MOLECULAR PHARMACOLOGY, 20:621-630

The Effect of Ethanol on the Activities of the Key Gluconeogenic and Glycolytic Enzymes of Rat Liver

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Received March 24, 1981; Accepted July 1, 1981

SUMMARY

A systematic study was conducted to determine the effects of an acute dose of ethanol (1–5 g/kg body weight) on all of the key regulatory enzymes of glucose metabolism in rat liver. The effect of ethanol on the activities of these enzymes varied depending on the dose of ethanol, whether or not the rats were fed or fasted, and whether or not the enzymes were assayed in the presence of their specific activators. In general, ethanol increased the total activities of the key gluconeogenic enzymes and decreased the total activities of the key glycolytic enzymes in the livers of fed rats. For example, ethanol increased the activities of pyruvate carboxylase and glucose 6-phosphatase and decreased the activities of glucokinase, hexokinase, phosphofructokinase, and pyruvate kinase. The extent of the inhibition of phosphofructokinase activity by ethanol was greater when this enzyme was assayed in the absence of its specific activators (70% inhibition) than when assayed in their presence (20% inhibition). Similarly, the inhibition of the activity of pyruvate kinase was greater when assayed in the absence of its activator. In fasted rats, ethanol decreased the activities of liver pyruvate carboxylase and phosphoenolpyruvate carboxykinase, and increased the activities of phosphofructokinase and pyruvate kinase. Ethanol-induced changes in glucose levels may be related to the differential effect of ethanol on these enzymes. Ethanol increased the ratio of the activities of the key gluconeogenic enzymes to glycolytic enzymes in the livers of fed animals. For example, with ethanol, 5 g/kg of body weight, the activity at the glucose 6-phosphatase/hexokinase + glucokinase step increased by 55%; at the fructose bisphosphatase/phosphofructokinase step, by 200%; and at the pyruvate carboxylase + phosphoenolpyruvate carboxykinase/pyruvate kinase step, by 88%. In contrast, in fasted rats, ethanol decreased the activity at the fructose bisphosphatase/phosphofructokinase step by 7%, and at the pyruvate carboxylase + phosphoenolpyruvate carboxykinase/pyruvate kinase step by 41%. These results suggest that ethanol-induced hyperglycemia in fed animals may be a consequence of an increase in the ratio of the activities of the key gluconeogenic enzymes to the glycolytic enzymes. A decrease in the ratio of the activities of these enzymes may be responsible for the ethanol-induced hypoglycemia in fasted animals. Ethanol may alter the activities of these enzymes by influencing the transition of the active-inactive forms or by affecting the total content of these enzymes.

INTRODUCTION

The effects of ethanol on glucose metabolism vary considerably with the nutritional state of animals. After a prolonged fast in human subjects, ethanol ingestion causes a profound hypoglycemia and a decrease in peripheral utilization of glucose (1–4). However, in fed human subjects and other animals, ethanol potentiates the hyperglycemia caused by glucose ingestion and may also by itself increase blood glucose and insulin concentrations (1).

Ethanol-induced hypoglycemia has been attributed to a decrease in the rate of gluconeogenesis. Measurement of the rate of ethanol utilization and acetate formation in perfused rat liver has shown that over 80% of the ethanol is metabolized via alcohol dehydrogenase and acetaldehyde dehydrogenase and is converted to acetate (5). This results in a decrease in NAD^+/NADH ratio, which lowers the concentration of pyruvate, a substrate for gluconeogenesis. The fall in the concentration of pyruvate lowers the rate of PC[1] reaction, one of the rate-limiting reactions

This work was supported by a grant from the Distilled Spirits Council of the United States of America, Inc.

[1] The abbreviations used are: PC, pyruvate carboxylase (EC 6.4.1.1); PFK, phosphofructokinase (EC 2.7.1.11); PK, pyruvate kinase (EC 2.7.1.40); Fru-P_2ase, fructose bisphosphatase (EC 3.1.3.11); PEPCK, phosphoenolpyruvate carboxykinase (EC 4.1.1.32); G6Pase, glucose 6-phosphatase (EC 3.1.3.9); HK, hexokinase (EC 2.7.1.1); GK, glucokinase (EC 2.7.1.2); Fru-P_2, fructose bisphosphate.

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in gluconeogenesis (6). This decrease in the concentration of pyruvate has been ascribed to an increase in the conversion of pyruvate to lactate (7). A decrease in the $NAD^+/NADH$ ratio may not be the only reason for the inhibition of gluconeogenesis. It is possible that a decrease in the concentration of pyruvate may be a result of the inhibition of the glycolytic enzymes by ethanol. Similarly, inhibition of gluconeogenesis may be associated with an inhibition by ethanol of the gluconeogenic enzymes. However, reports about the effect of ethanol on the activities of these enzymes are not consistent. Although Kiessling and Pilstrom (8) have reported an increase in the activity of rat liver PFK after ethanol ingestion, other reports have indicated a decrease in the enzyme activity caused by this agent (9, 10). Rat liver PK and Fru-P_2ase activities have also been reported to be unaffected or decreased by ethanol (9–11).

The discrepancies in these reports are due to various factors, e.g., assay conditions of the enzymes, use of only single doses of ethanol, route of administration, and nutritional state of the animals. There is no report in the literature that suggests whether the response of these enzymes to ethanol would differ if the enzymes were assayed in the presence or absence of their specific activators.

We here report that the response of the key rat liver gluconeogenic enzymes such as PC, PEPCK, Fru-P_2ase, and G6Pase, and the key glycolytic enzymes such as HK, GK, PFK, and PK to ethanol varies with the dose of ethanol, assay conditions of the enzymes, and dietary status of the animals. In general, ethanol increased the ratio of the activities of the key gluconeogenic enzymes to the glycolytic enzymes in fed animals, and decreased this ratio in the fasted animals.

MATERIALS AND METHODS

Chemicals

All of the chemicals and auxiliary enzymes used were purchased from Sigma Chemical Company (St. Louis, Mo.).

Rats

Male Sprague-Dawley rats weighing 200–250 g were normally fed or fasted for 48 hr.

Ethanol Administration

One part absolute ethanol was diluted with two parts isotonic NaCl and injected i.p. to rats in a final dose of 1, 3, or 5 g of ethanol per kilogram of body weight (11). The control rats were given 0.9% NaCl only. All of the rats were killed by decapitation 1 hr after the administration of ethanol or 0.9% NaCl.

Preparation of Liver Extracts

In a typical experiment, each dose of ethanol was given to at least three rats. Livers were pooled from the animals receiving the same dose of ethanol, washed with "homogenizing buffer" consisting of 0.25 M sucrose, 50 mM Tris-HCl (pH 7.4), and 50 mM β-mercaptoethanol. The livers were homogenized twice with 5 volumes of the same buffer in a Waring Blendor for 30 sec each time. The homogenate was used for the assay of G6Pase activity. The homogenate was centrifuged at 600 × *g* for 15 min at 4° to remove unbroken cells and nuclei. The supernatant obtained was centrifuged at 10,000 × *g* for 30 min to obtain the pellet containing mitochondria. The pellet was suspended in 10 ml of the same buffer and homogenized in a Polytron homogenizer for 30 sec. This mitochondrial preparation was used to assay PC activity. The 10,000 × *g* supernatant was used to assay all of the remaining enzymes. The extracts were stored at −90°, at which temperature all of the enzymes were stable.

