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EFFECT OF ETHANOL ON THE AMOUNT AND ENZYME ACTIVITIES OF HEPATIC ROUGH AND SMOOTH MICROSOMAL MEMBRANES

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SUMMARY

To study the effect of chronic ethanol administration on the hepatic endoplasmic reticulum, rough and smooth microsomal membranes were separated from the liver of female rats pair-fed a nutritionally adequate diet with 36% of total calories either as ethanol or carbohydrate (controls). 6 weeks of ethanol feeding increased protein and phospholipid contents in smooth but not in rough microsomes. Cytochrome P-450 also increased mainly in smooth microsomes. In controls, the activity of the microsomal ethanol-oxidizing system was distributed about equally among the subfractions, whereas after ethanol feeding, it increased mainly in smooth microsomes. Catalase activity after ethanol feeding did not increase; when expressed per mg of microsomal protein, it decreased in smooth microsomes. A reduction of dietary fat content from 35 to 10% of total calories did not influence the effect of ethanol on the hepatic endoplasmic reticulum.

INTRODUCTION

Morphological studies of the effects of chronic ethanol administration on the hepatic endoplasmic reticulum, especially the smooth membranes, have yielded conflicting results. Iseri *et al.*[1,2] reported that chronic ethanol feeding to rats results in a proliferation of the smooth endoplasmic reticulum and this was subsequently confirmed under a variety of conditions both in man[3–5] and in rats[6,7]. However, some other investigators failed to observe significant changes of this organelle after ethanol feeding[8]. Furthermore, Dobbins *et al.*[9,10] using a morphometric method, reported a 50% reduction of rough and smooth membranes after chronic ethanol administration.

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The controversy engendered by these morphologic studies prompted us to use biochemical methods. We also wished to elucidate the submicrosomal site of the microsomal ethanol oxidizing system and its response to ethanol feeding, as well as the concomitant changes in microsomal cytochrome P-450.

MATERIALS AND METHODS

Animal procedures

Sprague–Dawley (CD) rats purchased from Charles River Breeding Laboratories (Wilmington, Mass.) in groups of weanling female littermates were fed Purina laboratory chow and tap water *ad libitum* until the start of the study at which time they were housed in individual wired bottom cages.

In the first set of experiments, a group of nine pairs of female rats were pair-fed a nutritionally adequate liquid diet as previously described[11]. The regular diet provided 35% of total calories as fat, 11% as carbohydrate, 18% as protein and 36% either as ethanol or additional carbohydrate (controls). In the second set of experiments, five pairs of female rats were pair-fed a relatively low fat diet (10% of total calories as fat) with 36% of total calories as carbohydrate, 18% as protein and 36% either as ethanol or additional carbohydrate.

In both sets of experiments the rats were fed for a period of 6 weeks. All rats were fasted for 18 h before sacrifice (by decapitation). The livers were immediately perfused with ice-cold 0.25 M sucrose, quickly excised and weighed and all subsequent steps were performed at 0 to 4 °C. Liver homogenates were obtained with 3 vol. of 0.25 M sucrose using a glass homogenizer with a teflon pestle. The homogenates were centrifuged at 12000 × *g* for 20 min in a Sorvall RC2B refrigerated centrifuge. The supernatant fraction ("12000 × *g* supernate") was mixed with CsCl to a final concentration of 15 mM. Total microsomes were obtained by centrifugation of the "12000 × *g* supernate–15 mM CsCl" in a No. 50 titanium rotor at 105000 × *g* for 90 min and the pellet resuspended in 0.25 M sucrose.

Separation of rough and smooth microsomes of the liver

The rough and smooth microsomes were isolated according to Bergstrand and Dallner[12]: the "12000 × *g* supernate–15 mM CsCl" was layered over 1.3 M sucrose–15 mM CsCl and centrifuged in a No. 42.2 rotor at 105000 × *g* for 120 min; the clear upper phase above the fluffy double layer was discarded. The entire fluffy double layer together with a small amount of the 1.3 M sucrose solution was collected, diluted with both 0.25 M sucrose and distilled water in order to achieve a concentration of 0.25 M sucrose and then recentrifuged at 105000 × *g* for 90 min to obtain the smooth microsomes. The rough microsomes were obtained from the pellet formed by the first ultracentrifugation. The intermediate phase[12] between the fluffy double layer and the pellet was collected and subjected to biochemical analyses.

