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HEPATIC METABOLISM AND ENZYME ACTIVITY IN ACUTE ETHANOL ADMINISTRATION*

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Abstract—Administration of ethanol (5 g/kg) or isopropanol (4 g/kg) to normal fed rats resulted in a significant increase in hepatic glucose 6-phosphatase activity within 10–60 min. This increase in activity was observed with a dose as low as 3 g/kg of ethanol. In addition to an increased protein content of the microsomal fraction, there was an increase in glucose 6-phosphatase specific activity. Under these conditions, activities of hepatic fructose 1,6-diphosphatase, β-glucuronidase, and gulonolactone oxidase were either unchanged or decreased. Liver total lipids were increased 1 hr after ethanol administration and although the intracellular endoplasmic reticulum and the mitochondria exhibited extensive damage, mitochondrial function as demonstrated by the oxidation of labeled glucose, palmitate, and alanine, was impaired only slightly. The incorporation of label from alanine to glucose was accelerated in ethanol-treated animals, which suggests the presence of a functioning gluconeogenic pathway.

EFFECTS of ethanol on hepatic carbohydrate metabolism have been recognized for many years. Freinkel *et al.*[1] in a recent review of this subject have pointed out that ethanol may under proper circumstances stimulate hepatic glucose production as well as the better known and more often described inhibitory effect on gluconeogenesis.

Effects of ethanol on activities of various enzymes have also been described. In rats ethanol increases the activity of liver catalase,[2] lactic dehydrogenase, and phosphofructokinase.[3] Acute administration of ethanol to a patient with Von Gierke's disease has also been observed to increase the blood glucose response to glucagon.[4] This observation suggests that ethanol may in some way 'unmask' the activity of glucose 6-phosphatase. The activity of this enzyme is increased in diabetes or by treatment of animals with adrenal glucocorticoids.[5–8] Insulin, *in vivo*, causes a decrease in the activity of liver glucose 6-phosphatase.[6] Such changes in glucose 6-phosphatase activity occur over a period of 12–48 hr.[6] The present report deals with increases in the activity of this enzyme observed within 10–60 min after administration of alcohol.

MATERIALS AND METHODS

Male albino rats of the Wistar strain supplied by Holtzman (Madison, Wis.) were maintained on water and Purina laboratory chow *ad libitum*, and weighed 150–250 g at the time of the experiment.

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Homogenates were prepared from 1·0 g liver in 10 ml of isotonic KCl and centrifuged at 600 *g* for 10 min to remove unbroken cells and nuclei. The supernatant fluid obtained was used for the determination of enzyme activity. Microsomes were obtained by centrifugation at 12,000 *g* for 15 min to remove nuclei, cells, and mitochondria and the supernatant fraction then obtained was centrifuged at 105,000 *g* for 60 min. The resulting microsomal pellet was resuspended in 5 ml isotonic KCl and then used for the enzyme assays.

Experimental procedure

In vivo. Normal fed rats weighing approximately 150–200 g were injected i.p. with an intoxicating dose of 95% ethanol (5 g/kg body wt.), isopropanol and *N*-propanol (4 g/kg), and isobutanol (2·5 g/kg), diluted with isotonic NaCl in a ratio of 1:2. The animals were then sacrificed and the livers excised for enzymic studies.

In vitro. Liver slices were prepared with a Stadie–Riggs hand microtome and incubated in a Ringer bicarbonate medium equilibrated with 95% O_2–5% CO_2 for 90 min at 37°.[9] Approximately 1·0 g wet liver slices was incubated for 90 min with 6·0 ml of medium containing ethanol at concentrations of either 0·5 or 1 m-mole/ml. Liver slices were then homogenized in isotonic KCl, the microsomal fraction isolated, resuspended in KCl and assayed for glucose 6-phosphatase activity. The protein content of the microsomal fraction was determined by the method of Lowry,[10] and total liver lipid was determined gravimetrically after chloroform–methanol extraction.[11]

In metabolic studies liver slices were incubated in 4·0 ml of Ringer bicarbonate medium containing U-^{14}C-glucose (5 mM), U-^{14}C-palmitate (1·5 mM), and U-^{14}C-alanine (5 mM). After 90 min of incubation, glucose was isolated as the phenylglucosazone and CO_2 as the $BaCO_3$ and assayed for ^{14}C.[12]