Assay of Enzymes

All of the enzymes were assayed at 25° in the presence of 0.05 M Tris-HCl (pH 7.4) in a total volume of 1 ml. The remaining components of the assay mixtures are described below. For all of the enzymes, blank reactions were measured in the absence of their specific substrates and the rates were corrected accordingly. Protein was measured by using the Folin phenol reagent. The specific activities of all of the enzymes are expressed as micromoles of substrate consumed per minute per milligram of protein. One unit of enzyme activity is defined as micromoles of substrate consumed per minute. The results shown in Tables 1–5 and Figs. 1–8 are the average of three to six experiments; three rats were used for each dose of ethanol in every experiment. Each enzyme was assayed in triplicate; therefore, each value is the mean of at least 9–18 points. The standard deviation for each value was also determined.

PC assay. PC was assayed in forward direction by the method described by McClure *et al.* (12). The reaction mixture consisted of 8 mM sodium pyruvate, 21 mM $NaHCO_3$ (freshly made), 9 mM $MgSO_4$, 20 mM KCl, 2 mM ATP, 0.16 mM NADH, 0.16 mM acetyl CoA and 5 units of malate dehydrogenase. The oxidation of NADH was monitored at 340 nm in a Gilford recording spectrophotometer.

PEPCK assay. The assay system was a modification of the method described by Colombo *et al.* (13) and consisted of 2 mM phosphoenolpyruvate, 47 mM $NaHCO_3$ (freshly made), 1 mM inosine diphosphate 2.25 mM $MgCl_2$, 0.25 mM NADH, and 22 units of malate dehydrogenase.

Fru-P_2ase assay. The assay system was based on the method described by Tejwani *et al.* (14) and consisted of 0.2 mM Fru-P_2, 2 mM $MgCl_2$, 10 mM $(NH_4)_2SO_4$, 0.2 mM NADP, 0.8 unit of glucose 6-phosphate dehydrogenase, and 1.1 units of phosphoglucoseoisomerase. EDTA (0.1 mM) was used as an activator. Reduction of NADP was monitored at 340 nm.

G6Pase assay. The assay system described by Cori *et al.* (15) was modified in the following way. It consisted of 10 mM glucose 6-phosphate and 0.1% deoxycholate. Phosphatidylcholine (10 mM) was used as an activator. After the addition of homogenate the samples were incubated at 25° for 20 min. The reaction was stopped by adding trichloroacetic acid (10% final concentration) and the samples were centrifuged. The supernatant was diluted 1:10 with water and assayed for P_i using malachite green dye as described earlier (14).

HK and GK assays. HK and GK assay systems were based on the method described by Dileepan *et al.* (16).

HK activity was assayed in the presence of 2.5 mM glucose. The enzyme was also assayed in the presence of 100 mM glucose. GK activity was calculated by subtracting HK activity from the activity obtained in the presence of 100 mM glucose. The other components of the assay system were 5 mM ATP, 0.5 mM NADP, 7.5 mM $MgSO_4$, 100 mM KCl, 5 mM β-mercaptoethanol, and 0.3 unit of glucose 6-phosphate dehydrogenase.

PFK assay. The PFK assay system was based on the method described by Tejwani and Ramaiah (17). It consisted of 1 mM fructose 6-phosphate, 1 mM ATP, 5 mM $MgCl_2$, 0.15 mM NADH, and dialyzed auxiliary enzymes consisting of 0.4 unit of aldolase, 1 unit of α-glycerophosphate dehydrogenase, and 1 unit of triosephosphate isomerase. Activators used were 5 mM $(NH_4)_2SO_4$, 2.5 mM K_2HPO_4, and 0.5 mM AMP. These effectors activate PFK in a synergistic manner to give the maximal possible activity of the enzyme (17).

PK assay. The assay system was based on the method described by Cardenas and Dyson (18). It consisted of 1 mM phosphoenolpyruvate, 2 mM ADP, 8 mM $MgSO_4$, 75 mM KCl, 0.5 mM EDTA, 0.15 mM NADH, and 2 units of lactate dehydrogenase. Fru-P_2 (1 mM) was used as an activator.

RESULTS

Effect of Ethanol on Activities of Key Gluconeogenic Enzymes from Livers of Fed and Fasted Rats

Total activities. Ethanol increased the activity of PC by 33% in fed animals. This effect was more pronounced at the lower concentration of ethanol. However, in fasted animals ethanol decreased the activity of the enzyme by 37% (Fig. 1). Similarly, the activity of PEPCK was inhibited 33% by ethanol in fasted rats, whereas it was not significantly affected in fed animals (Fig. 2). However, ethanol decreased the activity of Fru-P_2ase in fed animals. This inhibition was 15% and 24% respectively, when the enzyme was assayed in the absence and presence of EDTA, an activator of the enzyme (Fig. 3A). In contrast, in fasted animals the activity of Fru-P_2ase was increased 13% and 30% by ethanol when the enzyme was assayed in the absence and presence of EDTA, respectively (Fig. 3B). The activity of G6Pase was not affected by ethanol to any significant extent in fed rats (Fig. 4A), but in fasted animals the activity of the enzyme increased by 10% and 25%, respectively, when it was assayed in the presence and absence of phosphatidylcholine, an activator of the enzyme (15).

Specific activities. Ethanol also affected the specific activities of these enzymes. In fed animals, ethanol increased the activity of the PC by 28%, whereas it decreased the activity of PEPCK by 25% (Table 1). The activity of Fru-P_2ase was inhibited significantly (by 28%) only when the enzyme was assayed in the presence of EDTA, and the activity of G6Pase was not affected significantly in fed animals (Table 1). In contrast, ethanol did not affect the activity of PC in fasted animals, whereas it increased PEPCK activity by 66% (Table 2). The specific activities of Fru-P_2ase and G6Pase increased by 50% and 32%, respectively, in ethanol-treated fasted rats (Table 2). The ethanol-induced increase in the specific activity of G6Pase may be due to its effect on the release of this enzyme from the hepatic microsomal membranes, as shown by Nelson *et al.* (11).