Recovery of microsomal preparations

Hepatic cytochrome P-450 content was determined in whole homogenates and in microsomal suspensions and the microsomal recovery was calculated according

to Greim[13]. Recovery of total microsomes was similar in ethanol-fed rats and controls (51 ± 4% *vs* 52 ± 3% in rats fed the 35% fat diet; and 59 ± 4% *vs* 54 ± 4% in rats fed the 10% fat diet). Losses were found to be mostly due to sedimentation during the preliminary 12000 × *g* centrifugation. The values of rough and smooth microsomes were corrected for their losses into the intermediate phase by the following equation:

$$Rx + Sy = I \quad (1)$$

$$x + y = 1 \quad (2)$$

where *R*, *S* and *I* are the respective RNA/protein ratios in each rough, smooth and intermediate fraction. *x* and *y* are the fraction of the total microsomal protein of the intermediate phase present as rough and smooth microsomes, respectively.

Enzyme assays

Ethanol oxidation in total microsomes and subfractions was measured as previously described[14] with the following modifications: aliquots of the microsomal suspension were incubated at 37 °C in a medium containing (per ml) 0.35 μmole of NADP, 6 μmoles of $MgCl_2$ 9 μmoles of sodium isocitrate, 22 μmoles of nicotinamide, 2.2 mg of isocitrate dehydrogenase (crude type I: Sigma Chemical Company, St. Louis, Mo.) and 80 μmoles of phosphate buffer (pH 7.4). Stoppered serum vials were preincubated for 5 min and the reaction started by addition of ethanol at a final concentration of 50 mM in a total incubation volume of 0.7 ml containing 2 mg of microsomal protein. The reaction was stopped with 5% $ZnSO_4$ (0.1 ml) and the vial immediately chilled on ice. Then 0.15 M $Ba(OH)_2$ (0.1 ml) and isopropanol (0.7 mg) were added and centrifuged at 5000 × *g* for 10 min at 2 °C. The supernatant fraction was used for acetaldehyde determination according to Roach and Creaven[15] with the following modifications: a Model 402 high efficiency gas chromatograph (Hewlett-Packard, Avondale, Pa.) with a flame ionization detector was used and helium gas served as the carrier gas at a flow rate of 100 ml/min. Flow rates of air and hydrogen were 300 and 37 ml/min, respectively. The temperatures were 100 °C for the column, 125 °C for the flash heater and 135 °C for the detector. A 6-ft U-shaped glass column (0.25 inch internal diameter) packed with Porapak Q (50/80 mesh, Hewlett Packard) was conditioned by heating for 18 h at 110 °C with carrier gas flow. Glass wool was packed at the injection port of the column and changed at regular intervals according to Majchrowicz *et al.*[16]. 2-3 μl of the supernate were injected into the column. The peak area was measured by a Model 3370 A Integrator (Hewlett-Packard). Catalase activity was measured spectrophotometrically according to Lück[17].

Chemical analyses

Cytochrome P-450 was assessed according to Omura and Sato[18] using an Aminco-Chance (American Instrument Company, Silver Spring, Md.) dual wavelength split-beam spectrophotometer in the split-beam mode. Protein was determined according to Lowry *et al.*[19] and phospholipids by the method of Bartlett[20]. RNA was extracted as described by Dallner *et al.*[21] and determined by the orcinol reaction[22]. In all experiments, each individual result was compared with its control. The individual differences were analyzed by the Student *t* test[23] for pairs.

RESULTS

Effect of chronic ethanol administration with a regular fat containing diet on the biochemical composition and enzyme activities of rough and smooth microsomal membranes of the liver

After 6 weeks of ethanol feeding, a significant increase in microsomal protein was observed in smooth microsomes whereas a significant decrease was found in rough microsomes (Table I). Total microsomal protein was slightly but not significantly higher than in controls. The phospholipid content significantly increased in total microsomes and in smooth microsomes but not in the rough fraction. The RNA content of rough microsomes decreased significantly after ethanol feeding and remained unchanged in total and smooth microsomes; the RNA:protein ratio remained unchanged in all of the subfractions after ethanol. In controls, the rough microsomes accounted for 56% of the microsomal proteins and the smooth microsomes for 44%. The phospholipids were distributed about equally between rough and smooth microsomes. RNA was mostly found in rough microsomes whether expressed per g of liver or per mg of microsomal protein. In the control livers, 60% of microsomal cytochrome P-450 content and activity of the microsomal ethanol oxidizing system was recovered from the rough microsomes and 40% from the smooth when expressed per g of liver (Table II); when expressed per mg of microsomal protein, there were no significant differences between the fractions.

