDL secretion and hepatocyte microtubule content after administration of the antimicrotubule agents colchicine or vinblastine. In alcohol-fed rats it was shown that the protein export defect was associated with a significant decrease in the content of polymerized tubulin, the major chemical component of microtubules [5]. That this biochemical change was indeed due to a decrease in microtubules has been also documented morphologically [99]. The microtubules of ethanol-fed rats were not only decreased in number, but they were shorter and thicker [99].

Incubation of hepatocytes with ethanol reproduced the microtubular alterations observed *in vivo*. These effects were prevented by pyrazole, indicating that they were linked to derangements generated by the oxidation of ethanol rather than to ethanol itself. Moreover, serial additions of acetaldehyde to maintain concentrations similar to those reported to occur *in vivo* (less than 200 μM) reproduced the effects of ethanol on both polymerized tubulin and visible microtubules. *In vivo*, the inhibitory effects of ethanol on either microtubules or polymerized tubulin were markedly exaggerated by pretreatment of the animals with disulfiram (an inhibitor of aldehyde dehydrogenase), incriminating acetaldehyde as the principal agent responsible for these effects of ethanol oxidation [6]. Acetaldehyde (but not ethanol or acetate) binds to tubulin, competes with colchicine for a similar binding site to tubulin, and inhibits polymerization of tubulin isolated from brain [6].

The disruption of liver microtubules produced by ethanol is associated with engorgement of the secretory vesicles of the Golgi complex [99], suggesting a concomitant impairment of secretion. Chronic alcohol consumption, which potentiated the disruptive effect of ethanol on microtubules, also enhanced the Golgi engorgement. Conversely, 4-methylpyrazole which decreased the effect of ethanol on microtubules, also decreased the engorgement of the Golgi. These various observations support the concept that integrity of microtubules is required for normal protein secretion and that the acetaldehyde mediated microtubular alteration is responsible, at least in part, for the impaired protein secretion after ethanol.

SEQUELLAE OF PROTEIN ACCUMULATION IN THE LIVER

EFFECTS OF ETHANOL ON PROTEIN CATABOLISM

It can be calculated from the data of Baraona *et al.* [5] that the difference between the rate of synthesis of proalbumin and the rate of release of albumin into the serum should result in an accumulation of albumin far greater than that actually measured. Thus, it is likely that most of the retained proteins undergo degradation. Morland and Bessesen [106] found no changes in the rate of proteolysis after the addition of ethanol. These results, however, may not pertain to the conditions of animals fed ethanol chronically, in whom the concentration of branched chain amino acids (such as leucine) increases both in liver and plasma [135]. In keeping with the possibility of increased proteolysis in ethanol-fed animals are the reports of increased urinary nitrogen excretion [64,127]. Furthermore, there was an increase in the hepatic content of amino acids, with preferential accumulation of those amino acids that are not normally catabolized by the liver (branched chain amino acids and α-amino-n-butyric acid (AANB)). Studies in the rat fed ethanol as 36% of total calories revealed increased levels of free AANB in the liver and plasma [136]. There was a gradient from liver to plasma suggesting hepatic production. Indeed, experiments in liver slices of ethanol fed animals demonstrate a 2–3 fold increase in the production of this amino acid [35]. Increased release from the liver was also shown in baboons fed ethanol as 50% of total calories, associated with a striking (7-fold) increase in plasma AANB after chronic but not acute ethanol consumption; a significant increase in plasma AANB was also found in volunteers given alcohol under metabolic ward conditions [135]. Figure 3 shows a hypothetical link between increased AANB, GSH depletion and increased acetaldehyde. AANB is a product of methionine, serine and threonine catabolism (Fig. 3). Chronic ethanol administration results in increased hepatic degradation of methionine [35]. This may possibly result from GSH depletion. As discussed before, the latter may reflect combination of acetaldehyde with either cysteine or GSH as well as increased free radicals formation by the "induced" microsomes and their detoxification by GSH (Fig. 3).

Investigations of plasma AANB in alcoholics revealed an increase related to chronic alcohol consumption [137]. However, the level in the plasma is also decreased by protein deficiency. Indeed, clinical protein malnutrition is associated with characteristic plasma amino acid abnormalities, including depression of branched-chain amino acids and AANB [138]. Thus, in alcoholic patients the level of AANB may reflect at least two factors: chronic alcohol consumption, which tends to increase this amino acid, and dietary protein deficiency, which tends to decrease it. In order to use the level of AANB in plasma as a reflection of chronic alcohol consumption it was, therefore, necessary to control for the nutritional factors. Since AANB and leucine are depressed to a similar degree by dietary protein restriction, the level of AANB was expressed relative to the branched-chain amino acids of which leucine was selected for convenience. The plasma AANB level relative to leucine was found to be increased in a large number of active alcoholics regardless of dietary protein deficiency or the presence of moderate liver injury [137]. This increase was reversible upon cessation of drinking, persisted for days to weeks, did not require the presence of alcohol in the blood and was not present in non-alcoholics with moderate liver injury of viral etiology [137].

Further studies have revealed that the level of AANB relative to leucine is not a linear relation over the range of leucine values and, therefore, replacement of the simple ratio by a normal curve was recommended [138]. In addition, determination of gamma-glutamyl transpeptidase was found to enhance the specificity and sensitivity of the test. In alcoholics with severe liver injury, however, AANB relative to leucine may be increased because of markedly depressed values of plasma leucine and result in false positive determinations [24, 104, 138]. However, this group does not usually present a problem with respect to differential diagnosis. Similarly, in patients with extremely severe metabolic abnormalities, such as diabetic ketoacidosis [33], massive obesity undergoing starvation [34] and subjects consuming an experimental diet with very low carbohydrate and high fat content [146], the AANB may increase relative to leucine. To exclude such subjects, the simple use of a "Dip Stix" test was recommended to detect acetone in the urine [138].

One study reported that alcoholics without liver damage who had been recently drinking did not have elevations in

the ratio of AANB to leucine [104]. However, recency of alcohol consumption and quantity of alcohol consumed were not specified. Furthermore, this study did not take into account the fact that the relationship of AANB to leucine is not linear over the entire range of leucine values [138]. The latter was also a shortcoming of a study of Ellingboe *et al.* [29]. In experimental animals, the increase in AANB relative to leucine was confirmed after chronic alcohol consumption in the absence of severe liver damage [145].

Although AANB may not offer a practical tool for alcoholism screening in populations which are heterogenous with regard to nutrition and degree of liver disease, there is now good evidence that in rehabilitation programs in which the patient can serve as his own control, measurement of AANB can be particularly useful for documentation of treatment success as well as for the detection of relapses in recovered alcoholics [141]. To that effect, it was much more useful than MCV (mean corpuscular volume of red blood cells) and more accurate than gamma-glutamyl transpeptidase. For such purposes, the subjects own AANB (at discharge) is used for comparison; therefore, no correction of AANB with leucine is needed and the absolute values of AANB can be usefully interpreted [141].

