Pathogenesis of Alcohol-Induced Accumulation of Protein in the Liver

E. Baraona, M. A. Leo, S. A. Borowsky, and C. S. Lieber

From the Laboratory of Liver Disease, Nutrition, and Alcoholism, Bronx Veterans Administration Hospital, Bronx, New York 10468, and Mount Sinai School of Medicine, of the City University of New York, New York 10029

ABSTRACT Alcohol feeding to rats produced hepatomegaly, associated with enlargement of the hepatocytes. The increase in liver dry weight was accounted for not only by fat but also by protein accumulation, primarily in microsomes and cytosol, with a selective increase in export proteins: concentrations of both immunoreactive albumin and transferrin were augmented in liver microsomes and cytosol of ethanol-fed rats. To investigate the mechanism of this protein accumulation, [14C]leucine was injected intravenously and its incorporation into both liver and serum proteins was measured after various time intervals. Rates of synthesis and export were assessed from protein labeling and specific activities of leucyl-tRNA. Synthesis of liver protein and proalbumin were enhanced by chronic ethanol feeding, but this was not associated with a corresponding rise in serum albumin output. Actually, there was a significant retention of the label in liver albumin and transferrin with delayed appearance in the serum of ethanol-fed rats. This indicated that, regardless of the changes in synthesis, the export of protein from the liver into the plasma was impaired. This alteration in export was associated with a decreased amount of polymerized tubulin in the liver of ethanol-treated animals. Thus, both enhanced protein synthesis and defective export contribute to the ethanol-induced accumulation of liver protein, and the decrease in liver microtubules represents a possible site for impairment of protein export.

INTRODUCTION

Enlargement of the liver is common in alcoholics. Hepatomegaly has been produced also in rats fed nutritionally adequate diets containing 36% of calories as ethanol. We recently showed, in a preliminary report (1), that the increase in liver dry weight was not merely the result of fat accumulation, but also of increased liver protein, with a selective accumulation of some proteins which are primarily exported from the liver. The present study was undertaken to expand the observation of protein accumulation in the liver and to assess its mechanisms, particularly the possible roles of increased hepatic synthesis and decreased disposition of proteins.

METHODS

Materials. L-[U-14C]-(325 mCi/mmol) and L-[N-4,5-3H]- (5 Ci/mmol)-leucine and [ring C, methoxy-3H]colchicine (18.45 mCi/mmol) were purchased from New England Nuclear (Boston, Mass.). The immunoglobulin G fractions of rabbit antirat serum albumin and antirat transferrin as well as rat serum albumin and transferrin were purchased from Cappel Laboratories, Inc. (Downingtown, Pa.) and shown to react specifically with the corresponding serum proteins by agarose immunoelectrophoresis. Horse spleen ferritin and rabbit antihorse ferritin were obtained from Miles Laboratories Inc. (Elkhart, Ind.). Proteins with an immunoelectrophoretic purity 95% were used as standards. Rat serum albumin was labeled "in vitro" with 14C by the method of Rice and Means (2), resulting in albumin-specific activities of $3 \times 10^9$ dpm/mg. Proalbumin was prepared from the immunoprecipitates of aceton powder extracts of liver microsomes by the procedure of Quinn et al. (3). The composition of the liquid diets used in this study has been previously described (4). They contain 18% of the total calories as protein, 35% as fat, 11% as carbohydrate, and 36% either as ethanol (alcohol diet) or as additional carbohydrate (control diet).

Animal procedures. Weanling CD male rat littermates, purchased from Charles River Breeding Laboratories (Wilmington, Mass.), were fed Purina chow (Ralston Purina Co., St. Louis, Mo.) until they reached a weight of 130–150 g. Then they were housed in individual cages and pair-fed the liquid diets described above for 4–6 wk. To minimize differences in the time of food intake preceding sacrifice, one-third of the amount of diet usually consumed was given at 9 a.m. and two-thirds at 5 p.m. the day before
sacrifice. On the following day (between 9–10 a.m.), rats were weighed and given 6 ml of diet/100 g body wt by gastric tube 90 or 150 (five pairs) min before sacrifice. This amount corresponds to one-fifth of the usual daily food intake of the pair-fed animals. In the case of the alcohol diet, this dose is equivalent to 3 g of ethanol/kg body wt and results in blood ethanol concentrations of 130–150 mg/100 ml at 90 min and 153±9 mg/100 ml at 120 min (5, 6).