TABLE I

EFFECT OF THE FEEDING OF ETHANOL AND A REGULAR FAT-CONTAINING DIET ON THE BIOCHEMICAL COMPOSITION OF ROUGH AND SMOOTH MICROSOMAL MEMBRANES OF THE LIVER

Female rats were pair-fed liquid diets with 35% of total calories as fat, 18% as protein, 11% as carbohydrate and 36% as ethanol or additional carbohydrate for 6 weeks. Each value represents the mean ± S.E. obtained from 9 animals.

		Total microsomes	*Rough microsomes*	*Smooth microsomes*
Protein				
mg/g liver	Control	29.4 ± 1.5	17.3 ± 0.5	13.4 ± 0.7
	Ethanol	31.3 ± 1.7	14.8 ± 0.5*	16.1 ± 1.1*
Phospholipids				
mg/g liver	Control	10.7 ± 0.7	5.5 ± 0.2	5.0 ± 0.3
	Ethanol	12.4 ± 0.7*	5.1 ± 0.3	6.6 ± 0.1*
mg/mg protein	Control	0.36 ± 0.01	0.32 ± 0.01	0.37 ± 0.01
	Ethanol	0.40 ± 0.01*	0.34 ± 0.01	0.41 ± 0.01*
RNA				
mg/g liver	Control	6.10 ± 0.23	5.31 ± 0.14	1.00 ± 0.07
	Ethanol	5.85 ± 0.31	4.56 ± 0.33**	1.11 ± 0.17
mg/mg protein	Control	0.21 ± 0.01	0.31 ± 0.01	0.07 ± 0.003
	Ethanol	0.19 ± 0.01	0.31 ± 0.01	0.07 ± 0.007

* ($P < 0.05$) compared to respective controls.
** ($P < 0.01$) compared to respective controls.

Ethanol feeding for 6 weeks resulted in an increase in cytochrome P-450 in

TABLE II

EFFECT OF THE FEEDING OF ETHANOL AND A REGULAR FAT-CONTAINING DIET ON CYTOCHROME P-450 AND THE ACTIVITIES OF THE MICROSOMAL ETHANOL-OXIDIZING SYSTEM AND OF CATALASE IN ROUGH AND SMOOTH MICROSOMAL MEMBRANES OF THE LIVER

Female rats were pair-fed liquid diets as described in Table I. Each value represents the mean ± S.E. obtained from 9 animals.

		Total microsomes	*Rough microsomes*	*Smooth microsomes*
Cytochrome P-450				
nmoles/g liver	Control	17.45 ± 0.95	9.99 ± 0.58	6.15 ± 0.39
	Ethanol	25.70 ± 1.53**	10.92 ± 0.61	13.57 ± 1.83*
nmoles/mg protein	Control	0.594 ± 0.028	0.577 ± 0.031	0.460 ± 0.028
	Ethanol	0.821 ± 0.038**	0.738 ± 0.031*	0.843 ± 0.067**
Microsomal ethanol-oxidizing system (nmoles ethanol per min)				
per g liver	Control	145.7 ± 7.0	87.5 ± 7.0	62.8 ± 5.4
	Ethanol	218.2 ± 10.0**	82.5 ± 6.2	111.7 ± 13.7*
per mg protein	Control	4.95 ± 0.34	5.06 ± 0.42	4.68 ± 0.58
	Ethanol	7.00 ± 0.28**	5.57 ± 0.39	6.94 ± 0.64*
Catalase				
units/g liver	Control	134.7 ± 15.4	101.7 ± 8.5	41.0 ± 4.0
	Ethanol	135.7 ± 13.5	105.2 ± 15.0	39.8 ± 5.5
units/mg protein	Control	4.58 ± 0.60	5.88 ± 0.59	3.10 ± 0.13
	Ethanol	4.34 ± 0.49	7.08 ± 0.75	2.47 ± 0.25*

* ($P < 0.01$) compared to respective controls.
** ($P < 0.001$) compared to respective controls.

total microsomes whether expressed per g of liver or mg of microsomal protein, this increase occurred predominantly in smooth microsomes when expressed per g of liver (Table II) and the increase was significant in both subfractions when expressed per mg of microsomal protein.