SWELLING OF THE HEPATOCYTE AND POSSIBLE RELATION TO NECROSIS

Two of the earliest and most conspicuous features of the hepatic damage produced by alcohol are the deposition of fat and the enlargement of the liver. This hepatomegaly was traditionally attributed to the accumulation of lipids. However, in animals fed alcohol-containing diets, it was shown that lipids account for only half the increase in liver dry weight [87], while the other half is almost totally accounted for by an increase in proteins [4]. The increase in protein was not associated with changes in concentration, indicating that water was retained in proportion to the increase in protein. The mechanism of the water retention is not fully elucidated, but the rise in both protein and amino acids, plus a likely increase in associated small ions, could account osmotically for a large fraction of the water increase. The increases in lipid, protein, amino acid, water and electrolytes result in increased size of the hepatocytes. The number of hepatocytes and the hepatic content of desoxyribonucleic acid (DNA) did not change after alcohol treatment and thus the hepatomegaly is entirely accounted for by the increased cell volume [4,5]. There is also an increase in the number of hepatic mesenchymal cells after ethanol feeding [4], but this increase does not significantly contribute to the hepatomegaly. The swelling of the hepatocytes after chronic alcohol administration was found to be associated with a reduction of the intercellular space and with portal hypertension [56]. Both in rats and baboons fed alcohol, and in alcoholic patients, the swelling is most conspicuous in the centrolobular (also now called perivenular) area which is the zone with the lowest oxygen tension. It has been postulated that ethanol, through enhancing the metabolic demand, may precipitate hypoxia and aggravate hepatic damage [55].

Subcellular Site of Protein Accumulation in the Liver

As discussed before, alcohol consumption results in proliferation of the smooth membranes of the endoplasmic reticulum [74]. The increase in microsomal protein, however, accounts for only 30% of the total protein increase. Mitochondria are also grossly altered after alcohol administration [54, 70, 129]. Swollen and giant mitochondria are commonly observed, but the mitochondrial proteins do not contribute significantly to the total increase in liver protein. More than half of the total increase in liver protein is actually due to increased soluble proteins of the cytosol [4].

Type of Proteins that Accumulate in the Liver after Chronic Alcohol Consumption

As discussed before, contrasting to the lack of changes in the concentration of total hepatic proteins, the concentration of some proteins (such as albumin and transferrin), which are primarily destined for export into the plasma, was found significantly increased in the liver of ethanol-fed rats, whereas the concentration of soluble constituent proteins (such as ferritin) decreased [4,5]. The increased concentration of export proteins reflects an even greater increase in the amount per total liver since alcohol administration produced hepatomegaly. Conversely, decreased concentration of the other proteins after alcohol-feeding may merely reflect dilution in enlarged protein and water pools.

It was shown, in the case of albumin, that the increase involves precursor proteins (such as proalbumin) as well as mature serum albumin [5]. The current theory is that proteins destined for export are synthesized by bound ribosomes, discharged into the cysterna of the rough endoplasmic reticulum and then transported to the smooth endoplasmic reticulum and Golgi apparatus. This theory, supported by biochemical evidence, has been disputed in the case of albumin because of electron microscopic evidence that indicates direct discharge of albumin from bound ribosomes into the cytosol [94]. Although immunoreactive albumin accumulates preferentially in the cytosol and transferrin in the microsomal fraction, after ethanol feeding the concentration of these proteins increases in both compartments. Since no evidence of leakage from the microsomes was obtained, the possibility that the cytosol could serve as a storage site of retained proteins had to be considered [5]. By immunoelectron microscopy, albumin was found to accumulate in the endoplasmic reticulum but not the cytosol of alcoholics [32]. In the latter compartment, however, the concentration of albumin may have been too small to be detectable by the technique used. It must also be pointed out that the increases in these two export proteins (albumin and transferrin) account for only a small fraction of the total increase in soluble proteins. Thus, the major contributor to the ethanol-induced accumulation of liver protein has not been identified as yet.

Alcoholic liver damage can also be associated with the formation of Mallory bodies, clusters of fibrillar material that resemble intermediary filaments normally present in various tissues. Although the severity of the lesions is usually somewhat greater in cases with Mallory bodies [47], no significantly worse prognosis was associated with their presence. These Mallory bodies, although helpful in establishing an etiological diagnosis, are not pathognomonic for alcoholic liver disease. The exact nature and pathogenesis of alcoholic hyaline has not been elucidated. These fibrils are morphologically and chemically different from microtubules and from microfilaments (actin) [41] and may represent prekeratin [23]. However, because antitubulins (such as griseolfulvin and colchicine) have the ability to induce the formation of Mallory bodies in mice [22], it is possible that the appearance of these bodies in alcoholic hepatitis may be linked to the

antitubulin effects of chronic alcohol ingestion. In any event, Mallory bodies may stimulate some immune responses which have been incriminated by Leevy *et al.* [71] in the progression of alcoholic liver injury, but this is still the subject of controversy. Even in the absence of Mallory bodies, one suspects that ballooning and associated gross distortion of the volume of the hepatocytes may result in severe impairment of key cellular functions. In alcoholic liver disease some cells not uncommonly have a diameter which is increased 2–3 times, and thereby their volume is increased about 4–10 fold. One may wonder to what extent this type of cellular disorganization, with protein retention and ballooning, may promote progression of the liver injury in the alcoholic. Indeed, there are other instances of protein retention in the liver such as alpha$_1$ antitrypsin deficiency, associated with progression to fibrosis and cirrhosis. By analogy, one may assume that the extent of protein retention may in some way also favor progression of liver disease in the alcoholic.

The present prevailing view is that alcoholic cirrhosis develops in response to alcoholic hepatitits. The latter is characterized not only by ballooning of the hepatocyte, but also by extensive necrosis and polymorphonuclear inflammation. It is understandable that necrosis and inflammation may trigger the scarring process of cirrhosis, but one must question whether this is the only mechanism involved. In some populations, particularly in Europe and Japan [53], cirrhosis commonly develops in alcoholics without an apparent intermediate stage of florid alcoholic hepatitis. It is noteworthy that other types of cirrhosis such as that seen in hemochromatosis can develop without an apparent inflammatory stage [124]. These observations raise the question of whether alcohol can promote development of cirrhosis without being preceded by alcoholic hepatitis. Indeed, in baboons fed alcohol, fatty liver developed, the hepatocytes increased in size, and there was some obvious ballooning. This ballooning was associated with some mononuclear inflammation but very few of the polymorphonuclear cells so characteristic of human alcoholic hepatitis [123]. Although some clumping was apparent in the cytoplasm, electron microscopy indicated that there was no alcoholic hyaline. Thus there was no picture of florid alcoholic hepatitis; yet in one-third of the animals, typical cirrhosis developed. If one can extrapolate from these baboons to humans, it appears that florid alcoholic hepatitis may not be a necessary intermediate step in the development of alcoholic cirrhosis.

This hypothesis in turn raises the question of which process, in the absence of alcoholic hepatitis, may initiate cirrhosis upon chronic consumption of ethanol. It is possible that cirrhosis develops in the presence of minimal inflammation and necrosis, which may suffice to trigger the fibrosis [75]. On the other hand, it is also possible that alcohol may have some more direct effect on collagen metabolism independent of the necrosis and inflammation as discussed subsequently.