Electron micrographs of rat liver tissue and microsomal fraction were obtained from normal rats after administration of ethanol (5 g/kg body wt.) *in vivo* by i.p. injection 60 min before sacrifice. Liver samples were obtained from the central part of the tissue to avoid any immediate or surrounding alcohol effect. The tissues were first fixed with 1% osmium tetroxide solution in phosphate buffer for 2 hr. After fixation the specimens were dehydrated in increasing concentrations of ethanol and finally in absolute ethanol. Samples were imbedded in Epon resin at 65° overnight. The lead citrate stain technique was employed after sectioning the blocks with a Porter–Blum microtome.

Enzyme assays

Glucose 6-phosphatase. Determination of glucose 6-phosphatase activity in both normal and ethanol-treated rats was carried out on homogenates and on the microsomal fractions. The incubations were in citrate buffer (pH 6·4) at 35° for 30 min[13, 14] and results have been expressed as μmole Pi released/g liver/30 min.

Fructose 1,6-diphosphatase. Fructose 1,6-diphosphatase activity was measured in liver homogenates incubated with fructose 1,6-diphosphate and Mg in citrate buffer (pH 9·4).[13] The results are expressed as μmoles Pi released/g liver/30 min.

β-Glucuronidase. Determination of β-glucuronidase activity was made on liver homogenates incubated in 0·1 M acetate buffer (pH 5·0) with 0·1 M phenolpthalein β-glucuronide solution as substrate.[15]

Gulonolactone oxidase. Liver homogenates were used in the determination of gulonolactone oxidase activity and assays were carried out by the determination of ascorbic acid produced, by the method of Roe and Kuether.[16]

RESULTS

Glucose 6-phosphatase activity in homogenates from livers of normal fed animals, with and without alcohol pretreatment, has been summarized in Table 1. Ethanol

TABLE 1. GLUCOSE 6-PHOSPHATASE ACTIVITY IN LIVER HOMOGENATES FROM NORMAL FED RATS GIVEN VARIOUS ALCOHOLS (5 G/KG FOR 60 MIN)

Treatment	No. of animals	Glucose 6-phosphatase activity	
		Control (μmole/g)	+ Alcohol (μmole/g)
Ethanol (5 g/kg)	4	248 ± 15·0	315 ± 10·3*
Isopropanol (4 g/kg)	4	247 ± 14·0	314 ± 13·2*
N-propanol (4 g/kg)	4	264 ± 7·1	269 ± 11·5
Isobutanol (2·5 g/kg)	4	259 ± 6·7	267 ± 2·5 P < 0·01*

* Data are represented as mean ±S.D. Significance levels were determined by Student's *t* test.

and isopropanol administration resulted in a significant increase in glucose 6-phosphatase activity (approximately 30 per cent), but no change in enzymatic activity was observed with *N*-propanol and isobutanol. (Fig. 1 summarizes data obtained *in vivo* and *in vitro* from liver microsomes of ethanol-treated and nontreated normal rats.) The studies of ethanol administration *in vivo* demonstrate a maximal increase in glucose 6-phosphatase activity within 10 min of the administration of the alcohol. Studies *in vitro* on liver slices for the same time periods show an increase only after 30 min of incubation with ethanol and maximal values within 60 min. An increase in glucose 6-phosphatase activity of approximately 30 per cent was observed after administration either *in vivo* or *in vitro* of alcohol. Fig. 2 summarizes the data on glucose 6-phosphatase activity in liver microsomes isolated after exposure of the tissue *in vivo* to increasing concentrations of ethanol. An increase in activity was found with doses as low as 2 and 3 g/kg and activity gradually increased as the alcohol concentration increased. The glucose 6-phosphatase activity induced by prior treatment with ethanol exhibited the same optimum pH as the enzyme of the unstimulated liver.