6 weeks of ethanol administration significantly enhanced the activity of the microsomal ethanol-oxidizing system in total microsomes as shown in Table II, and this was almost entirely due to the increase in smooth microsomes whether expressed per g of liver or mg of microsomal protein. Catalase activity of total microsomes did not increase following chronic ethanol administration. In controls, catalase activity was two times higher in rough than in smooth microsomes (Table II). This relation remained unchanged after chronic ethanol feeding except for a significant decrease in catalase activity in smooth microsomes when expressed per mg of microsomal protein.

Effect of the feeding of ethanol and a 10% fat diet on the biochemical composition and enzyme activities of rough and smooth microsomal membranes of the liver

After 6 weeks of feeding of a low fat (10%) diet with ethanol, no significant changes were observed in protein content of total and rough microsomes (Table III). There was, however, a significant increase in the protein content in smooth micro-

TABLE III

EFFECT OF THE FEEDING OF ETHANOL AND A LOW FAT DIET ON THE BIOCHEMICAL COMPOSITION OF ROUGH AND SMOOTH MICROSOMAL MEMBRANES OF THE LIVER

Females rats were pair-fed liquid diets with 10% of total calories as fat, 18% as protein, 36% as carbohydrate and 36% as ethanol or additional carbohydrate for 6 weeks. Each value represents the mean ± S.E. obtained from 5 animals.

		Total microsomes	*Rough microsomes*	*Smooth microsomes*
Protein				
mg/g liver	Control	36.2 ± 2.2	20.0 ± 1.2	13.7 ± 1.0
	Ethanol	39.9 ± 4.2	20.1 ± 2.2	18.0 ± 1.6*
Phospholipids				
mg/g liver	Control	11.1 ± 1.0	5.8 ± 0.3	4.5 ± 0.3
	Ethanol	13.4 ± 1.2	6.0 ± 0.5	6.9 ± 0.6*
mg/mg protein	Control	0.31 ± 0.02	0.29 ± 0.01	0.33 ± 0.02
	Ethanol	0.34 ± 0.03	0.30 ± 0.02	0.38 ± 0.03*
RNA				
mg/g liver	Control	6.26 ± 0.38	5.63 ± 0.42	0.90 ± 0.06
	Ethanol	6.43 ± 0.51	5.27 ± 0.45	1.24 ± 0.21
mg/mg protein	Control	0.17 ± 0.003	0.28 ± 0.01	0.07 ± 0.006
	Ethanol	0.16 ± 0.003	0.26 ± 0.01	0.07 ± 0.008

* ($P < 0.05$) compared to respective controls.

TABLE IV

EFFECT OF THE FEEDING OF ETHANOL AND A LOW FAT DIET ON CYTOCHROME P-450 AND THE ACTIVITY OF MICROSOMAL ETHANOL-OXIDIZING SYSTEM IN ROUGH AND SMOOTH MICROSOMAL MEMBRANES OF THE LIVER

Female rats were pair-fed liquid diets as described in Table III. Each value represents the mean ± S.E., obtained from 5 animals.