Obviously, the mechanisms whereby alcohol abuse leads to necrosis of the hepatocyte have not been fully elucidated. It is likely that a variety of factors are involved, such as toxic effects of acetaldehyde, consequences of injury to the endoplasmic reticulum, mitochondria and microtubules, as well as the physical damage resulting from the accumulation of fat and protein and the distention of the hepatocyte. Whatever its mechanism, recognition of the severity of the necrosis and inflammation may be of clinical import.

Liver biopsy is the most objective way of determining the degree of liver cell necrosis, but this is obviously not a practical tool for routine follow-up of a patient or for mass screening purposes. Therefore, spill-over in the blood of liver enzymes, especially transaminases, is commonly used as a marker of liver cell damage. However, blood transaminase values are a poor reflection of liver cell necrosis as revealed on biopsy [60], especially in alcohol-induced liver cell injury. In alcoholic hepatitis, for example, levels of transaminases are only moderately elevated and normal values can occasionally be found [8]. Gamma glutamyl transpeptidase (GGTP) is not more reliable, although some correlation with liver cell necrosis exists [154]. Elevation from non-hepatic origin is common and in some alcoholics elevated levels may only reflect microsomal induction [147]. In contrast with transaminases and GGTP, which show a considerable overlap between patients with and without significant cell necrosis on biopsy, glutamate dehydrogenase (GDH) more accurately reflects the degree of underlying cell damage [149]. The high liver content of this enzyme, its solely mitochondrial origin (mitochondrial lesions associated with alcoholic liver disease have been discussed before) [54, 100, 129], and its predominantly centrolobular localization [43] (the area which suffers the major impact of alcoholic liver injury) could explain the advantage of the enzyme as an index of liver cell damage in alcoholic patients. However, to help define the severity of alcoholic liver injury, the GDH determination must be carried out soon (within 1–2 days) after the alcoholic episode; thereafter, the values drop rapidly, whereas liver necrosis persists. Thus, except for the early stage, GDH offers no advantage over other enzymes [153].

COLLAGEN ACCUMULATION

Cellular injury is associated with extracellular deposition of the collagens, a family of proteins which are now well characterized. In alcoholic liver injury there is a great variability in the magnitude of collagen deposition. At the earlier stages, in the so-called simple or uncomplicated fatty liver, collagen is detectable by chemical means only [31]. When collagen deposition is sufficient to become visible by light microscopy, usually it appears around the central (also called terminal venules, resulting in so-called "pericentral" or "perivenular" sclerosis. This lesion is usually described in association with full blown alcoholic hepatitis. Less well recognized is the fact that this pericentral sclerosis can already occur at the fatty liver stage in the absence of hepatitis [27,148]. In a retrospective study of liver biopsies from hospitalized alcoholic patients that had been read by the pathologist as simple fatty liver, appropriate staining disclosed pericentral sclerosis in 40 percent [148]. Experimental studies in alcohol fed baboons show that in those animals which progressed to cirrhosis, pericentral sclerosis invariably occurred already at the fatty liver stage; by contrast, animals that did not show the lesion did not progress beyond the stage of fatty liver over the same period of time. These experimental data suggest that, at least in the baboon, pericentral sclerosis is a common and early warning sign of impending cirrhosis if drinking continues [148]. The concept of pericentral sclerosis as a precirrhotic lesion is further strengthened by preliminary findings in alcoholics [152]. What initiates the collagen deposition in the perivenular area is not known, but again some clues may be derived from the experimental model of cirrhosis in the baboon. Sequential biopsies in alcohol fed

baboons revealed in some animals already at the early fatty liver stage the appearance in the perivenular central areas of an increased number of lipocytes (Ito cells) or transitional cells between lipocytes, fibroblasts [98] and myofibroblasts (Nakano *et al.* unpublished). These cells eventually underwent deposition of abundant collagen bundles, first in the perivenular areas, leading to perivenular sclerosis and ultimately to diffuse fibrosis and cirrhosis. Histochemically procollagens and collagens types I and III are deposited with elastin and fibronectin; septa were accompanied by increases in laminin and collagen type IV in association with the appearance of myofibroblasts [44]. In cases of alcoholic hepatitis, Kent *et al.* [61] described an increased number of Ito cells. We now realize that even in the absence of alcoholic hepatitis, and prior to any fibrosis, some animals show an increased number of mesenchymal cells and these are the animals that ultimately progress to fibrosis. Thus, these cells appear to play a key role in the overabundant deposition of collagen. The composition of the fibrotic tissue suggests participation of a fibroblastic cell of wide biosynthetic potential distinct from fibroblasts which usually do not synthesize basement membrane proteins. It may be related to myofibroblasts described in human alcoholic cirrhosis [131]; as mentioned before, myofibroblasts were also observed in the alcohol-fed baboons in association with the development of fibrosis. In any event, these studies revealed close similarities of the major structural components and their distribution in human [45] and baboon [44] alcoholic fibrosis. However, the mechanism for the increase in the various proteins has not been elucidated, nor do we know the respective role of parenchymal and nonparenchymal cells in their production, particularly with regard to the collagens.

The accumulation of hepatic collagen during the development of cirrhosis could theoretically be accomplished by increased synthesis, decreased degradation, or both. The rate of hepatic fibrous tissue degradation has never been directly measured in human alcoholic liver disease or in any of the animal models of alcoholic liver disease. Therefore its role in the pathogenesis of hepatic fibrosis is unresolved. The cirrhosis of choline deficiency (to which the rat is highly susceptible and by which primates are much less affected) may be exacerbated in the rodent by administration of ethanol and in this context collagen degradation may be slowed [52].

The mechanisms of collagen degradation in the liver are complex. We have recently developed a quantitative assay for neutral collagenase activity in liver homogenate [113] and preliminary results suggest a paradoxical increase in the activity of this enzyme in animals fed ethanol [113], at least in early, precirrhotic stages.

The results observed with lysosomal enzyme activity are, in part, contradictory. After two weeks of ethanol administration (1 g/kg IP) the activities of lysosomal enzymes were decreased [122] whereas with larger, oral doses they were unchanged after one month and increased after six months [102]. Henley and Laughrey [51] reported that in ethanol treated rats inhibitors directed toward lysosomal peptidases (tested against radioactive collagen substrate at pH 4) are increased.