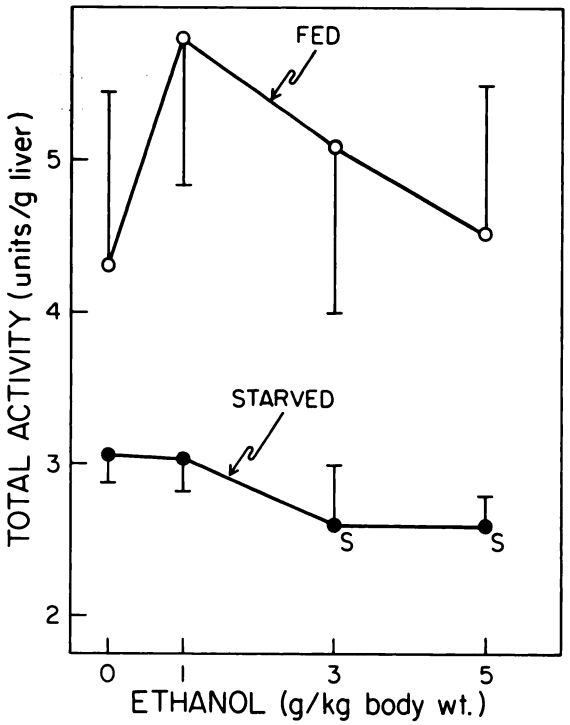

FIG. 1. *Total activity of PC from fed and fasted rats, 1 hr after i.p. injection of ethanol*

Liver extract was made as described under Materials and Methods. PC activity was assayed in a 1:10 diluted 10,000 × *g* "mitochondrial" pellet (0.01 ml) fraction. One unit of enzyme activity is defined as micromoles of substrate consumed per minute. Total activity is defined as units per gram of liver, wet weight. O——O, Normally fed rats; ●——●, rats fasted for 48 hr prior to ethanol injection; *S*, statistically significant difference from controls given only 0.9% NaCl solution ($p < 0.05$). Each *point* represents the mean of at least nine points; vertical bars indicate *n*-1 standard deviations.

Effect of Ethanol on Activities of Key Glycolytic Enzymes from Livers of Fed and Fasted Rats

Total activities. Ethanol inhibited the activity of GK in both fed rats (20%) and fasted rats (48%). Food deprivation by itself also decreased the activity of the GK from 0.89 to 0.5 unit/g of liver, wet weight (Fig. 5). However, HK activity was more inhibited by ethanol in fed animals (50%), than in animals deprived of food (15%, Fig. 6). The extent of inhibition of PFK activity by ethanol was greater (70%) when the enzyme was assayed in the absence of its activators. The inhibition was only 20% when the enzyme was assayed in the presence of AMP, P_i, NH_4^+, and K^+ ions (Fig. 7A). In contrast, in fasted animals, ethanol increased the activity of PFK from 0.6 to 1.7 units/g of liver, wet weight (Fig. 7B). The effect of ethanol on PK activity was similar to that on PFK activity. Ethanol inhibited the activity of PK by 50% in fed animals (Fig. 8A) but increased the activity of the enzyme by about 54% in fasted rats (Fig. 8B).

Specific activities. The activity of all of the glycolytic enzymes decreased in the ethanol-injected animals. The activity of GK and HK decreased 20–25% with ethanol, 5 g/kg of body weight (Table 3). The activity of PFK

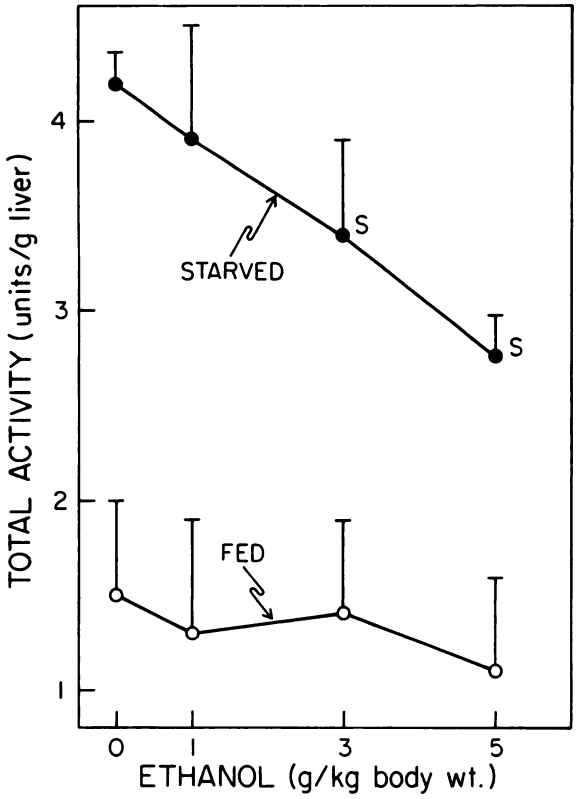

FIG. 2. *Effect of ethanol on the total activity of PEPCK*
Activity of the enzyme is based on the reversed conversion of phosphoenolpyruvate to oxaloacetate. Other details are the same as described in the legend to Fig. 1 and under Materials and Methods.

decreased by 50% when the enzyme was assayed in the absence of its activators. However, this inhibition was only 30% when the enzyme was assayed in the presence of its positive effectors such as AMP, P_i, NH_4^+, and K^+ ions. Similarly, the inhibition of PK activity by ethanol depended upon the presence of its activator, Fru-P_2, in the assay mixture. This inhibition was 17% and 38% when the enzyme was assayed in the presence and absence of Fru-P_2, respectively (Table 3).

The activity of both GK and HK decreased when the rats were deprived of food for 48 hr. As shown in Table 4, the activity of GK decreased further by 45% with ethanol, 5 g/kg of body weight, whereas the activity of HK was decreased in the rats given a low dose of ethanol (1–3 g/kg of body weight). Similarly, at the high dose of ethanol, the activity of PFK in the liver of fasted rats was increased by 20% and 40% when the enzyme was assayed in the absence and presence of the positive effectors, respectively. However, the activity of PK decreased by 20–25% in the fasted animals given ethanol (Table 4).

DISCUSSION

The effect of ethanol on glucose metabolism may occur as a result of modulation of the key reactions that determine the fluxes of metabolites throughout the various pathways. These key reactions are catalyzed by enzymes which are subject to stringent control. The rate of a key step or enzymatic reaction of a particular pathway can be regulated by (a) a change in the activity of the enzyme brought about by an alteration of the levels of its sub-

