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Pathways Responsible for the Adaptive Increase in Ethanol Utilization Following Chronic Treatment with Ethanol: Inhibitor Studies with the Hemoglobin-Free Perfused Rat Liver

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SUMMARY

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The perfused rat liver was chosen as a model to study the metabolism of the adaptive increase in ethanol metabolism resulting from chronic treatment with ethanol. This model allows for the continuous monitoring of intracellular pigments nondestructively (pyridine nucleotide and flavoprotein fluorescence; catalase-H_2O_2 absorption), which are qualitative monitors of the alcohol dehydrogenase and catalase pathways in the liver cell. The specificity of 4-methylpyrazole for alcohol dehydrogenase and of aminotriazole for catalase has been verified in this model with these techniques. Ethanol metabolism was activated 60% over controls as a result of chronic treatment with ethanol. Rates in both groups were nearly completely abolished (less than 16 μmoles/g/hr) by 4-methylpyrazole. Similar inhibition was observed with inhibitors of the mitochondrial respiratory chain (rotenone and antimycin A) and atractyloside, an inhibitor of adenine nucleotide translocase. The adaptive increase was completely abolished with ouabain, an inhibitor of the sodium-plus potassium-activated ATPase. Basal respiratory rates (127 μmoles/g/hr) were markedly elevated (205 μmoles/g/hr) as a result of treatment with ethanol. However, succinate-dependent respiration of isolated mitochondria was either unaffected or slightly depressed in livers from ethanol-treated animals. A direct relationship between oxygen uptake and ethanol oxidation in the perfused liver was observed. The data are consistent with the hypothesis that the primary event in enhanced ethanol metabolism following chronic treatment with ethanol is an increase in ATPase activity, most likely due to the sodium pump. The ADP produced from enhanced ion movement enters the mitochondrial space and stimulates electron transport and oxygen uptake. As a consequence of these events, a greater rate of NADH reoxidation occurs, resulting in a greater rate of production of NAD^+, which stimulates ethanol oxidation via alcohol dehydrogenase.

INTRODUCTION

When rats are fed ethanol chronically (1, 2), a marked increase in their ability to oxidize ethanol results (3-5). This adaptive increase in ethanol metabolism has also been observed in some alcoholic humans (6, 7).

A number of pathways involved in

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ethanol metabolism have been proposed to account for its accelerated rate of oxidation following chronic ethanol treatment. For example, the well-documented observation that chronic ethanol treatment produces proliferation of the endoplasmic reticulum, and therefore induction of the microsomal ethanol-oxidizing system, has been offered as an explanation for this phenomenon (8). However, the time courses of enhanced ethanol metabolism and induction of microsomal cytochrome P-450 are not identical (9), casting doubt on the involvement of the endoplasmic reticulum. Alternatively, ethanol oxidation by liver slices from animals treated chronically with ethanol was sensitive to an inhibitor of catalase, aminotriazole, suggesting that catalase may participate (10).

In addition to the possible involvement of these minor pathways, the predominant enzyme involved in hepatic ethanol metabolism is alcohol dehydrogenase. It has been reported that treatment with ethanol leads to an increase in the activity of this enzyme. On the other hand, recent evidence indicates that chronic treatment with ethanol either enhances, decreases, or has no effect on the activity of alcohol dehydrogenase *in vitro* (11). The activity of alcohol dehydrogenase is, however, of lesser importance, since the rate-limiting step for the alcohol dehydrogenase reaction is the supply of the cofactor NAD^+ (12–14).

Israel and his co-workers have demonstrated that ethanol oxidation and oxygen uptake of liver slices following chronic treatment with ethanol can be diminished by ouabain, an inhibitor of the sodium-plus potassium-activated ATPase. This ATPase produces intracellular ADP and stimulates mitochondrial respiration, which in turn oxidizes NADH and supplies more NAD^+ for the alcohol dehydrogenase reaction (15–18).

The purpose of the studies reported here was to evaluate these various hypotheses. The hemoglobin-free perfused rat liver was chosen as a model, since various spectral and fluorescent techniques can be applied nondestructively and qualitatively to determine the specificity of inhibitors with regard to the pathways involved. The interaction of nonspecific inhibitors with these pathways can then be monitored (19).

The data presented here indicate that the predominant enzyme system in the adaptive increase in ethanol metabolism following chronic treatment with ethanol is alcohol dehydrogenase. This most likely results from an increase in the rate of reoxidation of NADH, which is caused by enhanced activity of the sodium-plus potassium-activated ATPase. Preliminary accounts of this work have appeared elsewhere (20–22).

METHODS

Chronic Treatment with Ethanol

In these studies initial work was carried out with a semiliquid diet described by DeCarli and Lieber (2). Animals received the diet for 5 weeks, while controls received isocaloric amounts of sucrose. A second diet allowed animals to consume laboratory chow ad libitum; ethanol was administered in a sucrose solution as described by Porta and his co-workers (1), also for 5 weeks. There are advantages and disadvantages with both diets. For example, the Lieber diet allows pair feeding and control of alcohol intake, but is expensive and unstable. On the other hand, the Porta diet is stable and relatively inexpensive, but does not allow one to monitor caloric intake. Preliminary studies failed to detect any difference in activation of ethanol metabolism or sensitivity to 4-methylpyrazole between these two diets; thus the work reported here was performed employing the Porta diet (1). The animals were housed in individual cages.