Chronic ethanol consumption with adequate diets led to accumulation of hepatic collagen in rats and baboons, even when fibrosis was not yet histologically detectable. In the latter model, the role of increased collagen synthesis is suggested by increased activity of hepatic peptidylproline hydroxylase in rats and primates and increased incorporation of proline C^{14} into hepatic collagen in rat liver slices [31]. The role of increased collagen synthesis has been confirmed indirectly in man by autoradiographic techniques utilizing liver biopsies [14]. Using a slightly different alcohol feeding technique for rodents, which did not result in the expected hepatomegaly, Mezey *et al.* [103] were able to demonstrate hepatic collagen accumulation but could find no definite pathogenetic mechanism. A possible mechanism whereby alcohol consumption may be linked to collagen formation is the increase in tissue lactate secondary to alcohol metabolism [31]. Elevated concentrations of lactate have been associated with increased peptidylproline hydroxylase activity both *in vitro* [42] and *in vivo* [95]. The hepatic free proline pool size, which has been incriminated in the regulation of collagen synthesis [15] may be increased by ethanol [46] and is expanded in human portal cirrhosis [62]. Recently, it has again been postulated that lactate may play a role [63], this time through inhibition of proline oxidase [68]. This hypothesis illustrates once more the possible impact of ethanol induced redox changes on intermediary metabolism, including metabolism of protein, and in particular, collagen. Further studies, however, are needed to elucidate all mechanisms involved as well as to resolve some other important, but as yet unexplained, effects of ethanol, such as its possible interference with hepatic regeneration: whereas some found no effect on regeneration [17,39], others [150] report inhibition of H^3-thymidine incorporation but offer no explanation concerning the mechanism involved. The process of tissue formation and destruction, with the underlying alteration of protein synthesis and degradation, thus remains an interesting investigational challenge.

REFERENCES

1. Banks, W. L., E. S. Kline and E. S. Higgins. Hepatic composition and metabolism after ethanol consumption in rats fed liquid purified diets. *J. Nutr.* **100**: 581–593, 1970.
2. Baraona, E. and C. S. Lieber. Effects of ethanol on hepatic protein synthesis and secretion. In: *Currents in Alcoholism*, Vol. 1, edited by F. A. Seixas. New York: Grune and Stratton, Inc., 1977, pp. 33–46.
3. Baraona, E., R. C. Pirola and C. S. Lieber. Pathogenesis of post-prandial hyperlipemia in rats fed ethanol-containing diets. *J. clin. Invest.* **52**: 296–303, 1973.
4. Baraona, E., M. A. Leo, S. A. Borowsky and C. S. Lieber. Alcoholic hepatomegaly: Accumulation of protein in the liver. *Science* **190**: 794–795, 1975.
5. Baraona, E., M. A. Leo, S. A. Borowsky and C. S. Lieber. Pathogenesis of alcohol-induced accumulation of protein in the liver. *J. clin. Invest.* **60**: 546–554, 1977.
6. Baraona, E., Y. Matsuda, P. Pikkarainen, F. Finkelman and C. S. Lieber. Exaggeration of the ethanol-induced decrease in liver microtubules after chronic alcohol consumption. *Gastroenterology* **76**: 1274, 1979.
7. Baraona, E., P. Pikkarainen, M. Salaspuro, F. Finkelman and C. S. Lieber. Acute effects of ethanol on hepatic protein synthesis and secretion in the rat. *Gastroenterology* **79**: 104–111, 1980.
8. Beckett, A. G., A. V. Livingstone and K. R. Hill. Acute alcoholic hepatitis. *Br. Med. J.* **2**: 1113–1119, 1961.