To assess contamination of the liver extracts with plasma, 1 mg of the 14C-labeled rat serum albumin was injected in the dorsal vein of the penis, under ether anesthesia, 48 h before sacrifice. This interval was chosen to allow for equilibration with albumin of the extracellular space (7).

To study the time-course of labeling in liver and serum, [14C]leucine was injected in the dorsal vein of the penis at various times (0.3, 1, 2.5, 5, 7.5, 10, 30, 45, 90 and 120 min) before sacrifice.

Rats were sacrificed by exsanguination from the aorta, under ether anesthesia. For determinations of protein content and radioactivity in serum and liver, blood was obtained from the aorta and this was followed by perfusion of the liver with 50–100 ml of ice-cold saline through the portal vein to minimize contamination of liver extracts with blood. Liver perfusion was also required for the measurement of cytochrome P-450 in the liver homogenates (8). For determinations of specific activities of serum and liver leucine and those of leucine bound to transfer ribonucleic acid (leucyl-tRNA), liver perfusion was omitted, blood was obtained from the aorta, and this was followed by freezing of the liver in situ between aluminum blocks previously cooled in liquid nitrogen. Data on liver weight, specific gravity, morphology, and microtubules were also obtained in rats in which the liver perfusion was omitted. Liver volume was determined by displacement of water at 20°C. Samples of the liver were dried in a dessicator containing Drierite (Drierite Co., Xenia, Ohio). Liver specimens were fixed in neutral formalin (10 g/100 ml) and stained with hematoxylin and eosin for morphological assessment. Hepatocyte area was calculated by counting the number of hepatocytes per standard area. Blind assessment was not possible because the ethanol-fed rats had visible accumulation of liver lipids.

Subcellular fractionation. Livers were homogenized in 3 vol of ice-cold 0.15 M KCl or 0.25 M sucrose by two up and down strokes in a Potter-Elvehjem homogenizer with a motor driven teflon pestle. Because the bulk of liver protein is contained in mitochondria, microsomes, and cytosol, these fractions were isolated by centrifugation. Nuclei and cell debris were sedimented at 700 g. The supernate was further centrifuged at 9,000 g for 30 min. The sediment was used as a source of mitochondria, whereas the supernate was again centrifuged at 104,000 g for 60 min to produce a microsomal pellet and a cytosolic supernate. To correct for the recovery of these fractions, glutamate dehydrogenase activity (9) was used as a marker of mitochondria (10, 11); cytochrome P-450 (12), as a marker of microsomes (8); and alcohol dehydrogenase (13), as a marker of cytosol (10). The protein content of each fraction was calculated by dividing the activity or content of the markers in the initial homogenate by their specific activities (per milligram of protein) in the subcellular fractions.