		Total microsomes	*Rough microsomes*	*Smooth microsomes*
Cytochrome P-450				
nmoles/g liver	Control	16.53 ± 0.92	9.87 ± 0.36	4.89 ± 0.69
	Ethanol	24.12 ± 1.78*	11.09 ± 0.70	10.42 ± 0.96*
nmoles/mg protein	Control	0.457 ± 0.017	0.491 ± 0.027	0.357 ± 0.025
	Ethanol	0.605 ± 0.040**	0.552 ± 0.041	0.580 ± 0.029**
Microsomal ethanol-oxidizing system (nmoles ethanol per min)				
per g liver	Control	150.06 ± 12.66	95.03 ± 8.35	53.47 ± 3.38
	Ethanol	232.94 ± 31.22*	105.06 ± 12.60	104.79 ± 14.66*
per mg protein	Control	4.15 ± 0.43	4.75 ± 0.49	3.90 ± 0.26
	Ethanol	5.84 ± 0.38***	5.23 ± 0.24	5.82 ± 0.20**

* ($P < 0.05$) compared to respective controls.
** ($P < 0.01$) compared to respective controls.
*** ($P < 0.001$) compared to respective controls.

somes. The phospholipid content also increased significantly in smooth microsomes (but not in total and rough microsomes) whether expressed per g of liver or mg of microsomal protein. The RNA content expressed per g of liver or mg of microsomal protein did not change in any fraction.

6 weeks of feeding of ethanol with a low fat diet increased the cytochrome P-450 content per g of liver in total microsomes as well as in smooth microsomes (Table IV). The effect was similar to that achieved by ethanol and a regular fat diet (Table II). When expressed per mg of microsomal protein, the cytochrome P-450 content was also increased in total and smooth microsomes but to a lesser extent than in rats fed ethanol and a regular fat diet. Activity of the microsomal ethanol-oxidizing system after feeding ethanol and a low fat diet increased in total microsomes as well as in the smooth fraction whether expressed per g of liver or mg of microsomal protein. The changes were similar to those achieved after ethanol and a regular fat diet (Table II).

DISCUSSION

The present study substantiates and quantitates, by biochemical means, the increase in hepatic smooth endoplasmic reticulum and the decrease in rough endoplasmic reticulum following chronic ethanol administration previously reported by electron microscopy[2–7]. The smooth microsomal membranes consist mainly of phospholipids and proteins[24] and both increased in the hepatic smooth microsomal fraction of rats fed ethanol with adequate fat-containing diets. The reduction in protein and RNA content of rough microsomes found in this investigation is in agreement with the decrease in rough endoplasmic reticulum observed in some electron microscopic studies[4,5]. Other electron microscopic studies, however, yielded different findings. Thorpe *et al.*[8] observed no change in smooth endoplasmic reticulum after ethanol and Dobbins *et al.*[9,10] reported, with a morphometric method, a decrease in the surface area of both rough and smooth membranes after ethanol. In both these studies ethanol was added to the drinking water. This technique results in a limited alcohol intake insufficient to produce a fatty liver in the presence of an adequate diet[25]. Indeed, Thorpe *et al.*[8] reported no alteration in the hepatic lipid content in their study. Since the effects of ethanol on the endoplasmic reticulum could conceivably be affected by dietary fat, we decreased the fat content of the diet to 10% of total calories. However, ethanol still increased the mass of hepatic microsomal membranes (Table III) and this was associated with a rise in cytochrome P-450 content and activity of the microsomal ethanol-oxidizing system (Table IV). Similar results were observed in a more recent study with an ethanol diet containing only 5% of total calories as fat[26]. The fat content of the diet used by Dobbins *et al.*[10] was approximately 7.5% of total calories. Dobbins and Fallon[9] also found an increase in microsomal phospholipid per mg protein. It is difficult to reconcile their chemical findings with their morphologic observation of a decrease in endoplasmic reticulum. One possible explanation for this discrepancy may reside in the method used for morphometry[27]: the surface area of the endoplasmic reticulum was calculated by counting the intersection of test lines (grids) with endoplasmic reticular membranes. This fails to fully take into account differences in shape and thickness of the membranes.

The mechanism whereby ethanol increases the smooth membranes is not known. Tobon and Mezey[28] have reported that ethanol feeding decreases the rate of degradation of microsomal protein without changing the rate of incorporation of [^{14}C]leucine into microsomal protein. On the other hand, Kuriyama *et al.*[29] found a stimulation of protein synthesis in ribosomes after chronic ethanol feeding. By contrast, acute ethanol administration[29,30] as well as the perfusion of isolated liver with ethanol[31] depressed protein synthesis.

