Ethanol Effects on Hepatic Oxidations and Gluconeogenesis* (33957)

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Ethanol was shown to affect various biochemical systems, such as carbohydrate and lipid metabolism. In addition to the well-known ethanol-induced hepatic lipid deposition (1), ethanol produces impairment in hepatic oxidative metabolism (2) and gluconeogenesis (3). Freinkel *et al.* (2) demonstrated a decreased ability of ethanol-treated animals to metabolize carbohydrate and also a decreased ability to incorporate ^{14}C pyruvate and ^{14}C alanine to glucose. With ethanol, there is an increased conversion of pyruvate to lactate (4) which is associated with the increased $NADH_2$ levels. This rise in the lactate:pyruvate ratio is associated with a decreased gluconeogenesis (5, 6). Each of the oxidation steps in ethanol metabolism results in production of reduced NAD (7). Following ethanol administration there is an increase in the $NADH_2$:NAD ratio (8). Reoxidation of $NADH_2$ may occur by conversion of pyruvate to lactate, and by synthesis of fatty acids. To determine the effect of acute administration of ethanol on hepatic function, metabolism of ^{14}C glucose and ^{14}C alanine was studied at various time periods after alcohol administration.

Materials and Methods. Animals. Male albino rats of Wistar strain (fed *ad libitum*) were injected intraperitoneally with ethanol (5 g/kg of body wt), diluted with isotonic NaCl. After various time intervals the animals were sacrificed by stunning and decapitation and the livers were excised for metabolic studies.

Blood alcohol. Blood alcohol determinations were carried out on heparinized blood samples, from rats at various time periods (0–48 hr) after ethanol administration, diluted with an equal volume of an aqueous solution containing propyl acetate (0.50 mg/ml) as an internal standard. Blood alcohol levels were determined on an aerograph Hy-Fi, model 600 D, gas chromatograph with both flame ionization and electron capture detectors and equipped with an L and N Speedomax, 0.1 mV recorder. A 5 ft × 1/8-in. spiral stainless steel column containing 8% Hallcomid M-18 L on 80-100 mesh acid-washed chromosorb W was used in conjunction with a flame ionization detector. The injection port temperature was 150°, column temperature 85°; carrier gas (nitrogen) flow rate 22 ml/min; hydrogen and oxygen flow rates 77 and 143 ml/min, respectively. The assay parameter used was peak height ratio (ethanol:internal standard). Alcohol concentration (mg/100 ml) was calculated from peak height ratio and dilution factor and are summarized in Table I.

TABLE I. Blood Alcohol Concentrations in Animals at Various Time Periods after Ethanol Administration.

Time	No. of animals	Alcohol (mg/100 ml ± SD)[a]
(min)		
0	3	ND[b]
10	3	434 ± 42
15	3	440 ± 15
30	3	447 ± 56
60	3	420 ± 50
(hr)		
8	3	31 ± 12
12	3	ND
24	3	ND
48	3	ND

[a] Blood alcohol concentrations were determined by gas chromatographic analyses on normal animals at 0, 15, 30, and 60 min, and 12, 24, and 48 hr after ethanol administration (5 g/kg, ip). Controls were injected with isotonic NaCl (ip).

[b] Not detectable.

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TABLE II. Total Liver Lipid and Glycogen at Various Time Periods after Ethanol Administration.

Time after EtOH	No. of animals	(per g of liver ± SD) Glycogen (μmoles)	Lipid (mg)
Control 0	5	138 ± 5.2	68 ± 3.4
Ethanol 10 min	3	125 ± 7.1	75 ± 2.5
12 hr	3	50 ± 6.8	125 ± 5.0
24 hr	3	12 ± 6.8	76 ± 7.2
48 hr	3	78 ± 2.2	73 ± 2.8

Liver slices. Approximately 0.5-wet liver slices were prepared with a Stadie-Riggs hand microtome and incubated in 6.0 ml of Ringer bicarbonate medium equilibrated with 95% O_2–5% CO_2 as described previously (9). The medium contained 5 mmoles of U-^{14}C glucose, and 5 mmoles of U-^{14}C alanine to give an initial concentration of 1 mg/ml. After incubation for 90 min at 37° an aliquot of medium was analyzed for $^{14}CO_2$ and ^{14}C glucose. Glucose and glycogen were isolated as the phenylglucosazone and CO_2 as $BaCO_3$ for radioassay. The results of these studies are given in Tables III and IV.