Analytical procedures. Total liver lipids were extracted according to the method of Folch et al. (14) and measured by the method of Amenta (15). Liver proteins were extracted as described by the method of Lowry et al. (17), and their radioactivity determined by liquid scintillation counting in Aquasol (New England Nuclear, Boston, Mass.) DNA was measured in the liver by the method of Martin et al. (18). Albumin and transferrin were measured in serum and liver by radial immunodiffusion (19), using the corresponding serum proteins as standards. These proteins were extracted from particulate liver fractions with both 0.5% deoxycholate (20) and ultrasonic vibration (21). Total liver immunoreactive albumin was also calculated from the isotopic dilution of labeled serum albumin in the acid ethanol extracts of the immunoprecipitates, as described by Gordon and Humphrey (7). Hepatic proalbumin was determined by isotopic dilution according to the method of Dorling et al. (22). Liver ferritin was measured by radial immunodiffusion using horse spleen ferritin as standard, according to the method of Bjerkild and Helgeland (23). Plasma volume was calculated from the dilution of T-1824 (Evans blue), as described by Gregersen (24), except that the dye was measured after clearing the serum of particulate fat by ultra-centrifugation at 1.3 × 106 g/min, because turbidity due to hyperlipemia was present in the ethanol-fed rats. Leucine concentrations and radioactivities were measured by ion exchange chromatography in an automated amino acid analyzer (model 119, Beckman Instruments, Inc., Cedar Grove, N. J.) provided with stream splitter device. Leucine-specific activities were determined in serum (25), protein-free supernates of the liver (26), and in hepatic leucyl-tRNA at various time intervals. The method used by Wallyn et al. (27) for determination of valyl-tRNA-specific activity was applied to leucyl-tRNA after demonstration that leucine, similarly to valine, originated solely from the amino acyl-tRNA pool. There was no contamination of the leucine bound to tRNA with differently labeled leucine added to the initial homogenates either in the free state or incorporated into serum albumin.

Rates of protein synthesis were calculated according to Morgan and Peters (26), except that the integral of the specific activity curve of leucyl-tRNA was used instead of that of free leucine (26, 28) because of the reported amino acid compartmentation during protein synthesis (29, 30). For calculation of the synthesis of total liver protein and proalbumin, the incorporation after 10 min of intravenous injection of [14C]leucine was used. The values for leucine content of total liver protein (28) and proalbumin (3, 31) were obtained from the literature. Hepatic production of serum albumin was calculated from the data on leucine incorporation into serum albumin 120 min after injection, as described by Morgan and Peters (26).

Liver microtubules were assessed by the amount of polymeric tubulin, their major component. Free tubulin was measured by taking advantage of the ability of its dimer form to bind [3H]colchicine in a 1:1 molar ratio (32). Unbound colchicine was adsorbed on activated charcoal (33). Polymeric tubulin was calculated from the difference in colchicine binding when microtubule depolymerization was allowed to occur (total tubulin) and when it was prevented (free tubulin), as recently reported by Patzelt et al. (34), except that the method to preserve the microtubules was that of Filner and Behnke (35). Subsequent depolymerization of the preserved microtubules produced an amount of tubulin equivalent to 84% of that calculated from the difference between total and free tubulin.

Statistics. The values obtained in the alcohol-fed animals were compared to those obtained in their pair-fed controls and the means of the individual differences were tested by the Student’s t test (36). All values are expressed by their means±SEM.

RESULTS
Effects of ethanol feeding on body and liver weights. As previously reported (37), the ethanol-fed rats gained
less weight than their pair-fed controls. Thus, at the
time of 94% lower than those of pair-fed controls
(229±10 vs. 253±11 g in controls; 24 pairs, P < 0.001).
Despite the smaller body weight, the livers of the
ethanol-fed rats were significantly heavier than those of
controls (9.72±0.47 vs. 8.47±0.36 g; 24 pairs, P < 0.01). Expressed per 100 g of body wt, the liver
increased in weight by 27% in the rats fed ethanol.
This was associated with a parallel increase in dry
liver weight (1.32±0.39 vs. 1.01±0.20 g/100 g body wt,
in the controls; 24 pairs, P < 0.01). The wet weight/dry
weight ratios were similar in both groups of animals.
Ethanol feeding did not change the specific gravity of
the liver (1.009±0.010 vs. 1.016±0.017 g/ml in con-
controls; six pairs, NS). Thus, the changes in weight
were accompanied by proportional changes in volume.

