Biochemical Pharmacology, Vol. 31, No. 3, pp. 377–381, 1982.
Printed in Great Britain.

0006-2952/82/030377-05 $03.00/0

EFFECT OF CHRONIC ALCOHOL CONSUMPTION ON THE ACTIVITIES OF LIVER PLASMA MEMBRANE ENZYMES: GAMMA-GLUTAMYLTRANSFERASE, ALKALINE PHOSPHATASE AND 5′-NUCLEOTIDASE

MASANOBU NISHIMURA and ROLF TESCHKE

Alcohol Research Laboratory, Department of Medicine, Clinic D, University of Düsseldorf, 4000 Düsseldorf, Federal Republic of Germany

(*Received* 21 *April* 1981; *accepted* 30 *July* 1981)

Abstract—To study the effect of chronic alcohol administration on the activities of liver plasma membrane enzymes such as gamma-glutamyltransferase, alkaline phosphatase and 5′-nucleotidase, female rats were pair-fed for 6 weeks nutritionally adequate liquid diets containing either ethanol or isocaloric carbohydrates as controls. Compared to the control diet, chronic alcohol administration resulted in a significant enhancement of serum activities of gamma-glutamyltransferase, alkaline phosphatase and 5′-nucleotidase by 91% ($P < 0.005$), 80% ($P < 0.001$) and 65% ($P < 0.01$), respectively. Concomitantly, chronic alcohol intake led to a striking increase of gamma-glutamyltransferase activities in liver homogenates by 68% ($P < 0.001$), in liver plasma membranes rich in bile canaliculi by 80% ($P < 0.025$), and in liver plasma membranes free of bile canaliculi by 24% ($P < 0.02$). However, chronic ethanol consumption had no effect on alkaline phosphatase activities in liver homogenates and liver plasma membranes but significantly suppressed 5′-nucleotidase activities. These results therefore show that chronic intake of ethanol increases serum activities of enzymes originating from liver plasma membranes but has different effects on the enzyme activity in liver plasma membranes itself, suggesting that the alcohol-mediated increase of serum activities of various enzymes originating from liver plasma membranes might be due to different mechanisms.

A variety of studies have shown that chronic alcohol consumption may lead to structural alterations of the liver cell including mitochondria [1, 2], endoplasmic reticulum [1, 2], hepatic microtubules and Golgi apparatus [3, 4]. The latter two organelles are closely associated with plasma membranes [5, 6]. Indeed, some enzymes synthesized in the endoplasmic reticulum are translocated from the site of synthesis to plasma membranes via hepatic microtubules and Golgi apparatus [7]. However, little is known about the effect of alcohol on hepatic plasma membranes, except that chronic alcohol consumption has been reported to result in an enhancement of lipid peroxidation in liver plasma membrane fractions [8], whereas Na–K ATPase was found to be virtually unchanged [9].

The present study was undertaken to investigate the effect of chronic alcohol administration on the activities of various liver plasma membrane enzymes such as gamma-glutamyltransferase, alkaline phosphatase and 5′-nucleotidase. Moreover, the question was studied to what extent changes of serum enzyme activities may reflect the activity level of the corresponding plasma membrane enzyme in the liver.

MATERIALS AND METHODS

Animals. Female Sprague–Dawley rats were purchased from Zentralinstitut für Versuchstierzucht, Hannover, with a body weight of 130–150 g and fed Altrumin-R chow and tap water *ad libitum* until the start of the experiment. When the animals reached a body weight of 200–220 g, they were housed in individual wired bottom cages and fed for six weeks with liquid diets in drinking tubes as the only source of food and water.

Experimental procedures. The liquid diets were prepared essentially as described by DeCarli and Lieber [10]. The animals were divided into two groups. Each group of animals was pair-fed one of the nutritionally adequate liquid diets, namely either the alcohol diet or the control diet in which ethanol has been replaced isocalorically by carbohydrates for six weeks. The groups of pair-fed animals were killed by decapitation, and blood was collected from the neck vessels of the animals for the determination of serum enzyme activities.