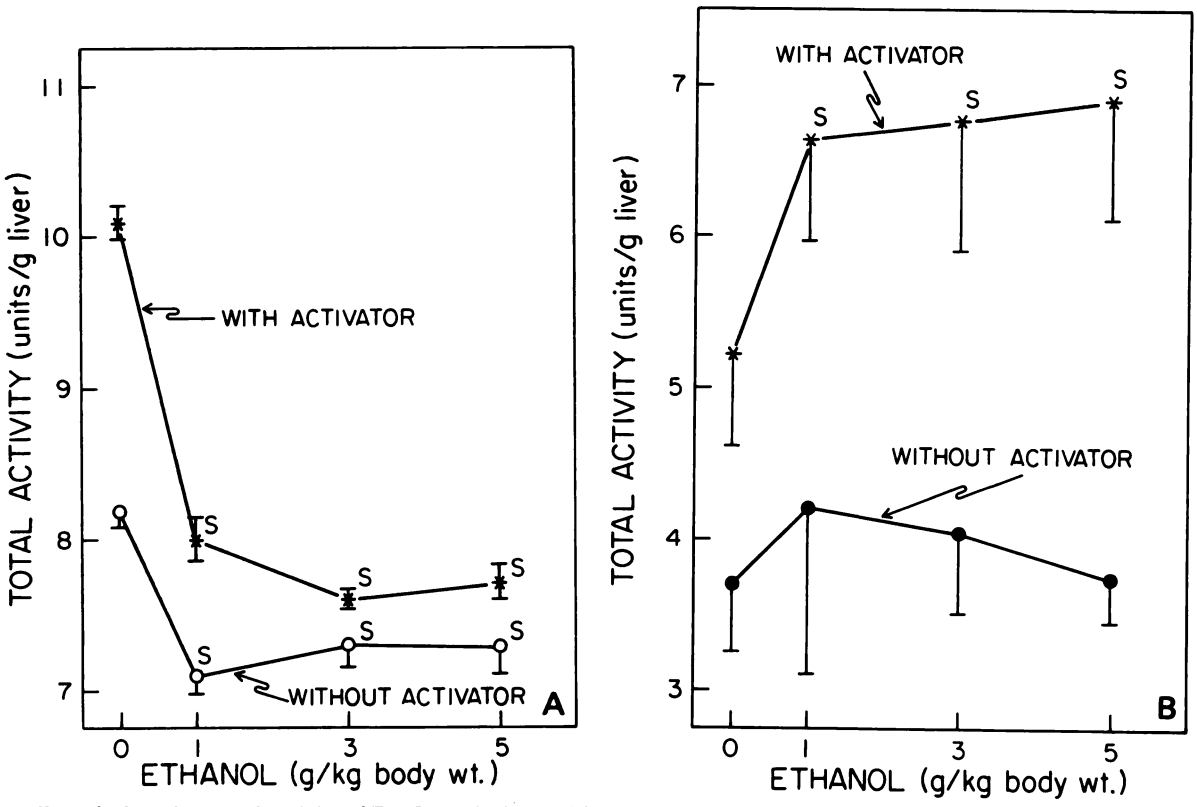

FIG. 3. *Effect of ethanol on total activity of Fru-P_2ase in fed and fasted rats*
A. Normally fed rats. EDTA was used as an activator. Other details are the same as described in the legend to Fig. 1 and under Materials and Methods.
B. Fasted rats. EDTA was used as an activator. Other details are the same as described in the legends to Figs. 1 and 3A.

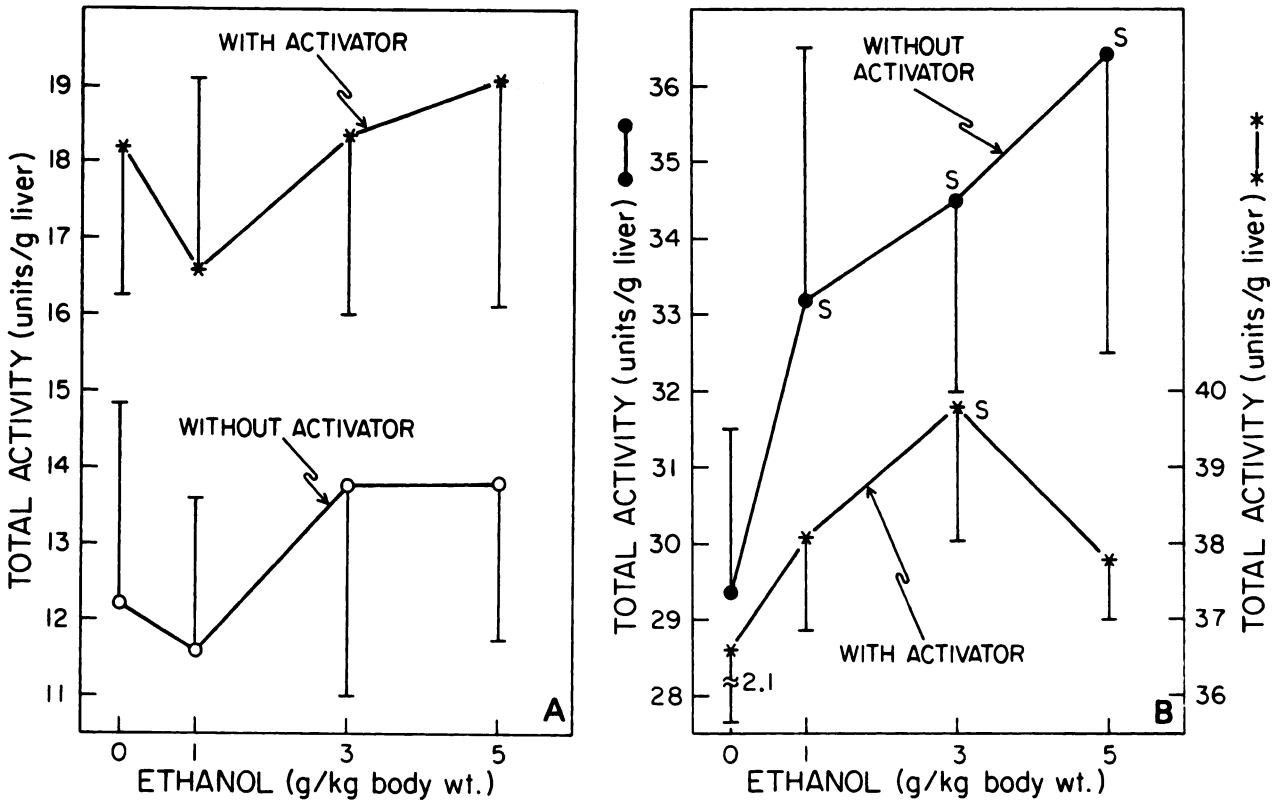

FIG. 4. *Effect of ethanol on total activity of G6Pase in fed and fasted rats*

A. Normally fed rats. G6Pase activity was assayed in a 1:10 diluted homogenate (0.1 ml) fraction of liver extract. Phosphatidylcholine was used as an activator. Other details are the same as described in the legend to Fig. 1 and under Materials and Methods.