Effects of ethanol feeding on liver lipids, pro-
tein, and DNA. As expected, ethanol feeding in-
creased total liver lipids (from 145±10 mg/100 g body
wt in the controls to 302±35 mg/100 g body wt, in
the ethanol-fed rats; 12 pairs, P < 0.01). Total liver
proteins also increased (from 573±23 mg/100 g body
wt in the controls to 706±40 mg/100 g body wt in the
ethanol-fed animals; 12 pairs, P < 0.01). In 12 additional pairs (Table I), in which
livers were perfused with saline before subcellular
fractionation, a similar increase in total protein was
found. The absolute increases in lipid (151±29 mg/100 g body wt) and in protein (142±24 mg/100 g
body wt) together accounted for almost the entire
increase in dry liver weight (304±40 mg/100 g body
wt). However, the concentration of protein in the liver
did not change (178±9 vs. 173±8 mg/g of liver in
controls; 12 pairs, NS), indicating that the increase in
protein was associated with a proportional increase in
water. By contrast, total liver DNA in ethanol-fed
rats was similar to that of controls (10.8±0.5 vs.
10.3±0.4 mg/100 g body wt in control; 10 pairs, NS),
with a corresponding decrease in concentration.
Histologic sections of the liver showed that hepatocytes
of ethanol-fed rats occupied a significantly larger
area than those of pair-fed controls (602±43 vs.
442±36 μm² in controls; six pairs, P < 0.01), thus
accounting for the hepatomegaly.

The recovery of mitochondria, microsomes, and
cytosol in ethanol-fed rats (26.4±2.4, 60.0±1.7, and 56.0
±5.2%, respectively) was similar to that of pair-fed
controls (28.7±1.6, 58.1±5.0, and 57.6±5.1%, respect-
ively). After correction for recovery, both microsomes
and cytosol contributed significantly to the increase in
liver protein induced by ethanol feeding (Table I).
The greatest contribution to the protein increase
was accounted for by the cytosol.

Effects of ethanol feeding on the composition of
liver proteins. Liver protein not only increased in
amount, but also changed in composition. Although
total protein concentration did not change, the con-
centrations of albumin and transferrin increased sig-
nificantly in the liver of ethanol-fed rats compared
to their pair-fed controls. Liver extracts from alcohol
and control rats were equally contaminated with plasma
(28.5±5.3 vs. 25.3±5.6 μg of liver in controls;
10 pairs, NS). After subtraction of the con-
taminating plasma proteins, albumin and transferrin
concentrations were found to be significantly in-
creased both in microsomes and cytosol, as well as
in the total liver extracts (Table II). Per 100 g body
wt, the increases in total liver albumin (3.38±0.32
vs. 2.08±0.11 mg in controls; P < 0.01) and in trans-
ferrin (1.57±0.09 vs. 1.04±0.06 mg in controls; P <
0.01) were greater than per gram of liver, because of
the hepatomegaly in the alcohol-treated animals.
Though the values for albumin concentration cal-
culated from the isotopic dilution of labeled serum
albumin in liver extracts were higher than those
measured by radial immunodiffusion, a similar in-
crease was observed in alcohol-fed rats (1.021±0.142
vs. 0.617±0.135 mg/g liver in controls, seven pairs,
P < 0.05). This rise was the result, at least in part, of
increased concentration of proalbumin after ethanol
treatment (0.453±0.055 vs. 0.315±0.031 mg/g liver
in controls, five pairs; P < 0.03).

By contrast, the hepatic concentration of ferritin, a
typical constituent protein, was significantly re-
duced (0.33±0.03 vs. 0.40±0.02 mg/g of liver in con-
controls; seven pairs, P < 0.02).