Glycogen and lipid. Total liver lipid was assayed gravimetrically following chloroform–methanol extraction (10), and glycogen was isolated by the method of Good *et al.* (11). The results are expressed as micromoles of glucose per gram of liver for glycogen and lipid as millgrams of lipid per gram of liver and are summarized in Table II.

Results. Table I summarizes the data on blood alcohol concentrations (mg/100 ml) obtained from animals 0–48 hr after an ethanol dose of 5 g/kg, (ip). Peak levels of ethanol were obtained within 10–30 min following injection and concentrations of less than 10–30 min following injection and concentrations of less than 10% of the peak were observed at 8 hr. Beyond this time, ethanol in blood was not detectable by the method used (minimal detection, 5 mg/100 ml).

The hepatic concentrations of glycogen and lipid at various time periods after ethanol are summarized in Table II. Glycogen concentration decreases steadily and reached a minimum level at 24 hr after ethanol administration. Lipid concentration, however, increased and reached a maximum level at 12 hr after ethanol, returning to near normal levels after 24 hr. These values compare favorably with results obtained by Mirone (12) on mouse livers after a single dose of ethanol, demonstrating maximal decrease in liver glycogen and an increase in total liver lipid in 8–16 hr after ethanol.

Oxidation of ^{14}C glucose and ^{14}C alanine to $^{14}CO_2$ by liver slices from normal and ethanol-treated rats is summarized in Table III. Liver slices from rats 10 min, 1, and 12 hr after ethanol administration exhibited impairment in the ability to oxidize ^{14}C glucose

TABLE III. Oxidation of ^{14}C Labeled Glucose and Alanine to $^{14}CO_2$ by Liver Slices from Normal and Ethanol-Treated Rats.[a]

Time after EtOH	U-^{14}C Glucose $^{14}CO_2$[b] (cpm/g)	U-^{14}C Glucose ^{14}C oxidized (%)	U-^{14}C Alanine $^{14}CO_2$[b] (cpm/g)	U-^{14}C Alanine ^{14}C oxidized (%)
Control 0	4638 ± 550[c]	0.39	169,200 ± 4562	15.4
Ethanol 10 min	1644 ± 600	0.13	165,600 ± 8500	15.2
1 hr	1548 ± 400	0.12	108,700 ± 3200	9.8
12 hr	4025 ± 200	0.30	147,878 ± 8150	13.4
24 hr	4722 ± 1214	0.39	126,500 ± 9000	11.5
48 hr	6309 ± 1222	0.50	165,580 ± 2400	15.1

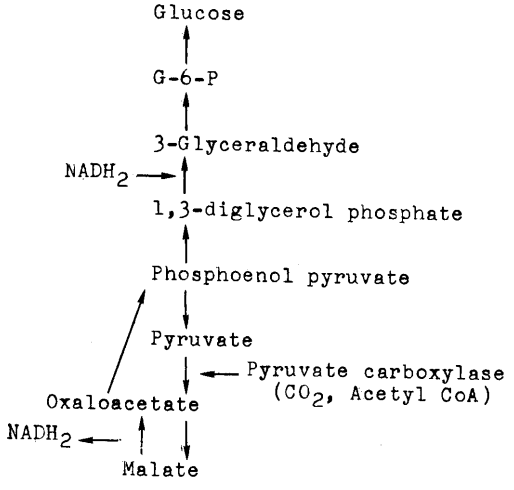
[a] Ethanol was administered in a dose of 5 g/kg ip; controls were injected with isotonic NaCl ip; animals were sacrificed after 60 min.

[b] Specific activity of $^{14}CO_2$ was determined from $Ba^{14}CO_3$ as described in the text.

[c] Each value is mean ± SE of 4 or more animals.