B. Fasted rats. Phosphatidylcholine was used as an activator. Other details are the same as described in the legends to Figs. 1 and 4A.

strates, activators, or inhibitors (19), or (b) by a change in the total content of enzyme, by altering either the rate of its synthesis or degradation, or both, to different extents (20). The changes in the concentrations of these effectors may also transform the enzyme to a less active or more active form (21). If the enzyme is assayed *in vitro* without any added effectors, the activity of the enzyme to a certain extent may reflect its *in vivo* activity. However, to detect a change in the total content of the enzyme, it is appropriate to assay the enzyme activity in

TABLE 1

Effect of ethanol on the specific activities of key gluconeogenic enzymes from livers of fed rats

Ethanol	Enzyme[a]			
	PC	PEPCK	Fru-P_2ase	G6Pase
g/kg body wt	*μmoles/min/mg protein*			
0	0.14 ± 0.017 (100)	0.071 ± 0.007 (100)	0.29 ± 0.001 (100) 0.36 ± 0.001[b] (100)[b]	0.38 ± 0.05 (100) 0.50 ± 0.10[b] (100)[b]
1	0.15 ± 0.022 (107)	0.061 ± 0.012 (86)	0.27 ± 0.001[c] (93) 0.26 ± 0.002[b, c] (72)[b]	0.30 ± 0.13 (79) 0.43 ± 0.19[b] (78)[b]
3	0.18 ± 0.02[c] (128)	0.065 ± 0.008[c] (92)	0.25 ± 0.001[c] (86) 0.30 ± 0.001[b, c] (83)[b]	0.39 ± 0.09 (103) 0.52 ± 0.29[b] (94)[b]
5	0.17 ± 0.02[c] (212)	0.053 ±0.004[c] (75)	0.27 ± 0.002[c] (93) 0.31 ± 0.005[b, c] (86)[b]	0.36 ± 0.06 (95) 0.6 ± 0.10[b] (109)[b]

[a] Values are means ± standard deviation of at least nine points. Values in parentheses indicate activities in percentages relative to control rats given no ethanol.

[b] Activities of the enzymes in the presence of their specific activators.

[c] Statistically significant difference from control ($p < 0.05$).

TABLE 2

Effect of ethanol on the specific activities of key gluconeogenic enzymes from livers of rats fasted for 48 hr

Ethanol	Enzyme[a]			
	PC	PEPCK	Fru-P_2ase	G6Pase
g/kg body wt	*μmoles/min/mg protein*			
0	0.168 ± 0.026 (100)	0.18 ± 0.036[b] (100)	0.20 ± 0.03[b] (100) 0.29 ± 0.05[c] (100)[c]	1.47 ± 0.02[b] (100) 1.47 ± 0.24[c] (100)[c]
1	0.185 ± 0.018 (110)	0.18 ± 0.05 (100)	0.22 ± 0.04 (101) 0.35 ± 0.03[c] (120)[c]	1.36 ± 0.38 (93) 1.62 ± 0.21[c] (110)[c]
3	0.163 ± 0.02 (97)	0.25 ± 0.05[d] (144)	0.26 ± 0.05 (130) 0.44 ± 0.06[c, d] (150)	1.69 ± 0.28 (115) 1.93[c, d] (132)[c]
5	0.175 ± 0.02 (104)	0.3 ± 0.03[d] (166)	0.21 ± 0.06 (100.5) 0.43 ± 0.05[c, d] (148)	1.15 ± 0.12[d] (78) 1.45 ± 0.05[c] (99)

[a] Values are means ± standard deviation of at least nine points. Values in parentheses indicate activities in percentages relative to control rats given no ethanol.

[b] Statistically significant difference from fed controls ($p < 0.05$).

[c] Activity of the enzymes in the presence of their specific activators.

[d] Statistically significant difference from control ($p < 0.05$).

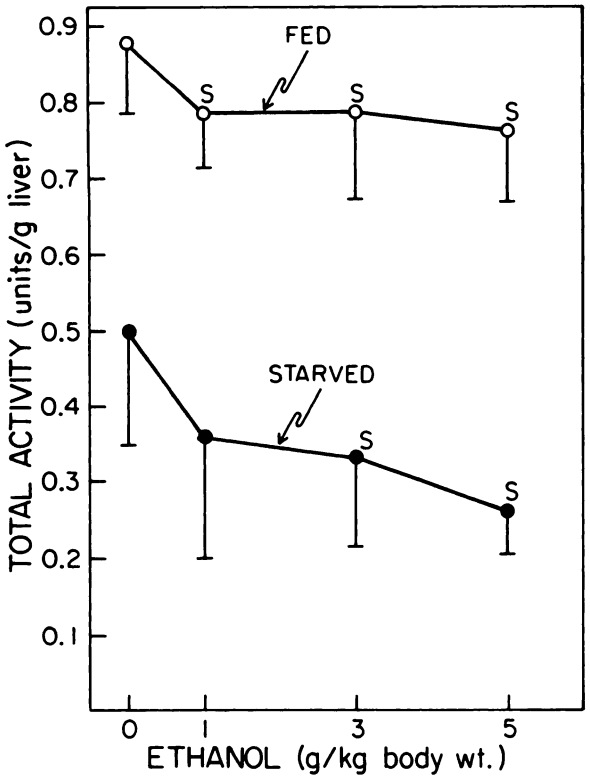

FIG. 5. *Effect of ethanol on the total activity of GK from fed and fasted rats*

Enzyme activity was assayed in presence of 100 mM glucose. GK activity was computed as the enzyme activity obtained with 100 mM glucose minus the activity of HK obtained with 2.5 mM glucose. Other details are the same as described in the legend to Fig. 1 and under Materials and Methods.

the presence of its activators (to obtain the maximal activity of the enzyme).

Ethanol has been shown to inhibit both gluconeogenesis (6) and glycolysis (22). Zakim (7) determined the effects of ethanol on the concentration of a number of gluconeogenic intermediates in rat liver. He reported that ethanol-induced inhibition of hepatic glucose production may be due to a decrease in the concentration of the gluconeogenic intermediates such as aspartate, 2-phosphoglycerate, 3-phosphoglycerate, Fru-P_2, fructose 6-phosphate, and glucose 6-phosphate. Williamson *et al.* (5) measured the concentration of all of the glycolytic intermediates and reported that ethanol in perfused rat liver decreased the concentrations of pyruvate and phosphoenolpyruvate by 71% and 52%, respectively, and increased the concentration of glucose 6-phosphate by 68%. The rate of PC and PK reactions would decrease as a result of a decrease in their substrates (pyruvate and phosphoenolpyruvate) concentrations. Similarly, the rate of HK reaction would decrease as a result of an increase in the concentration of glucose 6-phosphate, which has been shown to inhibit this enzyme in an allosteric manner (23). The rate of gluconeogenesis and glycolysis may also decrease as a result of the inhibition of the key enzymes by ethanol. For example, ethanol has been shown to inhibit the activities of PC, Fru-P_2ase, GK, PFK, and PK (9, 10). However, the mode of the inhibition of these enzymes by ethanol is still unknown.

The effects of ethanol on the blood glucose levels in animals depend on the nutritional state of the animals. In fed animals, ethanol causes hyperglycemia (1–4), which may be due to the increase in the activities of the key gluconeogenic enzymes or due to the inhibition of the key glucose-utilizing enzymes, or both. This is supported by the results shown in Figs. 1–8. Ethanol increased the total activity of PC (Fig. 1) and G6Pase (Fig. 4A) in fed animals, whereas it decreased the activities of glucose-utilizing enzymes such as GK (Fig. 5), HK (Fig. 6), PFK (Fig. 7A), and PK (Fig. 8A). In fasted animals, ethanol causes hypoglycemia which may be due to the inhibition of the key gluconeogenic enzymes or the increase in the key glycolytic enzymes, or both. This is supported by the ethanol-induced inhibition of the activity of PC (Fig. 1) and PEPCK (Fig. 2) in fasted animals. At the same time, ethanol increased the activities of the key glucose-utilizing enzymes such as PFK (Fig. 7B) and PK (Fig. 8B) in these animals.