Preparation of plasma membranes. Liver sections were obtained for histological examination, and the livers were subsequently perfused *in situ* through the portal vein with ice-cold buffer (1 mM $NaHCO_3$, pH 7.5) and surgically removed. The livers were weighted, minced with scissors on an ice plate and homogenized with a Teflon glass homogenizer. Liver cell plasma membranes were isolated by discontinuous sucrose density gradient ultracentrifugation according to a modification of the method of Song *et al.* [11] and Yousef *et al* [12]. Liver cell plasma membranes consist of a fraction rich in bile canalicular membranes (BCM) and of another one free from bile canaliculi (PM).

Enzyme assays. The activities of gamma-glutamyltransferase were measured in serum, liver homogenates, and plasma membranes by spectropho-

tometric assay at 25° according to the method of Szasz [13]. The activities of alkaline phosphatase were assayed in serum, liver homogenates, and plasma membranes at 25° according to the method of Hausamen *et al.* [14]. The activities of 5'-nucleotidase and glucose-6-phosphatase were determined at 37° according to the method of Solyom and Trams [15], and the liberated phosphate was determined according to Chen *et al.* [16]. Enzyme units were expressed as μmoles substrate metabolized per min. The activities of aspartate aminotransferase were measured by the method of Bergmeyer and Bernt [17]. The activities of alanine aminotransferase and of glutamate dehydrogenase were determined according to the method of Bergmeyer and Bernt [18] and Schmidt [19], respectively.

Protein. The determination of protein was performed according to the method of Lowry *et al.* [20], using crystalline bovine albumin as standard.

Statistical analysis. Each measurement was carried out at least in duplicate. The results obtained are expressed as means (±S.E.M.), and the significance of the differences was assessed by the Student's *t* test.

RESULTS

Gamma-glutamyltransferase (GGT)

Compared to animals fed the control diet, chronic alcohol consumption for 6 weeks led to a fatty liver by histological assessment and to a significant increase of serum gamma-glutamyltransferase activity (Table 1) which was associated with a striking enhancement of GGT activity in liver homogenates when expressed per g of liver wet weight, per g of liver protein or per 100 g of body weight (Table 2). Moreover, this regimen resulted in a significant increase of GGT activity in plasma membranes of the liver cell obtained by discontinuous sucrose density gradient ultracentrifugation (Table 3). In particular, the enhancement of GGT activity following chronic alcohol consumption could be demonstrated in both plasma membrane fractions, the bile canaliculi enriched and bile canaliculi free one. The specific activity of GGT was considerably higher in bile canaliculi enriched plasma membranes than in bile canaliculi free fractions, both in the alcohol-fed group and in their pair-fed controls (Table 3). Finally, compared to liver homogenates, the specific activity of GGT per g of liver protein was higher in the two fractions of plasma membranes, indicating successful enrichment of the enzyme during the course of preparation.

Alkaline phosphatase (ALP)

Following prolonged alcohol intake, a striking increase of activity of alkaline phosphatase was observed in the serum (Table 1). Conversely, no significant alterations could be demonstrated in liver homogenates, irrespective of whether the activity was expressed per g of liver wet weight, per g of liver protein or per 100 g of body weight (Table 2). Similarly, chronic alcohol consumption failed to significantly alter ALP activity in bile canaliculi enriched as well as free plasma membranes (Table 3). In the alcohol and the control group, the specific activity per g of protein was higher in the two fractions of plasma membranes compared to the corresponding liver homogenates. Moreover, compared to bile canaliculi free plasma membranes, ALP activity was strikingly higher in plasma membranes enriched in bile canaliculi both in alcohol-fed animals and in their pair-fed controls (Table 3).

5'-Nucleotidase

Serum 5'-nucleotidase activity was significantly increased in the serum after chronic alcohol consumption (Table 1). In liver homogenates, however, no significant alterations of 5'-nucleotidase activity could be demonstrated following prolonged ethanol administration, whether the activity was expressed per g of liver wet weight or per 100 g of body weight (Table 2). There was a slight but significant decrease of 5'-nucleotidase activity when calculated per g of liver protein. Moreover, chronic alcohol consumption resulted in a reduction of 5'-nucleotidase activities in bile canaliculi rich as well as free plasma membranes compared to those of the control group