9. Best, C. H., W. S. Hartroft, C. C. Lucas and J. H. Ridout. Liver damage produced by feeding alcohol or sugar and its prevention by choline. *Br. Med. J.* **ii**: 1101–1106, 1949.
10. Bhuyan, U. N., N. C. Nayak, M. G. Deo and V. Ramalingaswami. Effect of dietary protein on carbon tetrachloride-induced hepatic fibrogenesis in albino rats. *Lab. Invest.* **14**: 184–190, 1965.
11. Blomstrand, R., L. Kager and O. Lantto. Status on studies on the ethanol-induced decrease of fatty acid oxidation in rats and human liver. *Life Sci.* **13**: 113, 1973.
12. Cederbaum, A. I. and E. Rubin. Protective effect of cysteine on the inhibition of mitochondrial functions by acetaldehyde. *Biochem. Pharmac.* **25**: 963–973, 1976.
13. Cederbaum, A. I., C. S. Lieber and E. Rubin. The effect of acetaldehyde on mitochondrial function. *Archs Biochem. Biophys.* **161**: 26–39, 1974.
14. Chen, T. S. N. and C. M. Leevy. Collagen biosynthesis in liver disease of the alcoholic. *J. Lab. clin. Med.* **85**: 103–112, 1975.
15. Chvapil, M. and J. N. Ryan. The pool of free proline in acute and chronic liver injury and its effect on the synthesis of collagen-globular protein. *Agents Actions* **3**: 38–44, 1973.
16. Cohen, G. and M. Collins. Alkaloids from catecholamines in adrenal tissue: Possible role in alcoholism. *Science* **167**: 1749–1751, 1970.
17. Craig, J. Effects of ethanol and ethionine on DNA synthesis during experimental liver regeneration. *J. Stud. Alcohol* **36**: 148–157, 1975.
18. Craig, R. M., T. Neumann, K. N. Jeejeebhoy and H. Yokoo. Severe hepatocellular reaction resembling alcoholic hepatitis with cirrhosis after massive small bowel resection and prolonged total parenteral nutrition. *Gsatroenterology* **79**: 131–137, 1980.
19. Crow, K. E., N. W. Cornell and R. L. Veech. The rate of ethanol metabolism in isolated rat hepatocytes. *Alcoholism: Clin. Exp. Res.* **1**: 43–47, 1977.
20. Dajani, R. M. and C. Kouyoumjian. A probable direct role of ethanol in the pathogenesis of fat infiltration in the rat. *J. Nutr.* **91**: 535–539, 1967.
21. Davis, V. E. and M. J. Walsh. Alcohol, amines, and alkaloids: Possible biochemical basis for alcohol addiction. *Science* **167**: 1005–1007, 1970.
22. Denk, H. and R. Eckerstorfer. Colchicine-induced Mallory body formation in the mouse. *Lab. Invest.* **36**: 563–565, 1977.
23. Denk, H., W. W. Franke, R. Eckerstorfer, E. Schmid and D. Kerjaschki. Formation and involution of Mallory bodies ("alcoholic hyalin") in murine and human liver revealed by immunofluorescence microscopy with antibodies to prekeratin. *Proc. natn. Acad. Sci. U.S.A.* **76**: 4112–4116, 1979.
24. Dienstag, J. L., E. A. Carter, J. R. Wands, K. J. Isselbacher and J. E. Fischer. Plasma alpha-amino-n-butyric acid to leucine ratio: Nonspecificity as a marker for alcoholism. *Gastroenterology* **75**: 561–565, 1978.
25. DiLuzio, N. R. and A. D. Hartman. Role of lipid peroxidation on the pathogenesis of the ethanol-induced fatty liver. *Fedn Proc.* **26**: 1436–1442, 1967.
26. Domschke, S., W. Domschke and C. S. Lieber. Hepatic redox state: Attenuation of the acute effects of ethanol induced by chronic ethanol consumption. *Life Sci.* **15**: 1327–1334, 1974.
27. Edmondson, H. A., R. L. Peters, H. H. Frankel and S. Borowsky. The early stages of liver injury in the alcoholic. *Medicine* **46**: 119–129, 1967.
28. Elko, E. E., W. R. Wooles and N. R. DiLuzio. Alterations and mobilization of lipids in acute ethanol-treated rats. *Am. J. Physiol.* **201**: 923–926, 1961.
29. Ellingboe, J., J. H. Mendelson, C. C. Varanelli, D. Neuberger and M. Borysow. Plasma alpha amino-n-butyric acid: Leucine ratio. *J. Stud. Alcohol* **39**: 1467–1476, 1978.
30. Estler, C.-J. Comparison of hepatic triglyceride content and hepatic lipid secretion after various doses of ethanol. *Biochem. Pharmac.* **24**: 1871–1873, 1973.
31. Feinman, L. and C. S. Lieber. Hepatic collagen metabolism: Effect of alcohol consumption in rats and baboons. *Science* **176**: 795, 1972.
32. Feldman, G. and M. Maurice. Morphological findings of liver protein synthesis and secretion. In: *Membrane Alterations as Basis of Liver Injury*, edited by H. Popper, L. Bianchi and W. Reuter. Lancaster, England: MTP Press, 1977, pp. 61–76.
33. Felig, P., E. Marliss, J. L. Ohman and G. F. Cahill. Plasma amino acid levels in diabetic ketoacidosis. *Diabetes* **19**: 727–729, 1970.
34. Felig, P., O. E. Owen, J. Wahren and G. F. Cahill. Amino acid metabolism during prolonged starvation. *J. clin. Invest.* **48**: 584–594, 1969.
35. Finkelstein, J. D., J. P. Cello and W. E. Kyle. Ethanol-induced changes in methionine metabolism in rat liver. *Biochem. Biophys Res. Commun.* **61**: 475–481, 1974.
36. Fischel, P. and K. Oette. Experimentelle Untersuchungen an menschlichen Leberpunktaten und Rattenleberschnitten zur Oxidation von Fettsäuren mit unterschiedlicher Kettenlänge und unterschiedlicher Zahl von Doppelbindungen. *Res. exp. Med.* **163**: 1–16, 1974.
37. Forsander, O. A. Influence of the metabolism of ethanol on the lactate/pyruvate ratio of rat-liver slices. *Biochem. J.* **6**: 244–247, 1966.
38. Foy, H., A. Kondi and Y. Mbaya. Effect of riboflavine deficiency on bone marrow function and protein metabolism in baboons. *Br. J. Nutr.* **18**: 307–318, 1964.
39. Frank, W. O., A. N. Rayyes, A. Washington and P. R. Holt. Effect of ethanol administration upon hepatic regeneration. *J. Lab. clin. Med.* **93**: 402–413, 1979.
40. Freinkel, N. and R. A. Arkey. Effects of alcohol on carbohydrate metabolism in man. *Psychosom. Med.* **28**: 551–563, 1966.
41. French, S. W., J. S. Sim and K. E. Franks. Alcoholic hepatitis. In: *Alcohol and the Liver*, edited by M. M. Fisher and J. G. Rankin. New York-London: Plenum Press, 1976, pp. 261–286.
42. Green, H. and B. Goldberg. Collagen and cell protein synthesis by an established mammalian fibroblast line. *Nature* **204**: 347–349, 1964.
43. Guder, W. G., A. Habicht, J. Kleibl, U. Schmidt and O. H. Wielund. The diagnostic significance of liver cell inhomogeneity: Serum enzymes in patients with central liver necrosis and the distribution of glutamate dehydrogenase in normal human liver. *Z. Klin. Chem. Klin. Biochem.* **13**: 311–318, 1975.
44. Hahn, E. G., R. Timpl, M. Nakano and C. S. Lieber. Distribution of hepatic collagens, elastin and structural glycoproteins during the development of alcoholic liver injury in baboons. *Gastroenterology* **79**: 1024, 1980.
45. Hahn, E., G. Wick, D. Pencev and R. Timpl. Distribution of basement membrane proteins in normal and fibrotic human liver: Collagen type IV, laminin and fibronectin. *Gut* **21**: 63–71, 1980.
46. Häkkinen, H.-M. and E. Kulonen. Effect of ethanol on the metabolism of alanine, glutamic acid, and proline in rat liver. *Biochem. Pharmac.* **24**: 199–204, 1975.
47. Harinasuta, U. and H. J. Zimmerman. Alcoholic steatonecrosis. I. Relationship between severity of hepatic disease and presence of Mallory bodies in the liver. *Gastroenterology* **60**: 1036–1046, 1971.
48. Hashimoto, S. and R. O. Recknagel. No chemical evidence of hepatic lipid peroxidation in acute ethanol toxicity. *Exp. Molec. Pathol.* **8**: 225–242, 1968.
49. Hasumura, Y., R. Teschke and C. S. Lieber. Acetaldehyde oxidation by hepatic mitochondria: Its decrease after chronic ethanol consumption. *Science* **189**: 727–729, 1975.
50. Hasumura, Y., R. Teschke and C. S. Lieber. Characteristics of acetaldehyde oxidation in rat liver mitochondria. *J. Biol. Chem.* **251**: 4908–4913, 1976.
51. Henley, K. S. and E. G. Laughrey. Ethanol induced increase in neutral extractable hydroxyproline containing peptides, (NEHYP), in rat liver. *Alcoholism* **3**: 272, 1979.