Effects of ethanol feeding on the synthesis of liver
proteins. Ethanol-fed rats had significantly higher
concentration of leucine in the serum (235±16
nmol/ml) than their pair-fed controls (133±17 nmol/ml;
12 pairs, P < 0.001). Therefore, the radioactive leucine
injected intravenously on a body weight basis, be-
came significantly more diluted in the plasma
leucine of alcohol-treated rats (Fig. 1). Radioactive

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**Table I**

<table>
<thead>
<tr>
<th>Homogenate</th>
<th>Mitochondria</th>
<th>Microsomes</th>
<th>Cytosol</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg of liver protein/100 g of body wt</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethanol-fed rats</td>
<td>778±46</td>
<td>274±31</td>
<td>154±9</td>
</tr>
<tr>
<td>Pair-fed controls</td>
<td>638±19</td>
<td>233±12</td>
<td>125±11</td>
</tr>
<tr>
<td>P†</td>
<td>&lt;0.01</td>
<td>NS</td>
<td>&lt;0.01</td>
</tr>
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</table>

* Determinations were carried out in 12 male rat littersmates pair-fed either alcohol or control diet for 24 days. Livers were
perfused with saline. Degree of recovery of each fraction was
assessed by specific markers (see text).
† Paired comparisons.

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leucine was taken up very rapidly by the liver: maximal specific activities of both free leucine and leucyl-tRNA were reached at about 1 min after injection and the pulse labeling of these pools was almost completed within 10 min. Liver concentration of free leucine was also greater in ethanol-fed rats (273 ± 32 mmol/g of liver vs. 153 ± 15, in controls; 12 pairs, P < 0.02). This increased concentration of liver leucine represents an even greater increase in total liver leucine because of the hepatomegaly of alcohol-fed rats. Thus, further differences in leucine-specific activity were observed both in the liver-free leucine and leucyl-tRNA pools (Fig. 1). Leucine incorporation into liver proteins reached maximal levels by 10 min after leucine injection, followed by progressive decrease in protein radioactivity. At 10 min, leucine incorporation into total liver protein was similar in both groups of animals (1,343 ± 129 dpm x 10^9/100 g body wt in ethanol-fed rats vs. 1,446 ± 87, in controls; 12 pairs, NS), despite the lower specific activity of leucyl-tRNA in ethanol-fed rats. Thus, calculated rates of synthesis of total liver protein were significantly higher in ethanol-fed rats (22.4 ± 2.2 vs. 14.5 ± 1.2 mg/h per 100 g body wt in controls; 12 pairs, P < 0.01). Expressed per gram of liver, this difference was only barely significant (4.97 ± 0.39 vs. 3.94 ± 0.43 mg/h in controls; 12 pairs, P < 0.05).

During the first 10 min after intravenous injection, [14C]leucine was incorporated into liver albumin but not yet into serum albumin. At this time, albumin

### TABLE II
**Effects of Ethanol Feeding on Albumin and Transferrin Concentrations in Rat Liver Cell Fractions**

<table>
<thead>
<tr>
<th>Ethanol-fed rats</th>
<th>Pair-fed controls</th>
<th>Difference</th>
<th>Ethanol-fed rats</th>
<th>Pair-fed controls</th>
<th>Difference</th>
<th>Ethanol-fed rats</th>
<th>Pair-fed controls</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cytosol</strong></td>
<td></td>
<td></td>
<td><strong>Microsomes</strong></td>
<td></td>
<td></td>
<td><strong>Total liver extracts</strong></td>
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<tr>
<td>497</td>
<td>191</td>
<td>+306</td>
<td>270</td>
<td>206</td>
<td>+64</td>
<td>671</td>
<td>513</td>
<td>+158</td>
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<td>506</td>
<td>249</td>
<td>+257</td>
<td>200</td>
<td>144</td>
<td>+56</td>
<td>550</td>
<td>455</td>
<td>+95</td>
</tr>
<tr>
<td>33</td>
<td>53</td>
<td>-20</td>
<td>232</td>
<td>192</td>
<td>+40</td>
<td>659</td>
<td>530</td>
<td>+129</td>
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<td>+258</td>
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<td>415</td>
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<td>480</td>
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<td>498±97</td>
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<td>624±37</td>
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<tr>
<td><strong>II. Transferrin (μg/g of liver)</strong></td>
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<td>168</td>
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<td>229</td>
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<td></td>
<td>147±8</td>
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<td>101±9</td>
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<td>367±22</td>
<td>313±18</td>
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*Values were obtained in rats pair-fed either ethanol-containing or control diets for 4–6 wk. Determinations in total liver extracts and cytosol were carried out in nine pairs and were corrected for protein derived from serum contamination. Microsomal determinations were performed in five additional pairs; there was no detectable contamination of this fraction with serum proteins.