After ethanol administration glucose 6-phosphatase activity in liver microsomal fractions was increased from 5·8 ± 0·4 to 7·1 ± 0·3 μmole Pi released/mg protein ($P < 0·05$). In addition, an increase in the protein content of the liver microsomal fraction was observed after ethanol treatment (7·2 ± 0·3 to 9·2 ± 0·4 mg protein/g liver). The activities of glucose 6-phosphatase, fructose 1,6-diphosphatase, β-glucuronidase, and gulonolactone oxidase observed in liver homogenates are presented in Table 2. Glucose 6-phosphatase and gulonolactone oxidase are both microsomal enzymes, but they exhibited different responses to ethanol stimulation. Glucose 6-phosphatase increased in activity by 30 per cent, whereas the activity of gulonolactone oxidase decreased by 35 per cent. β-Glucuronidase, a lysozomal enzyme, and

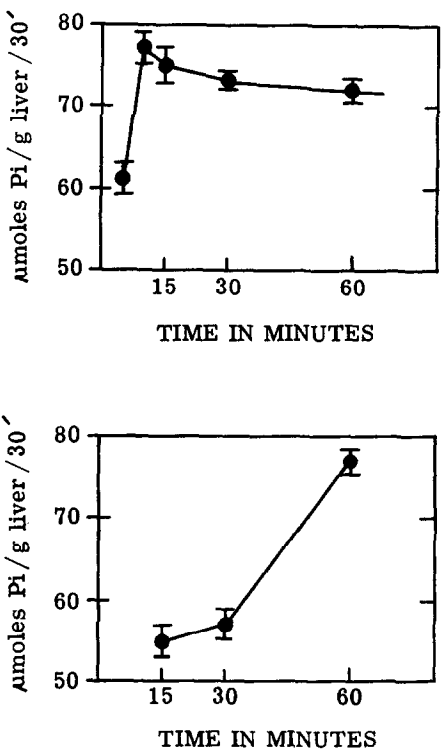

FIG. 1. Effect of i.p. injection of alcohol (5 g/kg) on glucose 6-phosphatase activity in rat liver microsomes *in vivo* and *in vitro*.

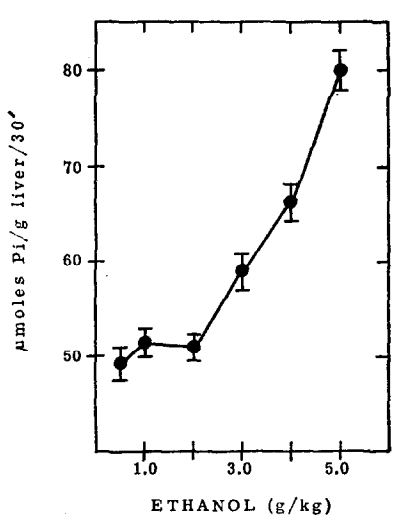

FIG. 2. Effect of ethanol concentration on glucose 6-phosphatase activity in rat liver microsomes *in vivo*.

fructose 1,6-diphosphatase, a soluble enzyme, do not appear to change in activity with ethanol stimulation.

Fig. 3 and 4 present electron micrographs of the liver cell and the liver microsomal fraction of untreated and ethanol-treated rats. A general disintegration of membranous structures in the endoplasmic reticulum and mitochondria, and an

TABLE 2. ACTIVITIES OF FOUR DISTINCT ENZYMES DETERMINED IN LIVER HOMOGENATES OF NORMAL AND ETHANOL-TREATED RATS (5 G/KG FOR 60 MIN)*

Rats	No. of animals	Glucose 6-phosphatase (μmole/g liver)	Fructose 1, 6-Diphosphatase (μmole/g liver)	β-Glucuronidase (μmole/mg protein)	Gulonolactone oxidase (μmole/mg protein)
Control	3	248 ± 15·0	165 ± 21·0	1·88 × 10^6 ± 5,650	278 ± 19·4
Ethanol-treated (60 min)	3	315 ± 10·3	167 ± 12·0	1·94 × 10^6 ± 17,400	207 ± 4·5
		P < 0·01	P > 0·05	P > 0·05	P < 0·01

* Data are represented as mean ±S.D. Significance levels were determined by Student's *t* test.

accumulation of electron dense particles in the cytoplasm of the alcohol-treated rat liver can be observed. The microsomal fraction exhibits complete membrane disorganization and an aggregation of the dense cytoplasmic particles. As might be expected, the dense particles are associated with lipid deposition, since total liver lipid increased from 20·2 ± 3·5 to 31·4 ± 2·1 mg/g liver (P < 0·05) in 60 min.