52. Henley, K. S., E. G. Laughrey, H. D. Appelman and K. Flecker. Effect of ethanol on collagen formation in dietary cirrhosis in the rat. *Gastroenterology* **72:** 502–506, 1977.
53. Inoue, E. The clinicopathological proteins of the alcoholic liver injury in Japan and its etiological relationship to hepatitis B virus. *Gastroenterol. Japonica* **12:** 230–240, 1966.
54. Iseri, O. A., C. S. Lieber and L. S. Gottfried. The ultrastructure of fatty liver induced by prolonged ethanol ingestion. *Am. J. Pathol.* **48:** 535–555, 1966.
55. Israel, Y., H. Orrego, J. M. Khanna, D. J. Stewart, M. J. Phillips and H. Kalant. Alcohol induced susceptibility to hypoxic liver damage: Possible role in the pathogenesis of alcoholic liver disease? In: *Alcohol and the Liver*, edited by M. M. Fisher and J. G. Rankin. New York: Plenum Press, 1977, pp. 323–346.
56. Israel, Y., P. G. Walfish, H. Orrego, J. Blake and H. Kalant. Thyroid hormones in alcoholic liver disease. Effect of treatment with 6-n-propylthiouracil. *Gastroenterology* **76:** 116–122, 1979.
57. Jeejeebhoy, K. N., A. Bruce-Robertson and U. Sodtke. The effect of ethanol on albumin and fibrinogen synthesis in vivo and in hepatocyte suspensions. In: *Alcohol and Abnormal Protein Synthesis*, edited by M. A. Rothschild, M. Oratz and S. S. Schreiber. New York: Pergamon Press, 1975, pp. 373–391.
58. Jeejeebhoy, K. N., M. J. Phillips, A. Bruce-Robertson and U. Sodtke. The acute effect of ethanol on albumin, fibrinogen and transferrin synthesis in the rat. *Biochem. J.* **126:** 1111–1126, 1972.
59. Johnson, O. Influence of the blood ethanol concentration on the acute ethanol-induced liver triglyceride accumulation in rats. *Scand. J. Gastroenterol.* **2:** 207–213, 1974.
60. Kallai, K., A. Hahn, V. Röder and V. Zupanic. Correlation between histological findings and serum transaminase values in chronic diseases of the liver. *Acta Med. Scand.* **175:** 49–56, 1964.
61. Kent, G., S. Gay, T. Inouye, R. Bahu, O. T. Minick and H. Popper. Vitamin A containing lipocytes and formation of type III collagen in liver injury. *Proc. natn. Acad. Sci. U.S.A.* **73:** 3719–3722, 1976.
62. Kershenobich, D., F. J. Fierro and M. Rojkind. The relationship between free pool of proline and collagen content in human liver cirrhosis. *J. clin. Invest.* **49:** 2246–2249, 1970.
63. Kershenobich, D., G. Garcia-Tsao and M. Rojkind. Relationship between serum lactic acid and serum proline in alcoholic liver cirrhosis. *Gastroenterology* **77:** A22, 1979.
64. Klatskin, G. The effect of ethyl alcohol on nitrogen excretion in the rat. *Yale J. Biol. Med.* **34:** 124–143, 1961.
65. Koivula, T. and K. O. Lindros. Effects of long term ethanol treatment on aldehyde and alcohol dehydrogenase activities in rat liver. *Biochem. Pharmac.* **24:** 1937–1942, 1975.
66. Korsten, M. A. and C. S. Lieber. Nutrition in the alcoholic. *Med. Clin. North Am.* **63:** 963–972, 1979.
67. Korsten, M. A., S. Matsuzaki, L. Feinman and C. S. Lieber. High blood acetaldehyde levels after ethanol administration in alcoholics. *New Engl. J. Med.* **292:** 386–389, 1975.
68. Kowaloff, E. M., J. M. Phang, A. S. Granger and S. J. Downing. Regulation of proline oxidase activity by lactate. *Proc. natn. Acad. Sci. U.S.A.* **74:** 5368–5371, 1977.
69. Kuriyama, K., P. Y. Sze and G. E. Rauscher. Effects of acute and chronic ethanol administration on ribosomal protein synthesis in mouse brain and liver. *Life Sci.* **10:** 181–189, 1971.
70. Lane, B. P. and C. S. Lieber. Ultrastructural alterations in human hepatocytes following ingestion of ethanol with adequate diets. *Am. J. Pathol.* **49:** 594–603, 1966.
71. Leevy, C. M., N. Kanagasundaram, K. Matsumoto *et al.* Alcoholic hyalin and immunologic reactivity. In: *Immune Reactions in Liver Disease*, edited by A. L. W. F. Eddleston, J. C. P. Weber and R. Williams. Kent, England: Pitman Medical Publishing Co., Ltd., 1979, pp. 195–207.
72. LeMarchand, Y., A. Singh, F. Assimacopoulos-Jeannet, L. Orci, C. Rouiller and B. Jeanrenaud. A role for the microtubular system in the release of very low density lipoproteins by perfused mouse livers. *J. Biol. Chem.* **248:** 6862–6870, 1973.
73. Leo, M. A., M. Sato, M. Arai and C. S. Lieber. Liver injury after vitamin A deficiency or excessive potentiation by ethanol. *Gastroenterology* **79:** 1034, 1980.
74. Lieber, C. S. Liver adaptation and injury in alcoholism. *New Engl. J. Med.* **288:** 356–362, 1973.
75. Lieber, C. S. *Pathogenesis of Alcoholic Liver Disease: An Overview*, Vol. 3, edited by M. M. Fisher and J. G. Rankin. New York: Plenum Press, 1977, pp. 197–255.
76. Lieber, C. S. Metabolism and metabolic effects of alcohol. *Sem. Hematol.* **17:** 85–99, 1980.
77. Lieber, C. S. and C. S. Davidson. Some metabolic effects of ethyl alcohol. *Am. J. Med.* **33:** 319–327, 1962.
78. Lieber, C. S. and L. M. DeCarli. Study of agents for the prevention of the fatty liver produced by prolonged alcohol intake. *Gastroenterology* **50:** 316–322, 1966.
79. Lieber, C. S. and L. M. DeCarli. Reduced nicotinamide-adenine dinucleotide phosphate oxidase: Activity enhanced by ethanol consumption. *Science* **170:** 78–80, 1970.
80. Lieber, C. S. and L. M. DeCarli. Hepatic microsomal ethanol-oxidizing system. In vitro characteristics and adaptive properties in vivo. *J. Biol. Chem.* **245:** 2505–2512, 1970.
81. Lieber, C. S. and L. M. DeCarli. Quantitative relationship between the amount of dietary fat and the severity of the alcoholic fatty liver. *Am. J. clin. Nutr.* **23:** 474–478, 1970.
82. Lieber, C. S. and L. M. DeCarli. The role of hepatic microsomal ethanol oxidizing system (MEOS) for ethanol metabolism in vivo. *J. Pharmac. exp. Ther.* **181:** 279–287, 1972.
83. Lieber, C. S. and L. M. DeCarli. An experimental model of alcohol feeding and liver injury in the baboon. *J. Med. Primatol.* **3:** 153–163, 1974.
84. Lieber, C. S. and L. M. DeCarli. Metabolic effects of alcohol on the liver. In: *Metabolic Aspects of Alcoholism*, edited by C. S. Lieber. Baltimore: University Press, 1977, pp. 31–79.
85. Lieber, C. S. and R. Schmid. The effect of ethanol on fatty acid metabolism: Stimulation of hepatic fatty acid synthesis in vitro. *J. clin. Invest.* **40:** 394–399, 1961.
86. Lieber, C. S. and N. Spritz. Effects of prolonged ethanol intake in man: Role of dietary, adipose, and endogenously synthesized fatty acids in the pathogenesis of the alcoholic fatty liver. *J. clin. Invest.* **45:** 1400–1411, 1966.
87. Lieber, C. S., D. P. Jones and L. M. DeCarli. Effects of prolonged ethanol intake: Production of fatty liver despite adequate diets. *J. clin. Invest.* **44:** 1009–1020, 1965.
88. Lieber, C. S., N. Spritz and L. M. DeCarli. Role of dietary, adipose and endogenously synthesized fatty acids in the pathogenesis of the alcoholic fatty liver. *J. clin. Invest.* **45:** 51–62, 1966.
89. Lieber, C. S., N. Spritz and L. M. DeCarli. Fatty liver produced by dietary deficiencies: Its pathogenesis and potentiation by ethanol. *J. Lipid Res.* **10:** 283–287, 1969.
90. Lieber, C. S., D. P. Jones, M. S. Losowsky and C. S. Davidson. Interrelation of uric acid and ethanol metabolism in man. *J. clin. Invest.* **41:** 1863–1870, 1962.
91. Lieber, C. S., D. P. Jones, J. Mendelson and L. M. DeCarli. Fatty liver, hyperlipemia and hyperuricemia produced by prolonged alcohol consumption, despite adequate dietary intake. *Trans. Ass. Am. Physns* **76:** 289–300, 1963.
92. Lieber, C. S., S. M. Leevy, S. W. Stein, W. S. George, G. R. Cherrick, W. H. Abelmann and C. S. Davidson. Effect of ethanol on plasma free fatty acids in man. *J. Lab. clin. Med.* **59:** 826–832, 1962.
93. Lieber, C. S., A. Lefevre, N. Spritz, L. Feinman and L. M. DeCarli. Difference in hepatic metabolism of long- and medium-chain fatty acids: The role of fatty acid chain length in the production of the alcoholic fatty liver. *J. clin. Invest.* **46:** 1451–1460, 1967.
94. Lin, C. T. and J. P. Chang. Electron microscopy of albumin synthesis. *Science* **190:** 465–467, 1975.
95. Linday, S., F. B. Pedersen, H. Turto and J. Uitto. Lactate, lactate dehydrongenase and protocollagen proline hydroxylase in rat skin autograft. *Hoppe-Seyler's Z. physiol. Chem.* **352:** 1113, 1971.