In order to investigate whether ethanol-induced hyperglycemia in fed animals and hypoglycemia in fasted animals is due to the reciprocal effect of ethanol on the activities of these enzymes, we determined the activities of all of the key enzymes in livers of rats given ethanol, 5 g/kg of body weight (Table 5). The activities of all of the enzymes were determined in the absence of their specific activators so that the activities obtained may reflect their activities *in vivo*. In fed animals, ethanol increased activity at the G6Pase/HK + GK step from

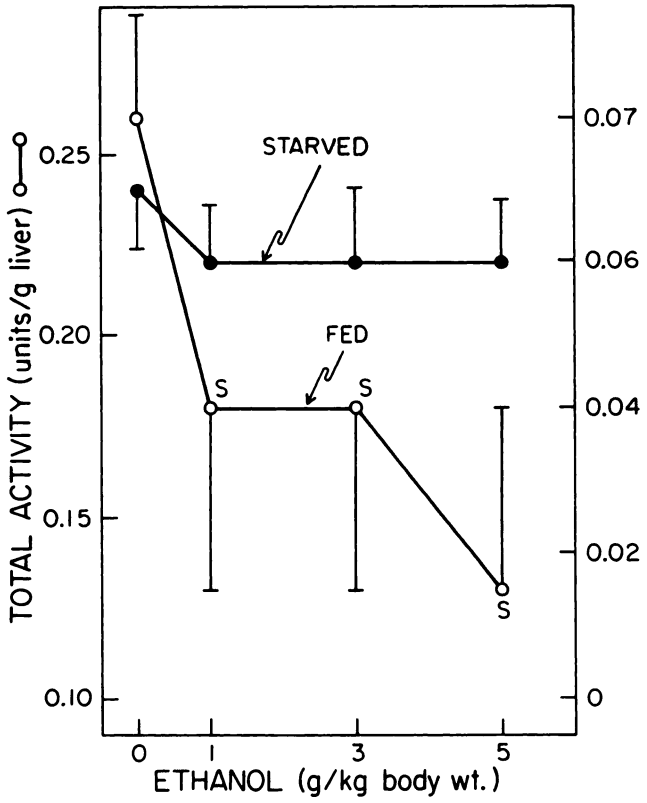

FIG. 6. *Effect of ethanol on the total activity of HK from fed and fasted rats*

Other details are the same as described in the legend to Fig. 1 and under Materials and Methods.

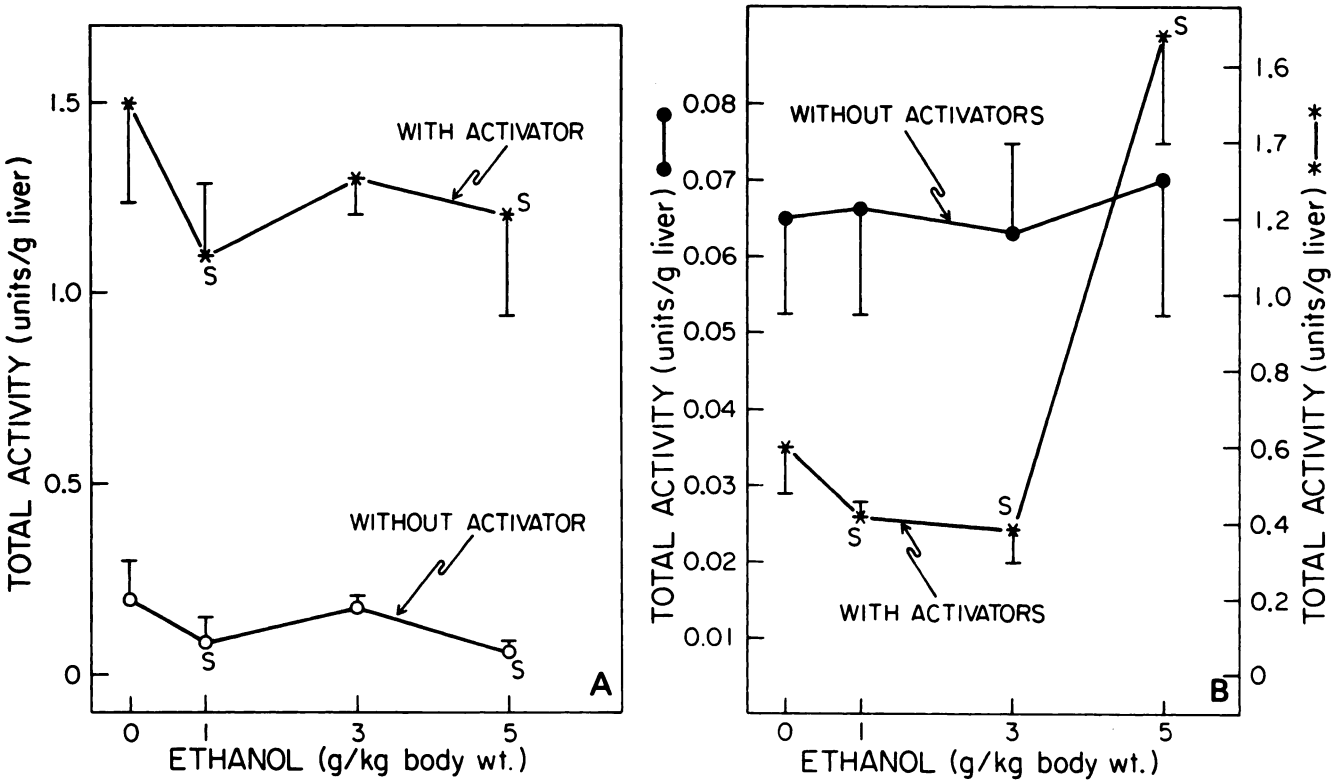

FIG. 7. *Effect of ethanol on total activity of PFK in fed and fasted rats*

A. Normally fed rats. Activators used were AMP (0.5 mM), $(NH_4)_2SO_4$ (5 mM), and K_2HPO_4 (2.5 mM). Other details are the same as described in the legend to Fig. 1 and under Materials and Methods.

B. Fasted rats. Other details are the same as described in the legend to Fig. 7A.

10.6 to 16.4 (55% increase), at the Fru-P_2ase/PFK step from 41 to 122 (200% increase), and at the PC + PEPCK/PK step from 0.85 to 1.6 (88% increase). In contrast, in fasted animals, ethanol decreased activity at the Fru-P_2ase/PFK step by 7% and at the PC + PEPCK/PK step by 41% (Table 5). These results suggest that ethanol may produce hyperglycemia in fed animals by increasing the ratio of the activities of the key gluconeogenic enzymes to the glycolytic enzymes in the livers of these animals. In fasted animals, however, a decrease in the ratio of the activities of these enzymes may be responsible for the ethanol-induced hypoglycemia.