(approx 55%) and ^{14}C alanine (approx 10%). The impairment becomes less marked after 24 and 48 hr and the decreased ability of liver slices from ethanol-treated rats to oxidize labeled substrates gradually returns to near normal control values in this time period. The incorporation of ^{14}C-labeled alanine into glucose is summarized in Table IV. Liver slices from rats 10 min, 1, and 12 hr after ethanol treatment incorporate more label into glucose than normal rat liver, but after 24 and 48 hr less label was incorporated.

Discussion. The increased incorporation of label in the initial time periods after ethanol treatment may be explained as a consequence of an increased levels of cofactors such as $NADH_2$ and acetyl CoA resulting from the metabolism of ethanol. These cofactors could influence gluconeogenesis at the steps outlined in scheme I.



An increase in cofactor levels produced while ethanol is being metabolized, might stimulate incorporation of label into glucose. Gluconeogenesis form lactate, pyruvate, glycerol, and amino acids is a major and essential function of the liver. The process maintains the supply of glucose for the nervous system at times when dietary carbohydrate is lacking and glycogen reserves are low; it plays an important role in the conservation of carbohydrate through resynthesis of glucose from lactate and pyruvate produced by muscle and erythrocytes and from the glycerol released by hydrolysis of fats in adipose tissue. The accumulation of gluconeogenic precursors and cofactors ($NADH_2$ and Acetyl CoA) produced by ethanol metabolism may stimulate the production of glucose from substances such as alanine.

TABLE IV. Incorporation of ^{14}C Alanine into Glucose by Liver Slices from Normal and Ethanol-Treated Rats.[a]

Time after EtOH	Glucose[b] (μmoles/g)	(cpm/g)[c]	^{14}C incorporation into glucose (%)
Control			
0	98 ± 6.2[d]	17,000 ± 1839	1.4
Ethanol			
10 min	115 ± 5.1	32,500 ± 1200	2.7
1 hr	87 ± 4.0	36,166 ± 5100	3.8
12 hr	40 ± 5.0	14,838 ± 1400	1.3
24 hr	48 ±11.0	10,689 ± 3000	0.97
48 hr	57 ± 4.0	10,280 ± 1900	0.9

[a] Ethanol was administered in a dose of 5 g/kg ip; controls were injected with isotonic NaCl (ip).

[b] Medium glucose was determined enzymatically by the glucose oxidase method (98, 99).

[c] Specific activity of ^{14}C glucose was determined from phenylglucosazones as described in the text.

[d] Each value is mean ± SE of 4 or more animals.

One of the primary steps in gluconeogenesis in rat liver involves carboxylation of pyruvate (PEP) by PEP carboxykinase (14–16). PEP, aspartate, melate, α-ketoglutarate, and some citrate can diffuse from mitochondria (15, 16). In the extramitochondrial compartment of the cell, oxidation of malate and transamination of aspartate yield oxaloacetate which can be converted to (PEP) by the soluble PEP carboxykinase. The oxidation of malate continually forms $NADH_2$, and this $NADH_2$ may serve for the reduction of 1, 3-diphosphoglycerate to triose phosphate. Ethanol is metabolized at 0.5 g/kg/hr (1), and hence almost all the alcohol absorbed from the 5 g/kg dose should be metabolized in 10 to 12 hr. Gas chromatographic analyses of blood alcohol in ethanol-treated rats showed a drop from 420 mg/100 ml in 1 hr to 31 mg/100 ml in 8 hr. There was no detectable alcohol at 12 hr. This correlates

well with decreased gluconeogenesis observed at 12 hr, as the incorporation of label into glucose from a maximum incorporation at 1 hr (3.8%) returns to near normal (1.3%) levels after 12 hr.

Summary. Effect of ethanol administration of hepatic metabolism was studied. After the ethanol administration (5 g/kg ip) blood alcohol concentration reached a maximum level in 60 min and there was no detectable alcohol after 12 hr. Glycogen concentration decreased after ethanol administration. Total liver lipid exhibited a maximal increase in 12 hr after ethanol administration. Oxidation of ^{14}C alanine and ^{14}C glucose was impaired at 10 min, 1, and 12 hr after ethanol administration. However, the incorporation of ^{14}C alanine into glucose was increased in 1–12 hr.

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