96. Lundquist, F., N. Tygstrup, K. Winkler, K. Mellemaard and S. Munck-Petersen. Ethanol metabolism and production of free acetate in the human liver. *J. clin. Invest.* **41**: 955–961, 1962.
97. Maijer, A. J. and J. R. Williamson. Transfer of reducing equivalents across the mitochondrial membrane. I. Hydrogen transfer mechanisms involved in the reduction of pyruvate to lactate in isolated liver cells. *Biochim. biophys. Acta* **333**: 1–11, 1974.
98. Matsuda, Y., T. Worner and C. S. Lieber. Ultrastructure and pathogenesis of perivenular sclerosis (PVS) in alcoholic liver injury. *Gastroenterology* **77**: A26, 1979.
99. Matsuda, Y., E. Baraona, M. Salaspuro and C. S. Lieber. Effects of ethanol on liver microtubules and Golgi apparatus: Possible role in altered hepatic secretion of plasma proteins. *Lab. Invest.* **41**: 455–453, 1979.
100. Matsuzaki, S. and C. S. Lieber. Increased susceptibility of hepatic mitochondria to the toxicity of acetaldehyde after chronic ethanol consumption. *Biochem. Biophys. Res. Commun.* **75**: 1059–1065, 1977.
101. Mendenhall, C. L. Origin of hepatic triglyceride fatty acids: Quantitative estimation of the relative contributions of linoleic acid by diet and adipose tissues in normal and ethanol fed rats. *J. Lipid Res.* **13**: 177–183, 1972.
102. Mezey, E., J. J. Potter and R. A. Ammon. Effect of ethanol administration on the activity of hepatic lysosomal enzymes. *Biochem. Pharmac.* **25**: 2663–2667, 1976.
103. Mezey, E., J. J. Potter, R. J. Slusser and W. Abdi. Changes in hepatic collagen metabolism in rats produced by chronic ethanol feeding. *Lab. Invest.* **36**: 206–214, 1977.
104. Morgan, M. Y., J. P. Milsom and S. Sherlock. Ratio of plasma alpha amino-n-butyric acid to leucine as an empirical marker of alcoholism: Diagnostic value. *Science* **197**: 1183–1185, 1977.
105. Morland, J. Effects of chronic ethanol treatment on tryptophan oxygenase, tyrosine aminotransferase and general protein metabolism in the intact and perfused rat liver. *Biochem. Pharmac.* **73**: 71–75, 1974.
106. Morland, J. and A. Bessesen. Inhibition of protein synthesis by ethanol in isolated rat liver parenchymal cells. *Biochem. biophys. Acta* **474**: 312–320, 1977.
107. Morland, J., M. A. Rothschild, M. Oratz, J. Mongelli, D. Donor and S. S. Schreiber. The lack of effect of ethanol on protein export in isolated rat hepatocytes. *Gastroenterology* **77**: A29, 1979.
108. Moura, D., I. Azevedo and W. Osswald. Hepatotoxicity of the condensation product of adrenaline with acetaldehyde. *J. Pharm. Pharmac.* **29**: 255–256, 1977.
109. National Academy of Sciences, National Research Council. *Nutrient Requirements of Nonhuman Primates*. Washington, D.C., 1978, Vol. 14.
110. National Academy of Sciences. *Recommended Daily Allowance*, 9th edition, Washington, D.C., 1979.
111. Nikkila, E. A. and K. Ojala. Role of hepatic L-a-glycerophosphate and triglyceride synthesis in production of fatty liver by ethanol. *Proc. Soc. exp. Biol. Med.* **113**: 814–817, 1963.
112. Ontko, J. A. Effects of ethanol on the metabolism of free fatty acids in isolated liver cells. *J. Lipid Res.* **14**: 78–86, 1973.
113. Okazaki. I., L. Feinman and C. S. Lieber. Hepatic mammalian collagenase: Development of an assay and demonstration of reversal activity after ethanol consumption. *Gastroenterology* **73**: 1236, 1977.
114. Patek, A. J., Jr., J. Post and O. D. Ratnoff. Dietary treatment of cirrhosis of the liver. *J. Am. Med. Ass.* **138**: 543–549, 1948.
115. Patek, A. J. Jr., S. Bowry and K. C. Hayes. Cirrhosis of choline deficiency in the rhesus monkey. Possible role of dietary cholesterol. *Proc. Soc. exp. Biol. Med.* **148**: 370–374, 1975.
116. Patek, A. J., E. G. Toth, M. G. Saunders, G. A. M. Castro and J. J. Engel. Alcohol and dietary factors in cirrhosis. *Archs Int. Med.* **135**: 1053–1057, 1975.
117. Perin, A. and A. Sessa. In vitro effects of ethanol and acetaldehyde on tissue protein synthesis. In: *The Role of Acetaldehyde in the Action of Ethanol*, edited by K. O. Lindros and C. J. P. Eriksson. 6th Int. Congr. Pharmac. Helsinki: The Finnish Foundation for Alcohol Studies, 1975, pp. 105–122.
118. Perin, A., G. Scalabrino, A. Sessa and A. Arnaboldi. In vitro inhibition of protein synthesis in rat liver as consequence of ethanol metabolism. *Biochim. biophys. Acta* **366**: 101–108, 1974.
119. Peters, R. L., T. Gay and T. B. Reynolds. Postjejunoileal bypass hepatic disease. Its similarity to alcoholic hepatic disease. *Am. J. clin. Pathol.* **63**: 318–331, 1975.
120. Pikkarainen, P. H., M. P. Salaspuro and C. S. Lieber. A method for the determination of "free" acetaldehyde in plasma. *Alcoholism: Clin. Exp. Res.* **3**: 259–261, 1979.
121. Pikkarainen, P., E. Baraona, F. Finkelman and C. S. Lieber. Mechanism of the ethanol-induced inhibition of hepatic protein synthesis *in vitro*. *Gastroenterology* **77**: A32, 1979.
122. Platt, D., U. Stein and H. Heissmeyer. Untersuchungen zum katabolen Mucopolysaccharid-proteinstoffwechsel der Rattenleber nach Gabe von Athonol. *Z. Klin. Chem. U. Klin. Biochem.* **9**: 126–129, 1971.
123. Popper, H. and C. S. Lieber. Histogenesis of alcoholic fibrosis and cirrhosis in the baboon. *Am. J. Pathol.* **98**: 695–716, 1980.
124. Powell, L. W., M. L. Bassett and J. W. Halliday. Hemochromatosis: 1980 Update. *Gastroenterology* **78**: 374–384, 1980.
125. Reaven, E. P. and G. M. Reaven. Evidence that microtubules play a permissive role in hepatocyte very low density lipoprotein secretion. *J. Cell Biol.* **84**: 28–39, 1980.
126. Renis, M., A. Giovine and A. Bertolino. Protein synthesis in mitochondrial fractions from rat brain and liver after acute and chronic ethanol administration. *Life Sci.* **16**: 1447–1458, 1975.
127. Rodrigo, C., C. Antezana and E. Baraona. Fat and nitrogen balance in rats with alcohol-induced fatty liver. *J. Nutr.* **101**: 1307–1310, 1971.
128. Rothschild, M. A., M .Oratz, J. Mongelli and S. S. Schreiber. Alcohol induced depression of albumin synthesis: Reversal by tryptophan. *J. clin. Invest.* **50**: 1812–1818, 1971.
129. Rubin, E. and C. S. Lieber. Early fine structural changes in the human liver induced by alcohol. *Gastroenterology* **52**: 1–13, 1967.
130. Rubin, E., D. S. Beattie and C. S. Lieber. Effects of ethanol on the biogenesis of mitochondrial functions. *Lab. Invest.* **23**: 620–627, 1970.
131. Rudolph, R., W. J. McClure and M. Woodward. Contractile fibroblasts in chronic alcoholic cirrhosis. *Gastroenterology* **76**: 704–709, 1979.
132. Salaspuro, M. P. and C. S. Lieber. Metabolic consequences of chronic alcohol consumption: Attenuation of hepatic redox changes despite enhanced capacity to eliminate ethanol. In: *Currents in Alcoholism: Biological, Biochemical and Clinical Studies*, Vol. V, guest editor, M. Galanter. New York: Grune and Stratton, 1979, pp. 109–118.
133. Salaspuro, M. P. and C. S. Lieber. Accelerated ethanol metabolism after chronic consumption with special references to nonlinearity of blood ethanol elimination and associated redox changes. In: *Proceedings of the National Institute on Alcohol Abuse and Alcoholism Workshop "Alcohol and Nutrition"*, edited by T. K. Li, S. Schenker and L. Lumeng. Research Monograph No. 2, DHEW Publication (ADM) 79-780. Washington, D.C.: U.S. Gov. Printing Office, 1979, pp. 349–366.
134. Seakins, A. and D. S. Robinson. Changes associated with the production of fatty livers by white phosphorus and by ethanol in the rat. *Biochem. J.* **92**: 308–312, 1964.
135. Shaw, S. and C. S. Lieber. Plasma amino acid abnormalities in the alcoholic: Respective role of ethanol, nutrition and liver injury. *Gastroenterology* **74**: 677–682, 1978.
136. Shaw, S. and C. S. Lieber. Incrreased hepatic production of alpha amino-n-butyric acid in rats and baboons. *Gastroenterology* **78**: 108–113, 1980.