Table 1. Effect of chronic ethanol consumption on serum enzyme activities

Assay	Alcohol (A) diet	Control (C) diet	A/C	P
	(U/l)	(U/l)		
Gamma-glutamyl-transferase	4.33 ± 0.60	2.27 ± 0.14	1.91	<0.005
Alkaline phosphatase	279 ± 22	155 ± 17	1.80	<0.001
5'-Nucleotidase	270 ± 27	164 ± 19	1.65	<0.01
Glutamate dehydrogenase	6.94 ± 0.94	3.92 ± 0.57	1.77	<0.02
Aspartate aminotransferase	106 ± 6.3	100 ± 6.2	1.06	NS
Alanine aminotransferase	60.6 ± 3.4	24.9 ± 2.1	2.43	<0.001

Female Sprague–Dawley rats were fed for 6 weeks nutritionally adequate liquid diets containing either ethanol (36% of total calories) or isocaloric dextrin as controls. The enzyme activities were determined in the serum and expressed as U/l. The values are means of 11 experimental animals. NS: not significant.

Table 2. Effect of chronic ethanol consumption on liver enzyme activities

Assay	Alcohol (A) diet	Control (C) diet	A/C	P
Gamma-glutamyltransferase				
U/g of liver wet weight	0.15 ± 0.02	0.08 ± 0.01	1.89	<0.001
U/g of liver protein	1.23 ± 0.09	0.81 ± 0.07	1.52	<0.005
U/100 g of body weight	0.74 ± 0.08	0.36 ± 0.03	2.06	<0.001
Alkaline phosphatase				
U/g of liver wet weight	0.41 ± 0.03	0.39 ± 0.04	1.05	NS
U/g of liver protein	3.56 ± 0.23	3.64 ± 0.35	0.98	NS
U/100 g of body weight	2.13 ± 0.23	1.86 ± 0.18	1.15	NS
5′-Nucleotidase				
U/g of liver wet weight	4.38 ± 0.20	5.55 ± 0.53	0.79	NS
U/g of liver protein	38.67 ± 2.17	54.00 ± 3.33	0.72	<0.005
U/100 g of body weight	22.45 ± 1.5	23.15 ± 1.88	0.97	NS
Glucose-6-phosphatase				
U/g of liver wet weight	10.97 ± 0.33	8.35 ± 0.35	1.31	<0.001
U/g of liver protein	96.5 ± 5.33	82.33 ± 2.67	1.17	<0.05
U/100 g of body weight	55.72 ± 2.81	37.93 ± 2.13	1.47	<0.001
Glutamate dehydrogenase				
U/g of liver wet weight	170 ± 81	128 ± 8	1.33	<0.005
U/g of liver protein	1497 ± 88	1229 ± 111	1.22	NS
U/100 g of body weight	871 ± 44	575 ± 38	1.51	<0.001
Aspartate aminotransferase				
U/g of liver wet weight	98.4 ± 3.1	71.8 ± 2.4	1.37	<0.001
U/g of liver protein	858 ± 39	734 ± 49	1.16	NS
U/100 g of body weight	508 ± 15	378 ± 12	1.34	<0.001
Alanine aminotransferase				
U/g of liver wet weight	19.9 ± 0.8	11.8 ± 0.8	1.69	<0.001
U/g of liver protein	174 ± 10	117 ± 6	1.49	<0.001
U/100 g of body weight	102 ± 4	53 ± 3	1.92	<0.001

Female Sprague–Dawley rats were fed for 6 weeks nutritionally adequate liquid diets containing either ethanol (36% of total calories) or isocaloric dextrin as controls. The enzyme activities were determined in the 25% liver homogenates. The data in liver homogenates were expressed as U/g of liver wet wt, U/g of liver protein and U/100 g of body wt. The values are derived from 11 experimental animals in each group and are given as means ±S.E.M. NS: not significant.

Table 3. Effect of chronic ethanol consumption on plasma membrane enzyme activities and microsomal enzyme activity in liver homogenates and liver plasma membranes