The metabolism of labeled substrates is summarized in Table 3. The ability of liver slices from alcohol-treated rats to oxidize substrates to CO_2 was impaired by approximately 30–50 per cent with labeled glucose and alanine and 20 per cent with palmitate.

TABLE 3. METABOLISM OF LABELED SUBSTRATES BY LIVER SLICES FROM NORMAL UNTREATED AND ALCOHOL-TREATED RATS (5 G/KG FOR 60 MIN)*

Animal	No. of animals	Glucose (μmole/g)	(cpm/g)	Per cent ^{14}C incorporation	CO_2 (cpm/g)	Per cent ^{14}C incorporation
				Glucose (5 mM)		
Control	3				806 ± 106	0·7
Ethanol	3				387 ± 98	0·3
				Palmitate (1·5 mM)		
Control	3	94·2 ± 5·0	130 ± 11	0·02	125 ± 9·7	0·01
Ethanol	3	95·0 ± 5·0	148 ± 21	0·02	102 ± 8·4	0·01
				Alanine (5 mM)		
Control	3	82 ± 2·0	43,000 ± 52	9·7	120,750 ± 4000	27
Ethanol	3	87 ± 4·0	93,000 ± 210	20·4	88,500 ± 3200	18

* Data are represented as mean ±S.D. Significance levels were determined by Student's *t* test.

Glucose production from alanine was increased by 50 per cent in liver slices from ethanol-treated rats. Changes in medium glucose concentration (Table 3) primarily reflect glycogenolysis, since only 4–5 μmole of alanine were incorporated into glucose even in the presence of ethanol.

DISCUSSION

These studies indicate that ethanol increases glucose 6-phosphatase activity in rat liver within 10–60 min, which is in contrast to the 12–48 hr required *in vivo* to change the enzyme activity in conditions such as diabetes and starvation.[6, 7] This specific effect of ethanol and possibly of isopropanol responds to increased alcohol concentrations, in comparison with previous studies which have demonstrated a non-specific inhibition by aliphatic alcohols on glucose and fructose metabolism.[17]

In studies on the role of ethanol on hepatic fatty acid metabolism, Scheig and Isselbacher[18] demonstrated that the stimulatory effect of ethanol on lipid synthesis *in vivo* is at the level of the hepatic microsome, since microsomal triglyceride synthesis was increased 5-fold in a period of 4 hr after ethanol administration. A significant increase in incorporation of ^{14}C-leucine by liver microsomes into liver protein after ethanol administration has also been reported.[19] These changes would be consistent with an increase in microsomal enzyme function after ethanol. That the alcohol effect is not general for all microsomal enzymes is evident by the observation that the activity of gulonolactone oxidase (Table 2) is decreased by alcohol administration.

The ethanol-induced 'unmasking' or activation of glucose 6-phosphatase appears to be more rapid than the hormone-mediated changes reported previously. This effect may be related to changes in the microsomal fraction observed in electron microscopy. Electron micrographs of the liver cell and microsomal fraction (Fig. 4) reveal an extensive disruption of cellular organelles, especially membrane integrity. This finding is supported by Rubin and Lieber,[20] but is in contrast to the results of ultrastructure studies that have demonstrated no extensive damage with an oral ethanol dose of 4·5 g/kg[21] and 6·0 g/kg.[22] In a similar manner, an oral ethanol dose of 6·0 g/kg in a 50 per cent solution administered via intragastric intubation produced no observable increase in glucose 6-phosphatase activity in liver homogenates (260 ± 22 to 256 ± 17). However, an oral ethanol dose of 12 g/kg did produce an increase in enzyme activity ($256 \pm 19{\cdot}0$–$302 \pm 11{\cdot}5$ μmole Pi/g liver, $P < 0{\cdot}01$). Doses of ethanol required to elicit changes in hepatic glucose 6-phosphatase activity are approximately the LD_{50} (5 g/kg i.p. and 13·5 g/kg i.g.). In view of these findings, it is suggested that the enzymic and metabolic effects of ethanol are dependent upon dose and route of administration. The structural disruption of the endoplasmic reticulum itself may enhance the activity of glucose 6-phosphatase and similarly inhibit gulonolactone oxidase.