I t = (mean difference)/(standard error of the differences) (36).
labeling was similar in ethanol-treated and control rats (Fig. 2). Over 90% of the radioactivity was recovered in proalbumin (105±19 dpm × 10^9/100 g body wt in ethanol-fed rats vs. 116±19, in controls; five pairs, NS). Most of the incorporation occurred in microsomes, where 82 and 69% of the total albumin labeling was recovered in alcohol and control rats, respectively. By contrast, only 1.2 and 1.3% of the total albumin labeling was recovered in the cytosol of alcohol and control rats, respectively. Because of greater isotopic dilution of the labeled leucine, calculated rates of proalbumin synthesis were greater in ethanol-fed rats than in controls (1.46±0.276 vs. 0.907±0.169 mg/h per 100 g body wt in controls; five pairs, P < 0.01).

**Effects of ethanol feeding on the export of protein from the liver.** At 30 and 45 min after leucine injection, when serum albumin was labeled at maximal rates, total liver albumin labeling became significantly greater in ethanol-fed rats than in controls (Fig. 2). This increased incorporation of leucine into the liver albumin coincided with delayed appearance of radioactivity into serum albumin in the rats fed ethanol as compared to their controls. In controls, serum albumin reached near maximal labeling by 30 min, whereas it took 60–90 min for the ethanol-fed rats to reach comparable levels of serum albumin labeling. Similarly, labeling of serum transferrin was delayed, whereas incorporation into liver transferrin was increased in ethanol-fed rats (Fig. 2).

However, the feeding of ethanol for several weeks did not change the serum concentration of either albumin (33.2±0.8 vs. 30.4±2.4 mg/ml in controls; 12 pairs, NS) or transferrin (3.63±0.13 vs. 3.42±0.01 mg/ml in controls; 12 pairs, NS). Plasma volume was also similar in alcohol and control rats (3.66±0.42 vs. 3.56±0.35 ml/100 g body wt in controls; six pairs, NS). Rates of serum albumin production calculated from serum albumin labeling at 120 min and the specific activity of leucyl-tRNA, were similar in both groups of animals (1.025±0.129 vs. 0.817±0.049 mg/h per 100 g body wt in the pair-fed controls; six pairs, NS).

**Effect of ethanol feeding on liver microtubules.** The concentration of total tubulin was significantly decreased in the hepatic cytosol of ethanol-fed rats compared to their controls, whereas free tubulin remained unaffected (Table III). Thus, polymerized tubulin was significantly decreased, not only per gram of liver (Table III), but also per total liver (938±158 vs. 1,168±108 µg/100 g body wt in controls; six pairs, P < 0.02).

**DISCUSSION**

In the present study we confirm and extend our preliminary report (1), which showed that alcohol-induced hepatomegaly in the rat is the result not merely of accumulation of fat, but also of a striking increase in liver protein. Protein accumulation after alcohol feed-
ing is partially accounted for by proliferation of sub-
cellular organelles which we described before (38,
39); we now find, however, that the greatest increase
in cellular protein occurs in the soluble fraction or
cytosol. Moreover, the increase in protein is associ-
ated with a change in its composition characterized
by increased concentration of proteins normally ex-
ported into the plasma. Indeed, the hepatic concen-
tration of albumin and transferrin was significantly
increased in ethanol-fed rats when contamination
with extracellular proteins was subtracted. It has been
shown that liver proteins other than albumin are precipitated by antibodies against serum albumin
(40, 41). The material present in the immunoprecipi-
tates has properties compatible with those of an al-
bumin precursor—proalbumin (31, 42, 43). The in-
crease in immunoreactive albumin in the liver of
ethanol-fed rats was due, in part, to a parallel in-
crease in proalbumin. Because of the hepatomegaly
in ethanol-fed rats, the differences in concentration
of albumin and transferrin reflect an even larger in-
crease in their total hepatic content. Contrasting with
the increased hepatic concentration of these proteins,
total liver protein concentration did not change. The
concentration of soluble constituent proteins (such as
ferritin, organic anion-binding proteins [44], and
alcohol dehydrogenase [45]) is unchanged or decreased
in ethanol-fed rats.