Assay	Alcohol (A) diet	Control (C) diet	A/C	P
	(U/g of protein)	(U/g of protein)		
Gamma-glutamyltransferase				
homogenate	1.29 ± 0.10	0.77 ± 0.07	1.68	<0.005
plasma membranes				
—bile canaliculi enriched	18.7 ± 5.1	6.7 ± 1.5	2.80	<0.025
—bile canaliculi free	3.47 ± 0.64	1.55 ± 0.29	2.24	<0.02
Alkaline phosphatase				
homogenate	3.63 ± 0.28	3.65 ± 0.42	0.99	NS
plasma membranes				
—bile canaliculi enriched	48.3 ± 6.3	47.6 ± 9.1	1.02	NS
—bile canaliculi free	10.38 ± 1.31	8.33 ± 1.55	1.25	NS
5′-Nucleotidase				
homogenate	39.83 ± 2.66	54.17 ± 4.00	0.74	<0.005
plasma membranes				
—bile canaliculi enriched	280.83 ± 26.5	396.83 ± 35.17	0.71	<0.02
—bile canaliculi free	37.8 ± 6.83	75.5 ± 17.33	0.50	<0.05
Glucose-6-phosphatase				
homogenate	94.5 ± 6.0	81.67 ± 3.17	1.16	<0.001
plasma membranes				
—bile canaliculi enriched	42.52 ± 6.67	53.00 ± 8.83	0.80	NS
—bile canaliculi free	17.83 ± 1.83	16.83 ± 2.17	1.06	NS

Female Sprague–Dawley rats were fed for 6 weeks nutritionally adequate liquid diets containing either ethanol (36% of total calories) or isocaloric dextrin as controls. The enzyme activities were determined in the 25% liver homogenate and in liver plasma membranes. The data in liver homogenates were expressed as U/g of liver protein and those in plasma membranes as U/g of plasma membrane protein. The values are means of 9 experimental animals each group. NS: not significant.

(Table 3). Upon isolation of plasma membranes, a striking increase of 5'-nucleotidase activity could be demonstrated only in bile canaliculi enriched plasma membranes compared to the corresponding values in liver homogenates, both in the alcohol and the control groups (Table 3).

Glucose-6-phosphatase

Following chronic alcohol consumption, there were no significant alterations of glucose-6-phosphatase activities, a marker enzyme of the microsomal fraction of the hepatocyte, in either plasma membrane fraction (Table 3). The specific activity of glucose-6-phosphatase was relatively low in the two plasma membrane fractions compared to the activity in the liver homogenate (Table 3). On the other hand, the enzyme activity in liver homogenates was significantly increased following chronic alcohol consumption (Table 2).

Glutamate dehydrogenase (GDH)

Glutamate dehydrogenase activity increased in the serum after chronic alcohol consumption (Table 1). Some enhancement of GDH activity was also observed in liver homogenates. This increase, however, was only significant when the activity was expressed per g of liver wet weight or per 100 g of body weight but not per g of liver protein (Table 2).

Aspartate aminotransferase (AST)

The activity of aspartate aminotransferase remained unchanged following prolonged alcohol intake both in the serum (Table 1) and liver when the latter activity was expressed per g of liver protein (Table 2). There was some increase of activity, however, when the data are given per g of liver wet weight or per 100 g of body weight (Table 2).

Alanine aminotransferase (ALT)

Alanine aminotransferase showed a striking increase of the serum activity due to chronic alcohol pretreatment (Table 1). Similarly, a striking rise could be demonstrated for the hepatic activity, irrespective of whether the results are given per g of liver wet weight, per g of liver protein or per 100 g of body weight (Table 2).

DISCUSSION

The present study shows that chronic alcohol consumption enhances the activities of serum enzymes such as gamma-glutamyltransferase, alkaline phosphatase or 5'-nucleotidase (Table 1). All these enzymes are also liver plasma membrane enzymes, but alcohol has a different effect on the activity level in these membranes. Indeed, chronic alcohol intake strikingly enhances gamma-glutamyltransferase activities, has no effect on alkaline phosphatase activities and leads to a suppression of 5'-nucleotidase activities in liver plasma membranes, both in bile canaliculi-rich and bile canaliculi-free fractions (Table 3). Similar results are obtained when the activities of these enzymes were determined in the liver homogenates (Table 2).

In accordance with previous reports [21, 22], the data obtained in this study confirm that gamma-glutamyltransferase, alkaline phosphatase and 5'-nucleotidase are all localized in the plasma membranes of the hepatocytes (Table 3). A variety of studies have indicated that gamma-glutamyltransferase activities of plasma membranes are increased by the administration of drugs such as phenobarbital [23], findings similar to the data obtained in the present study for ethanol (Table 3). On the other hand, phenobarbital depresses 5'-nucleotidase activity [23], and a similar effect has been demonstrated also for ethanol (Table 3).