Since our study reveals that the increase in smooth endoplasmic reticulum is associated with a decrease in the rough membranes, one must consider the possibility that the increase in the smooth fraction may result merely from the detachment of ribosomes from the rough membranes. However, it is unlikely that this fully accounts for our results since we also found a preferential increase in the content of cytochrome P-450 and activity of the microsomal ethanol-oxidizing system in smooth microsomes. Furthermore, the ratio of RNA over protein in microsomal subfractions remained unchanged after ethanol. A significant ribosomal detachment due to ethanol should have resulted in a contamination of the smooth fraction by free ribosomes with a corresponding increase in the RNA:protein ratio[12,32].

Drugs other than ethanol, such as phenobarbital, also produce an increase in microsomal protein content and in the activity of hepatic microsomal enzymes both in smooth and rough microsomal fractions with a predominance of the effect in the smooth membranes[33,34]. This agrees with the morphologic observations of the proliferation of the smooth endoplasmic reticulum[33,35]. However, in contrast to ethanol, phenobarbital was reported to produce no appreciable morphological changes in the rough endoplasmic reticulum[33,36]. On the other hand, a wide variety of hepatotoxic and carcinogenic compounds such as ethionine[37] and 3'-methyl-4-dimethylaminoazobenzene[38], increases the smooth microsomal membranes and produces a loss of parallel arrays of the rough membranes and swelling of the mitochondria[2,4]. These changes of the hepatic organelles resemble those produced by ethanol which, in that respect, can be considered to exert a hepatotoxic effect.

Drug-induced hypertrophy of smooth endoplasmic reticulum is generally accompanied by an increase in cytochrome P-450 and hepatic microsomal drug-metabolizing enzymes[39]. Similarly, ethanol administration has been found to increase the activity of drug-metabolizing enzymes[6,40], cytochrome P-450 content[6] and activity of the microsomal ethanol-oxidizing system[14]. The data in Table II indicate that the ethanol-induced increase in cytochrome P-450 content and activity of the microsomal ethanol-oxidizing system occurred preferentially in smooth microsomes. In view of the recent suggestion that catalase may be involved in microsomal oxidation of ethanol[41–43], the activity of this enzyme was determined. Although some minor role of catalase for microsomal oxidation of ethanol cannot be ruled out on the basis of the present study, a rate-limiting function of catalase is unlikely in view of the lack of parallelism between microsomal ethanol oxidation and catalase activity in the smooth microsomal fraction after ethanol feeding. Indeed, the increase in ethanol oxidation in the smooth fraction was accompanied by a significant decrease in catalase activity when expressed per mg of microsomal protein. Studies of the effects of catalase inhibitors on microsomal ethanol oxidation have yielded conflicting interpretations, ranging from a clear-cut differentiation of the activity of the microsomal ethanol oxidation from catalase[14] to the opinion that ethanol oxidation reflects

contaminating catalase[42,43]. However, more recent studies[44,45] have succeeded in separating the activity of the microsomal ethanol-oxidizing system from that of catalase by column chromatography.