Increased protein in the liver could result either
from increased synthesis or from decreased export
into the plasma or both. Acute ethanol administra-
tion to animals has been reported to inhibit hepatic
synthesis of both constituent (46–48) and export (49–
52) proteins in vivo and in vitro. Other investigators
have reported that chronic ethanol administration
increases protein synthesis in ribosomes (46) and
microsomes (53), whereas acute administration pro-
duces opposite effects. In our study, synthesis of total
liver protein was significantly enhanced in ethanol-
fed rats compared to pair-fed controls. It must be
pointed out, however, that the incorporation of
\[^{14}\text{C}\]leucine into liver proteins was not affected, but
the specific activity of leucine and leucyl-tRNA was
markedly reduced because of the higher concentra-
tions of both serum and liver leucine in ethanol-fed
rats. Comparable increases in the serum concentra-
tion of branched chain amino acids has been reported
in baboons chronically fed alcohol (54). Similarly,
proalbumin synthesis was also increased in ethanol-
fed rats. However, there is no evidence to suggest
that albumin or transferrin was synthesized at a
faster rate than other proteins, the concentration of
which did not change. Furthermore, if increased al-
bumin synthesis were the primary cause of the ac-
cumulation, one could expect this to be associated
with a parallel increased output into the plasma.
However, 30 and 45 min after \[^{14}\text{C}\]leucine in-
jection, when secretion of newly labeled albumin was
taking place at maximal rates, ethanol feeding pro-
duced opposite changes in liver and serum albumin
labeling (Fig. 2). At 30 and 45 min after \[^{14}\text{C}\]leucine
injection, incorporation into liver albumin was sig-

![Figure 2: Incorporation of intravenously injected \[^{14}\text{C}\]leucine into liver and serum albumin and transferrin at various time intervals in rats pair-fed either ethanol-containing or control diet for 4–6 wk. Each point represents the average of three to six animals. Labeling of liver albumin (30–45 min after injection) and of liver transferrin (30–90 min) was significantly greater in ethanol-fed rats, whereas labeling of serum albumin and transferrin (same time periods) was significantly reduced in these rats, compared to controls (P < 0.01; paired comparisons). After 60 min in the case of serum albumin, and at 120 min in the case of transferrin, the differences in labeling between both types of animals were not statistically significant.](image)

### Table III

**Effects of Ethanol Feeding on Hepatic Microtubular Protein**

<table>
<thead>
<tr>
<th></th>
<th>Total tubulin</th>
<th>Free tubulin</th>
<th>Polymerized tubulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol-fed rats</td>
<td>338±40</td>
<td>187±12</td>
<td>196±33</td>
</tr>
<tr>
<td>Pair-fed controls</td>
<td>423±33</td>
<td>184±10</td>
<td>289±21</td>
</tr>
<tr>
<td><em>p</em></td>
<td>&lt;0.01</td>
<td>NS</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

* Determinations were carried out in 12 male rat littermates of rats pair-fed either alcohol or control diet for 24 days.