While determining the effect of ethanol on the activities of these enzymes, it is important to specify whether or not the activators of the enzymes are included in the assay system. Previous studies have not determined the effect of ethanol on the activities of any of the enzymes of glucose metabolism by assaying their activities in the presence and absence of specific activators of the enzymes (8–11). The effect of ethanol on the activities of some of these enzymes depends on the assay conditions. For example, there was a greater inhibition of the activities of PFK and PK by ethanol in fed animals when the enzymes were assayed in the absence of their activators (Figs. 7A and 8A; Table 3). The total activity of PFK decreased by 20% with ethanol, 5 g/kg of body weight, when the enzyme was assayed in the presence of AMP, P_i, K^+, and NH_4^+. In their absence, the activity decreased by 70% (Fig. 7A). Similarly, ethanol decreased the specific activity of PFK by 29% and 50% when the enzyme was assayed in the absence and presence of its activators (Table 3). In the presence of these activators,the maximal activity of the enzyme is obtained (17, 21, 23) and thus any decrease in the activity of the enzyme, assayed in the presence of these activators, would reflect a decrease in the total content of the enzyme. Ethanol may also convert the active form of liver PFK to the less active form. In the presence of these activators the less active form of the enzyme is converted back to the active form (21), which would explain the difference in the degree of the inhibition of the enzyme activity. In fasted rats, the enzymes seem to be more sensitive to inhibition by ethanol. For example, with ethanol, 5 g/kg of body weight, specific activity of glucokinase is inhibited 46% in fasted rats (Table 3) as compared with 21% in fed rats (Table 4). HK and PFK seem to be inhibited at lower concentration than at the higher concentration of ethanol (Table 4). A similar biphasic effect of ethanol on rat liver tyrosine-2-oxoglutarate aminotransferase activity has recently been reported by Badawy *et al.* (24).

The existence of futile (i.e., ATP-dissipating) substrate cycles in glucose metabolism has been established *in vivo* and *in vitro* at the glucose/glucose 6-phosphate and fructose 6-phosphate/Fru-P_2 steps. Rognstad and Katz (25) observed in isolated hepatocytes that the cycle between fructose 6-phosphate/Fru-P_2 was greatly decreased by ethanol. Topping *et al.* (26) observed that ethanol did not inhibit the extent of cycle at the glucose/glucose 6-phosphate step in perfused livers. An increase in the activity at the G6Pase/HK + GK step caused by

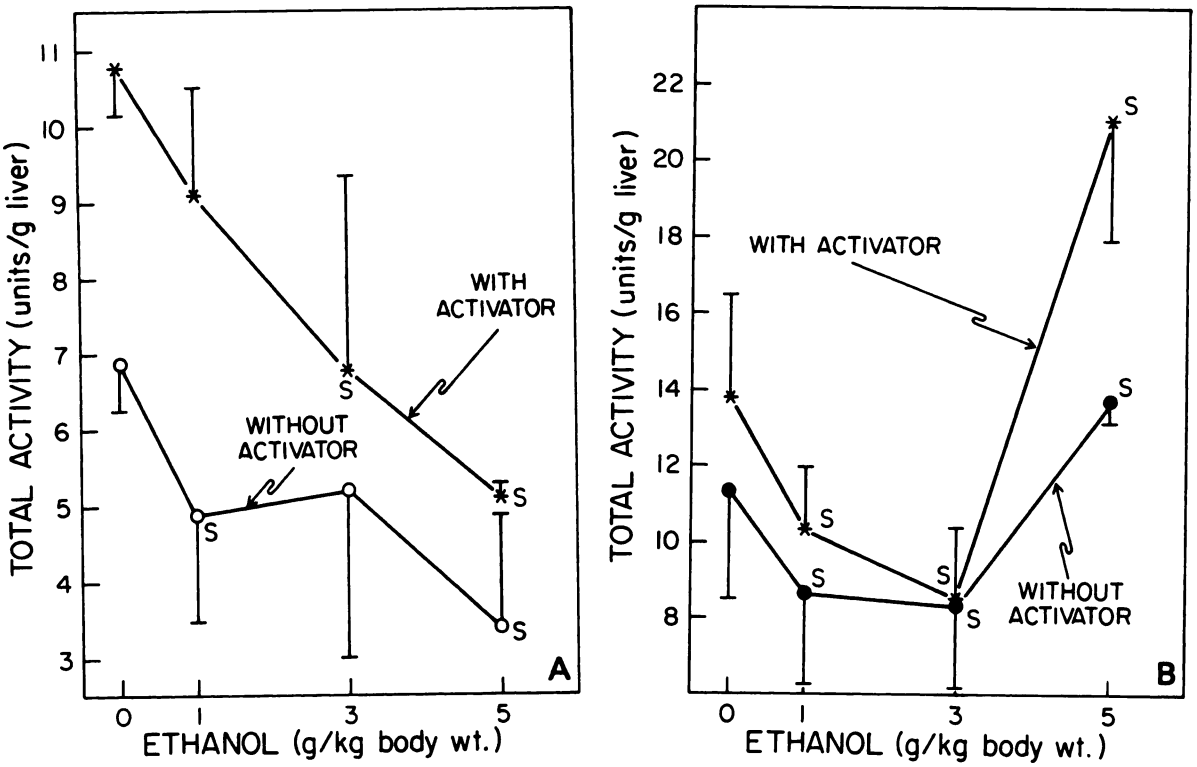

FIG. 8. *Effect of ethanol on the total activity of PK in fed and fasted rats*

A. Normally fed rats. Fru-P_2 (1 mM) was used as an activator. Other details are the same as described in the legend to Fig. 1 and under Materials and Methods.

B. Fasted rats. Other details are the same as described in the legends to Figs. 1 and 8A.

ethanol (Table 5) would maintain the cycle between glucose and glucose 6-phosphate. Topping *et al.* (26) also concluded that with ethanol in fed animals there was very little metabolism of glucose below the level of fructose 6-phosphate in the glycolytic pathway. This may be due to more than 70% inhibition in the activity of PFK caused by ethanol in fed animals (Fig. 7A).

During ethanol intake there is a tendency toward both metabolic and respiratory acidosis. The extent of metabolic acidosis is greater in fasted animals. Metabolic acidosis has been shown to facilitate PEPCK activity and to increase gluconeogenesis (27). We observed that

TABLE 3

Effect of ethanol on the specific activities of key glycolytic enzymes from livers of fed rats

Ethanol	Enzyme[a]			
	GK	HK	PFK	PK
g/kg body wt	*μmoles/min/mg protein*			
0	0.033 ± 0.002 (100)	0.017 ± 0 (100)	0.006 ± 0.003 (100)	0.29 ± 0.05 (100)
			0.059 ± 0.013[b] (100)[b]	0.40 ± 0.05[b] (100)[b]
1	0.034 ± 0.005 (103)	0.013 ± 0[c] (76)	0.005 ± 0.002 (83)	0.22 ± 0.095 (76)
			0.038 ± 0.011[b] (64)[b]	(0.36 ± 0.086[b] (90)[b]
3	0.032 ± 0.004 (97)	0.012 ± 0.004[c] (71)	0.006 ± 0.002 (100)	0.22 ± 0.11 (76)
			0.051 ± 0.006[b] (86)[b]	0.4 ± 0.053[b] (100)[b]
5	0.026 ± 0.002 (79)	0.013 ± 0 (76)	0.003 ± 0.001[c] (50)	0.18 ± 0.12 (62)
			0.042 ± 0.006[b, c] (71)[b]	0.33 ± 0.086[b, c] (83)[b]

[a] Values are means ± standard deviation of at least 18 points. Values in parentheses indicate activities in percentages relative to control rats given no ethanol.