In spite of the damage to the liver tissue by ethanol, the formation of glucose from labeled alanine (Table 3) suggests the presence of a functioning gluconeogenic pathway in ethanol-treated rats. Further, it should be noted that respiratory function is present although impaired. Kiessling and Pilstrom[3] have also shown that there is a relationship between ethanol-induced structural disorganization of mitochondria and impaired ability to oxidize various substrates. In this study, mitochondrial oxidation of labeled glucose and alanine are decreased only 50 per cent and 20 per cent with labeled palmitate. This suggests that the operation of the tricarboxylic acid cycle is only partially inhibited by ethanol.

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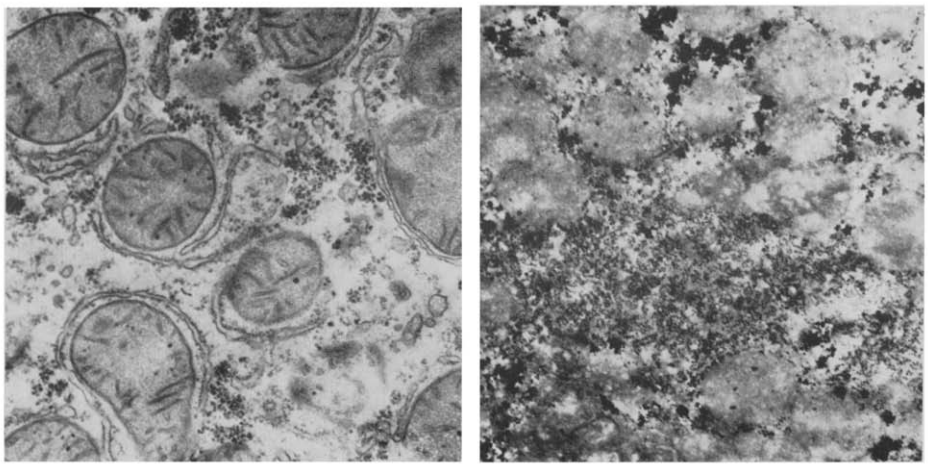

FIG. 3. Electron micrographs of liver cell from normal and ethanol-treated rats (5 g/kg for 60 min). Magnification ×21,440.

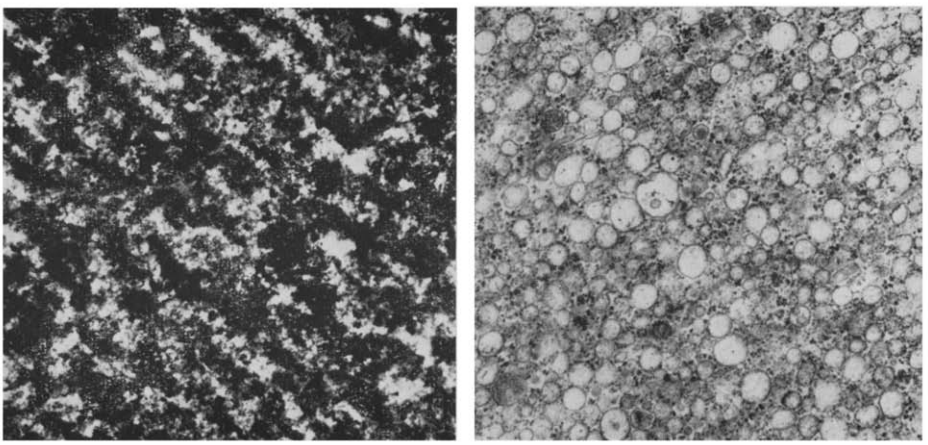

FIG. 4. Electron micrographs of liver microsomal fraction from normal and ethanol-treated rats (5 g/kg for 60 min). Magnification ×16,000.

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