**Pair** Comparisons.
significantly increased, whereas incorporation into serum albumin was significantly decreased. Only after 60 and 90 min did serum albumin labeling reach levels comparable to those of controls. This delay cannot be attributed to an enlargement of the serum albumin pool, because both plasma volume and serum albumin concentrations were similar in alcohol-treated and control rats. Thus, the triad of increased albumin concentration in the liver, delayed transport of newly labeled albumin into the serum, and retention of albumin label in the liver, indicates that ethanol feeding interferes with the export of albumin, leading to its accumulation in the liver. These alterations are similar to those reported (22) after rats were given colchicine, an inhibitor of protein secretion (55–59). The modest increase in proalbumin after colchicine was attributed to concomitant inhibition of proalbumin synthesis (22). This was not the case in our rats chronically fed alcohol, in which the synthesis of proalbumin was increased rather than decreased. Nevertheless, the amount of albumin retained after several weeks of ethanol feeding appears much smaller than that expected from the difference between the calculated rates of proalbumin and albumin synthesis. This suggests that most of the retained albumin in these rats may be disposed by proteolysis. This possibility is consistent with the increased urea excretion previously reported in ethanol-fed rats (60) and with the increased concentrations of branched chain amino acids observed in this study. Because these amino acids are not significantly oxidized in the liver, their production has been used as an indicator of hepatic proteolysis (30). The effects of ethanol feeding on liver and serum transferrin labeling were similar to those on albumin (Fig. 2). Because the time required for transferrin to be secreted is longer than that for albumin (26), the differences in transferrin labeling were more persistent than those in albumin.

Export proteins are produced by ribosomes attached to the membranes of the rough endoplasmic reticulum (61, 62), from where they are thought to be released into the microsomal cisterna (20, 21), though the electron microscope evidence is conflicting (63, 64). It is therefore intriguing that, after alcohol feeding, albumin and transferrin concentrations increased not only in the microsomal fraction but also in the cytosol despite subtraction of the extracellular albumin or transferrin contaminating the liver extracts. Retention of albumin in the liver cytosol has also been reported in rats with chronic uremia (65). The possibility that microsomes from ethanol-fed rats could leak out more of their content during centrifugation is not likely because after administration of labeled leucine, the newly labeled albumin remained in the microsomal fraction, with only minimal labeling of the cytosolic albumin. One must then wonder whether the cytosol becomes a storage site for retained export proteins.

The alteration of the export proteins from the liver produced by ethanol feeding could take place at many possible sites. Inasmuch as it appears to affect more than a single protein of the export class, a common site in their transport pathways should be looked for. One such possible site could be the microtubular system. This system has been postulated to play a role in the export of proteins in the liver as well as in other organs (55–59). The evidence is based mainly on the effects of colchicine and other related alkaloids which bind the microtubular protein (tubulin) and interfere with its assembly into microtubules (66) as well as inhibit the export of proteins from the liver (55–59). From the difference in colchicine binding prior and after microtubule depolymerization, it was calculated that ethanol feeding markedly reduces the amount of polymerized tubulin as well as that of total tubulin. Microtubules decreased not merely in concentration, but also when expressed per total liver; thus, this decrease cannot be accounted for by dilution. The mechanism of this hitherto unrecognized effect of ethanol remains to be elucidated.

Thus, the alteration in microtubules induced by ethanol feeding may account, at least in part, for the delayed export of serum protein from the liver. This alteration in export leads to intrahepatic accumulation of protein. The contribution of this retention of export proteins to the total increase in liver protein is difficult to assess from the present data because only two proteins of the export class have been measured and the sum of both increases (1.83 mg/100 g body wt) accounts for only a small portion of the total increase in soluble proteins. The possible contribution of other secretory or constituent proteins awaits further assessment. In any event, at this stage of alcoholic liver damage, the delay in export is not sufficient to change serum protein pool or total output of albumin into the serum. Increased synthesis of nascent albumin in the liver may reflect a homeostatic compensation to maintain plasma levels. In the course of advancing alcoholic liver damage, decreased concentration of serum protein could result from either progressive deterioration of the secretory mechanism and/or concomitant failure in protein synthesis.

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552 E. Baraona, M. A. Leo, S. A. Borowsky, and C. S. Lieber
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E. Baraona, M. A. Leo, S. A. Borowsky, and C. S. Lieber