Recent studies suggested that enzymes of liver plasma membranes such as 5'-nucleotidase and alkaline phosphatase are synthesized in the endoplasmic reticulum of the hepatocyte and subsequently translocated from their site of synthesis via Golgi apparatus to the plasma membranes [24, 25]. A similar mechanism may apply for gamma-glutamyltransferase [26]. Previous experiments from our laboratory have shown that gamma-glutamyltransferase activity was induced in the microsomal fraction of the hepatocyte after chronic alcohol administration [27], although microsomal fractions may be contaminated to some extent by plasma membranes during subcellular fractionation. It is conceivable that the hepatic induction of gamma-glutamyltransferase activity after ethanol administration occurs primarily at the site of the endoplasmic reticulum. The enzyme could then be translocated from the endoplasmic reticulum to plasma membranes, possibly by means of the Golgi apparatus. Of particular interest was the observation of the present study that chronic alcohol consumption increased the serum activities of gamma-glutamyltransferase, alkaline phosphatase and 5'-nucleotidase under these experimental conditions (Table 1). These data implicate that alcohol may exert effects on synthesis in the endoplasmic reticulum, intracellular translocation and/or possibly solubilization at the site of the plasma membranes which are quite different between each of the plasma membrane enzymes studied in the present experiments. Theoretically, extrahepatic origin of enzymes could also contribute to increased serum activities. Further studies are therefore necessary to study the underlying mechanism.

In a single experimental study with rats, a reduction of hepatic activity of gamma-glutamyltransferase has been reported following chronic alcohol consumption [28], findings not substantiated in other studies [27, 29–31]. Furthermore, the enhancement of hepatic gamma-glutamyltransferase activity following chronic intake of alcohol has been attributed to dietary imbalance by some [32], an interpretation strongly opposed by others [27, 29, 30, 33].

Gamma-glutamyltransferase is a component of the gamma-glutamyl cycle in liver cells and may play a significant role for the transport of extracellular amino acids through plasma cell membranes [34]. Previous studies in rats have shown that chronic ethanol intake leads to an increase of the hepatic content of reduced glutathione which is also a component of the gamma-glutamyl cycle in the liver cell [35, 36]. Therefore, the increase of plasma membrane gamma-glutamyltransferase activity found after chronic ethanol consumption (Table 3) may explain, at least in part, alterations of hepatic amino

acid and protein metabolism commonly observed under these experimental conditions [3, 37]. The physiological role of 5′-nucleotidase and alkaline phosphatase in plasma membranes is not yet fully understood. It has been speculated that alkaline phosphatase may play some role in the transport of substances into the liver cell [38], and 5′-nucleotidase might be involved in the metabolism of ATP [22].

In conclusion, chronic alcohol administration increased serum enzyme activities of gamma-glutamyltransferse, alkaline phosphatase and 5′-nucleotidase. Concomitantly, gamma-glutamyl transferase activities were strikingly enhanced in liver homogenates and liver plasma membranes, but no effect on alkaline phosphatase activity and a suppression of 5′-nucleotidase in liver homogenates and liver plasma membranes was observed. These results suggest that ethanol has different effects on the activities of liver plasma membrane enzymes.

Acknowledgements—The expert technical assistance of Ms. H. Landmann and U. Hennigs is gratefully acknowledged. One of us (M. N.) is recipient of a fellowship from Heinrich-Hertz-Foundation.