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REFERENCES

1 O. A. Iseri, L. S. Gottlieb and C. S. Lieber, *Fed. Proc.*, 23 (1964) 579.
2 O. A. Iseri, C. S. Lieber and L. S. Gottlieb, *Am. J. Pathol.*, 48 (1966) 535.
3 B. P. Lane and C. S. Lieber, *Am. J. Pathol.*, 49 (1966) 593.
4 E. Rubin and C. S. Lieber, *Gastroenterology*, 52 (1967) 1.
5 C. S. Lieber and E. Rubin, *Am. J. Med.*, 44 (1968) 200.
6 E. Rubin, F. Hutterer and C. S. Lieber, *Science*, 159 (1968) 1469.
7 N. Carulli and F. Manenti, in G. A. Martini and Ch. Bode, *Metabolic Changes Induced by Alcohol*, Springer-Verlag, New York, 1971, p. 93.
8 M. E. C. Thorpe and C. D. Shorey, *Am. J. Pathol.*, 48 (1966) 557.
9 W. O. Dobbins, III and H. J. Fallon, *Gastroenterology*, 60 (1971) 176.
10 W. O. Dobbins, III, E. L. Rollins, S. G. Brooks and H. J. Fallon, *Gastroenterology*, 62 (1972) 1020.
11 L. M. DeCarli and C. S. Lieber, *J. Nutr.*, 91 (1967) 331.
12 A. Bergstrand and G. Dallner, *Anal. Biochem.*, 29 (1969) 351.
13 H. Greim, *Naunyn Schmiedeberg Arch. Exp. Pathol.*, 266 (1970) 261.
14 C. S. Lieber and L. M. DeCarli, *J. Biol. Chem.*, 245 (1970) 2505.
15 M. K. Roach and P. J. Creaven, *Clin. Chim. Acta*, 21 (1968) 275.
16 E. Majchrowicz and J. H. Mendelson, *Science*, 168 (1970) 1100.
17 H. Lück, in H. U. Bergmeyer, *Methods of Enzymatic Analysis*, Academic Press, New York, 1963, p. 885.
18 T. Omura and R. Sato, *J. Biol. Chem.*, 239 (1964) 2370.
19 O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. Biol. Chem.*, 193 (1951) 265.
20 G. R. Bartlett, *J. Biol. Chem.*, 234 (1959) 466.
21 G. Dallner, P. Siekevitz and G. Palade, *J. Cell Biol.*, 30 (1966) 73.
22 W. Mejbaum, *Z. Phys. Chem.*, 258 (1939) 117.
23 G. W. Snedecor and W. G. Cochran, *Statistical Methods*, The Iowa University Press, Iowa City, Iowa, 1967.
24 G. Dallner and L. Ernster, *J. Histochem. Cytochem.*, 16 (1968) 611.
25 G. Klatskin, H. M. Gewin and W. A. Krehl, *Yale J. Biol. Med.*, 23 (1951) 317.
26 L. M. DeCarli, H. Ishii, Y. Hasumura and C. S. Lieber, in *Proc. Ninth Int. Congr. Nutr.*, S. Karger, New York, in the press.
27 E. R. Weibel, G. S. Kistler and W. F. Scherle, *J. Cell Biol.*, 30 (1966) 23.
28 F. Tobon and E. Mezey, *J. Lab. Clin. Med.*, 77 (1971) 110.
29 K. Kuriyama, P. Y. Sze and G. E. Rauscher, *Life Sci.*, 10 (1971) 181.
30 M. A. Rothschild, M. Oratz, J. Mongelli and S. S. Schreiber, *J. Clin. Invest.*, 50 (1971) 1812.
31 K. N. Jeejeebhoy, M. J. Phillips, A. Bruce-Robertson, J. Ho and U. Sodtke, *Biochem. J.*, 126 (1972) 1111.
32 T. C. Lee, D. C. Swartzendruber and F. Snyder, *Biochem. Biophys. Res. Commun.*, 36 (1969) 748.
33 H. Remmer and H. J. Merker, *Science*, 142 (1963) 1657.
34 S. Orrenius and L. Ernster, *Biochem. Biophys. Res. Commun.*, 16 (1964) 60.
35 T. E. Gram, L. A. Rogers and J. R. Fouts, *J. Pharmacol. Exp. Ther.*, 157 (1967) 435.
36 J. R. Fouts and L. A. Rogers, *J. Pharmacol. Exp. Ther.*, 147 (1965) 112.
37 R. L. Wood, *Am. J. Pathol.*, 46 (1965) 307.
38 K. R. Porter and C. Bruni, *Cancer Res.*, 19 (1959) 997.
39 A. H. Conney, *Pharmacol. Rev.*, 19 (1967) 317.
40 E. Rubin and C. S. Lieber, *Science*, 162 (1968) 690.

41 M. K. Roach, W. N. Reese, Jr and P. J. Creaven, *Biochem. Biophys. Res. Commun.*, 36 (1969) 596.
42 K. J. Isselbacher and E. A. Carter, *Biochem. Biophys. Res. Commun.*, 39 (1970) 530.
43 R. G. Thurman, H. G. Levy and R. Scholz, *Eur. J. Biochem.*, 25 (1972) 420.
44 E. Mezey, J. J. Potter and W. D. Reed, *Clin. Res.*, 20 (1972) 461.
45 R. Teschke, Y. Hasumura, J.-G. Joly, H. Ishii and C. S. Lieber, *Biochem. Biophys. Res. Commun.*, 49 (1972) 1187.