[b] Activities of the enzymes in the presence of their specific activators.

[c] Statistically significant difference from control ($p < 0.05$).

TABLE 4

Effect of ethanol on the specific activites of key glycolytic enzymes from livers of rats fasted for 48 hr

Ethanol	Enzyme[a]			
	GK	HK	PFK	PK
g/kg body wt	*μmoles/min/mg protein*			
0	0.022 ± 0.004[b] (100)	0.0037 ± 0.0004[b] (100)	0.005 ± 0.002 (100)	0.79 ± 0.13[b] (100)
			0.054 ± 0.015[c] (100)[c]	1.07 ± 0.11[b, c] (100)[c]
1	0.017 ± 0.002[d] (77)	0.0028 ± 0.0008 (76)	0.004 ± 0.001 (80)	0.78 ± 0.11 (99)
			0.043 ± 0.015[c] (78)[c]	0.84 ± 0.14[c, d] (79)[c]
3	0.016 ± 0.002[d] (72)	0.0034 ± 0.0009 (92)	0.005 ± 0.0005 (100)	0.7 ± 0.085 (89)
			0.054 ± 0.017[c] (100)[c]	0.77 ± 0.12[c, d] (72)[c]
5	0.012 ± 0.001[d] (54)	0.0037 ± 0.0009 (100)	0.006 ± 0.002 (120)	0.65 ± 0.085[c] (82)
			0.076 ± 0.012[c, d] (140)[c]	0.82 ± 0.12[c, d] (77)[c]

[a] Values are means ± standard deviation of at least 18 points. Values in parentheses indicate activities in percentages relative to control rats given no ethanol.

[b] Statistically significant difference from fed control ($p < 0.05$.)

[c] Activity of the enzymes in the presence of their specific activators.

[d] Statistically significant difference from control ($p < 0.05$).

TABLE 5

Effect of ethanol (5 g/kg body weight) on the total activities of the key gluconeogenic and glycolytic enzymes in rat liver

All of the enzymes were assayed in the absence of their specific activators. Data are taken from Figs. 1–8; for standard deviation and significance see these figures.

Enzymes	Fed animals		Fasted animals	
	Control	+ Ethanol	Control	+ Ethanol
	units/g liver, wet wt			
G6Pase	12.2	13.8	29.3	36.3
HK + GK	0.89 ± 0.26	0.71 + 0.13	0.5 + 0.07	0.26 + 0.06
	(10.6 ± 2.5)[a]	(16.4 ± 3.3)	(51.4 ± 12)	(113.4 ± 26)
Fru-P_2ase / PFK	8.2 / 0.2	7.3 / 0.06	3.73 / 0.065	3.76 / 0.07
	(41 ± 20)	(121.6 ± 24)	(57.4 ± 16)	(53.7 ± 16)
PC + PEPCK / PK	4.36 + 1.5 / 6.9	4.5 + 1.1 / 3.4	4.24 + 3.05 / 11.34	2.7 + 2.6 / 13.71
	(0.85 ± 0.25)	(1.6 ± 0.17)	(0.64 ± 0.12)	(0.38 ± 0.02)

[a] Numbers in parentheses indicate ratio of the activity of the gluconeogenic enzymes to the glycolytic enzymes ± standard deviation.

ethanol decreased the specific activity of PEPCK in fed rats (Table 1) but increased the specific activity of the enzyme in fasted rats (Table 2). This increase in the activity of the enzyme may be due to the greater extent of metabolic acidosis observed during food deprivation. This may be a compensatory mechanism in the body to increase gluconeogenesis under conditions when glucose is needed most (i.e., during fasting).

Ethanol elimination *in vivo* is believed to proceed almost entirely by oxidation in the liver. Fed animals eliminate ethanol faster than fasted animals. Ylikahri and Maenpaa (28) studied the rate of elimination of ethanol from blood after i.p. injection of ethanol, 2 g/kg of body weight. They reported ethanol elimination rates of 36 and 21 mg/100 ml of blood per hour in fed rats and rats fasted for 48 hr, respectively. Therefore, it is expected that the blood ethanol levels of fasted animals would be higher than those of fed animals. However, some time is required to see such differences. Ylikahri and Maenppa (28) reported that, 1 hr after i.p. injection of ethanol, 2 g/kg of body weight, a level of about 200 mg of ethanol per 100 ml of blood was achieved in both fed rats and rats fasted for 48 hr. In the studies reported here, we used similar conditions of injecting ethanol to the animals; therefore, the differences in the effects of ethanol on the activities of these enzymes in fed and fasted animals may not be due to any differences in ethanol metabolism. Moreover, Lumeng *et al.* (29) recently measured the concentration of ethanol in livers of fed and fasted rats 1 hr after i.p. injection of ethanol, 2 g/kg of body weight, and found it to be almost the same in both groups. They reported ethanol concentrations of 35 and 39 mM in the livers of fed rats and rats fasted for 48 hr, respectively. About 3 hr are required for some differences in the blood ethanol levels in fed and fasted animals to become apparent. For example, Ylikahri and Maenpaa (28) reported blood ethanol levels of about 130 and 150 mg/100 ml of blood in fed and 48-hr fasted rats, 3 hr after i.p. injection of ethanol, 2 g/kg of body weight. Similarly, Smith and Newman (30) reported blood ethanol levels of 192 and 214 mg/100 g of blood in fed and fasted rats, 3 hr after i.p. injection of ethanol, 3 g/kg of body weight.

In conclusion, it appears that more than one mechanism may be involved in ethanol-induced hypoglycemia and decreased glucose utilization: (a) an increase in the NADH/NAD^+ ratio (1); (b) a decrease in the concentrations of substrates and cofactors of the key regulatory enzymes of glucose metabolism (5–7); (c) conversion of active forms of the enzymes to less active forms; and (d) a decrease in the total content of the enzymes.

An increase in the activities of PC and G6Pase and a decrease in the activities of GK, HK, PFK, and PK in the livers of ethanol-treated fed rats may contribute to hyperglycemia in these animals. A decrease in the activities of PC and PEPCK and an increase in the activities of PFK and PK observed in the livers of ethanol-treated fasted rats may contribute to hypoglycemia in these animals. It would be of interest to determine the effect of ethanol on the turnover of these enzymes and to determine whether the total content of the enzymes is changed because of alteration in their rates of synthesis or degradation, or both.

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