REFERENCES

1. O. A. Iseri, C. S. Lieber and L. S. Gottlieb, *Am. J. Path.* **48**, 535 (1966).
2. K.-H. Kiessling and L. Pilström, *Cytobiologie* **4**, 339 (1971).
3. E. Baraona, M. A. Leo, S. A. Borowsky and C. S. Lieber, *J. Clin. Invest.* **60**, 546 (1977).
4. Y. Matsuda, E. Baraona, M. Salaspuro and C. S. Lieber, *Lab. Invest.* **41**, 455 (1979).
5. W. G. Whaley, M. Dauwalder and J. E. Kephart, *Science* **175**, 596 (1972).
6. G. L. Nicolson and G. Poste, *New Engl. J. Med.* **295**, 197 (1976).
7. D. J. Morré, in *Membrane Alterations as Basis of Liver Injury* (Eds. H. Popper, L. Bianchi and W. Reutter), p.15. MTP Press, Lancaster (1977).
8. S. Shaw, E. Jayatilleke, W. A. Ross and C. S. Lieber, *Gastroenterology* **77**, A41 (1979).
9. R. L. Ricci, S. S. Crawford and P. B. Miner, Jr., *Gastroenterology* **80**, 1445 (1981).
10. L. M. DeCarli and C. S. Lieber, *J. Nutr.* **91**, 331 (1967).
11. C. S. Song, W. Rubin, A. B. Rifkind and A. Kappas, *J. Cell Biol.* **41**, 124 (1969).
12. I. M. Yousef, D. L. Bloxam, M. J. Phillips and M. M. Fisher, *Can. J. Biochem.* **53**, 989 (1975).
13. G. Szasz, *Clin. Chem.* **15**, 124 (1969).
14. T. U. Hausamen, R. Helger, W. Rick and W. Gross, *Clin. Chim. Acta* **15**, 241 (1967).
15. A. Solyom and E. G. Trams, *Enzyme* **13**, 329 (1972).
16. P. S. Chen, Jr., T. Y. Toribara and H. Warner, *Analyt. Chem.* **28**, 1756 (1956).
17. H. U. Bergmeyer and E. Bernt, in *Methoden der Enzymatischen Analyse* (Ed. H. U. Bergmeyer), p. 685. Verlag Chemie, Weinheim (1970).
18. H. U. Bergmeyer and E. Bernt, in *Methoden der Enzymatischen Analyse* (Ed. H. U. Bergmeyer), p. 717. Verlag Chemie, Weinheim (1970).
19. E. Schmidt, in *Methoden der Enzymatischen Analyse* (Ed. H. U. Bergmeyer), p. 607. Verlag Chemie, Weinheim (1970).
20. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
21. G. D. Smith and T. J. Peters, *Biochem. Soc. Trans.* **6**, 543 (1978).
22. M. H. Wisher and W. H. Evans, in *Membrane Alterations as Basis of Liver Injury* (Eds. H. Popper, L. Bianchi and W. Reutter), p. 127. MTP Press, Lancaster (1977).
23. N.-E. Huseby, *Clin. Chim. Acta* **94**, 163 (1979).
24. J. J. M. Bergeron, M. V. Berridge and W. H. Evans, *Biochim. biophys. Acta* **407**, 325 (1975).
25. K. Oda and Y. Ikehara, *Biochim. biophys. Acta* **640**, 398 (1981).
26. E. Köttgen, Ch. Bauer, W. Reutter and W. Gerok, *Klin. Wochenschr.* **57**, 151 (1979).
27. R. Teschke, A. Brand and G. Strohmeyer, *Biochem. biophys. Res. Commun.* **75**, 718 (1977).
28. J. S. Singer and M. M. Kaplan, *Gastroenterology* **74**, 263 (1978).
29. H. Ishii, S. Yasuraoka, Y. Shigeta, S. Takagi, T. Kamiya, F. Okuno, K. Miyamoto and M. Tsuchiya, *Life Sci.* **23**, 1393 (1978).
30. S. Shaw and C. S. Lieber, *Gastroenterology* **77**, A41 (1979).
31. R. Teschke, J. Rauen, M. Neuefeind, A. S. Petrides and G. Strohmeyer, in *Advances in Experimental Medicine and Biology Vol.* 132: *Alcohol and Aldehyde Metabolizing Systems*—4 (Ed. R. G. Thurman), p. 647. Plenum Publishing, New York (1980).
32. J. Mørland, N.-E. Huseby, M. Sjøblom and J. H. Strømme, *Biochem. biophys. Res. Commun.* **77**, 1060 (1977).
33. A. S. Petrides and R. Teschke, *Rev. Française Gastroentérologie* **159**, 22 (1980).
34. A. Meister, in *Glutathione* (Eds. L. Flohe, H. Ch. Benöhr, H. Sies, H. D. Waller and A. Wendel), p. 56. Thieme, Stuttgart (1974).
35. J.-G. Joly, L. Yelle and C. Hétu, *Clin. Res.* **25**, 688 (1977).
36. M. Nishimura, H. Stein, W. Berges and R. Teschke, *Biochem. biophys. Res. Commun.* **99**, 142 (1981).
37. S. Shaw and C. S. Lieber, in *Biochemistry and Pharmacology of Ethanol* (Eds. E. Majchrowicz and E. P. Noble), Vol. 1, p. 383. Plenum Publishing, New York (1979).
38. M. M. Kaplan, *Gastroenterology* **62**, 452 (1972).