137. Shaw, S., B. Stimmel and C. S. Lieber. Plasma alpha amino-n-butyric acid to leucine ratio. An empirical biochemical marker of alcoholism. *Science* **194**: 1057–1058, 1976.
138. Shaw, S., S-L. Lue and C. S. Lieber. Biochemical tests for the detection of alcoholism: Comparison of plasma alpha-amino-n-butyric acid with other available tests. *Alcoholism* **2**: 3–7, 1978.
139. Shaw, S., E. Jayatilleke and C. S. Lieber. Hepatic lipid peroxidation: Potentiation by chronic alcohol feeding and attenuation by methionine. *Clin. Res.* **28**: 232, 1980.
140. Shaw, S., E. Jayatilleke, W. A. Ross and C. S. Lieber. Hepatic lipid peroxidation and glutathione depression after alcohol. *Gastroenterology* **77**: 41, 1979.
141. Shaw, S., T. Worner, M. Borysow, R. E. Schmitz and C. S. Lieber. Comparative diagnostic value of MCV, GGTP, and AANB. *Alcoholism* **4**: 297–301, 1979.
142. Sorrell, M. F. and D. J. Tuma. Selective impairment of glycoprotein metabolism by ethanol and acetaldehyde in rat liver slices. *Gastroenterology* **75**: 200, 1978.
143. Sorrell, M. F., D. J. Tuma and A. J. Barak. Evidence that acetaldehyde irreversibly impairs glycoprotein metabolism in liver slices. *Gastroenterology* **73**: 1138–1141, 1977.
144. Sprince, H., C. M. Parker, G. Smith and L. J. Gonzales. Protection against acetaldehyde toxicity in the rat by L-cysteine, thiamine and L-2-methyl-thiazolidene-4-carboxylic acid. *Agents Actions* **4**: 125–130, 1974.
145. Stanko, R. T., E. L. Morse and S. A. Adibi. Prevention of effects of ethanol on amino acid concentrations in plasma and tissues by hepatic lipotropic factors in rats. *Gastroenterology* **76**: 132–138, 1979.
146. Swendseid, M. E., C. Yamada, E. Vinyard, W. G. Figueroa and E. J. Drenick. Plasma amino acid levels in subjects fed isonitrogenous diets containing different proportions of fat and carbohydrate. *Am. J. clin. Nutr.* **20**: 52–55, 1967.
147. Teschke, R., A. Brand and G. Strohmeyer. Induction of hepatic microsomal gamma-glutamyltransferase activity following chronic alcohol consumption. *Biochem. Biophys. Res. Commun.* **75**: 718–724, 1977.
148. Van Waes, L. and C. S. Lieber. Early perivenular sclerosis in the alcoholic fatty liver: An index of progressive liver injury. *Gastroenterology* **73**: 646–650, 1977.
149. Van Waes, L. and C. S. Lieber. Glutamate dehydrogenase, a reliable marker of liver cell necrosis in the alcoholic. *Br. J. Med.* **2**: 1508–1510, 1977.
150. Wands, J. R., E. A. Carter, N. L. R. Bucher and K. J. Isselbacher. Inhibition of hepatic regeneration in rats by acute and chronic ethanol intoxication. *Gastroenterology* **77**: 528–531, 1979.
151. Wilgram, G. F. Experimental Laennec type of cirrhosis in monkeys. *Ann. intern. Med.* **51**: 1134–1158, 1959.
152. Worner, T. M. and C. S. Lieber. Prognostic value of perivenular sclerosis in the progression of alcoholic liver injury. *Gastroenterology* **75**: 995, 1978.
153. Worner, T. M. and C. S. Lieber. Plasma glutamate dehydrogenase: Clinical application in patients with alcoholic liver disease. *Alcoholism: Clin. Exper. Res.* **4**: 431–434, 1980.
154. Wu, A., G. Slavin and A. J. Levi. Elevated serum gamma-glutamyl-transferase (transpeptidase) and histological liver damage in alcoholism. *Am. J. Gastroenterol.* **65**: 318–323, 1976.