Hemoglobin-Free Liver Perfusion

A perfusion system described previously (22, 23) was used with slight modifications. The perfusion fluid consisted of 65 ml of Krebs-Henseleit bicarbonate buffer, pH 7.4 (24), containing 2 g of bovine serum albumin per 100 ml (fraction V, Sigma). This solution was saturated in a temperature-regulated (37°) disc oxygenator with a mixture of oxygen and carbon dioxide (95:5). The perfusion fluid was pumped through the liver via a cannula inserted in the portal vein. It left the liver via a cannula inserted in the vena cava and passed

an oxygen electrode before returning to the oxygenator (23).

Female albino rats, Sprague-Dawley strain 150–200 g were anesthetized with pentobarbital (50 mg/kg). Details of the surgical procedure are described elsewhere (25).

Oxygen concentration in the effluent perfusate was monitored polarographically with a Clark platinum electrode.

Ethanol Utilization

Samples of perfusate were collected every 15 min, deproteinized with perchloric acid (2%, final concentration), and neutralized with potassium carbonate (0.25 M, final concentration). Ethanol was subsequently determined enzymatically by standard procedures (26).

Surface Fluorometry and Absorption Spectroscopy of Hemoglobin-Free Perfused Rat Liver

Surface fluorescence of pyridine nucleotides and flavoproteins and absorption of catalase-H_2O_2 were determined as described by Scholz *et al.* (23) and Sies and Chance (27), respectively.

Materials

All enzymes and coenzymes used in this study were purchased from Sigma Chemical Company. All chemicals were reagent grade from standard sources. 4-Methylpyrazole was purchased from Research Plus Laboratories, Denville, N. J.

RESULTS

Effect of Chronic Ethanol Treatment on Ethanol Utilization

Treatment of animals for 5–7 weeks with ethanol produced a 40–60% increase in the rate of ethanol utilization (Tables 1 and 2).

Effect of L-Alanine on Ethanol Utilization

The addition of L-alanine to perfused livers from control and ethanol-treated animals stimulated ethanol utilization in both groups (Table 1).

TABLE 1

Effect of L-alanine on ethanol metabolism in perfused livers from normal and ethanol-treated animals

Livers from normal and ethanol-treated animals (fed) were perfused with 65 ml of Krebs-Henseleit bicarbonate buffer, pH 7.4 (24). Ethanol (25 mM) was added after 10 min of perfusion, followed after 45 min by L-alanine (15 mM). Samples (0.5 ml) were taken every 15 min, and ethanol was determined enzymatically. The data represent rates over the treatment period, with the initial (transient) period excluded. Values are means ± standard errors (n = 8–10).

Rats	Ethanol utilized	
	Control	L-Alanine
	μmoles/g/hr	
Normal	55 ± 4	66 ± 5
Ethanol-treated	78 ± 6	104 ± 8

TABLE 2

Effects of inhibitors on ethanol utilization by perfused livers from normal and ethanol-treated rats

Livers were perfused as described in Table 1. Inhibitors were added after 45 min: 4-methylpyrazole, 4 mM; antimycin A, 30 μM; atractyloside, 1 mM; rotenone, 20 μM; ouabain, 2 mM. Values are means ± standard errors (n = 30–34 for control values; n = 4–7 for inhibitors).

Inhibitor	Ethanol utilization	
	Normal	Ethanol-treated
	μmoles/g/hr	
None (control)	58 ± 4	85 ± 5
4-Methylpyrazole	16 ± 3	16 ± 3
Rotenone	23 ± 3	25 ± 2
Antimycin A	24 ± 6	32 ± 6
Atractyloside	32 ± 5	38 ± 2
Ouabain	47 ± 3	53 ± 7

Inhibitors of Ethanol Utilization in Perfused Livers from Normal and Ethanol-Treated Rats

Effect of 4-methylpyrazole. Pyrazole and pyrazole derivatives are effective inhibitors of alcohol dehydrogenase both *in vitro* and *in vivo* (28, 29); however, differing degrees of inhibition have been reported in perfusion studies and *in vivo*, suggesting that pyrazole may not be the optimal inhibitor in intact cells. For example, Grunnett

et al. (30), working with isolated liver cells, Pappenberg (31), working with perfused liver, and Teschke *et al.* (32), working with liver slices, observed approximately 50% inhibition of ethanol utilization with pyrazole. On the other hand, 4-methylpyrazole has been clearly and consistently demonstrated to inhibit 80–90% of ethanol utilization (33, 34). Thus 4-methylpyrazole was chosen for these studies. Furthermore, the failure of ethanol to produce the characteristic reduction in pyridine nucleotides in the presence of 4-methylpyrazole (Fig. 2) substantiates the efficiency of this pyrazole derivative as an inhibitor of alcohol dehydrogenase in the perfused liver. At the same time, 4-methylpyrazole had no effect on oxygen uptake in these livers (Table 4), indicating that mitochondrial respiration was unaffected in spite of the report that pyrazole inhibits mitochondrial respiration (35).

Effect of aminotriazole. Aminotriazole is an effective inhibitor of catalase. It reacts with catalase-H_2O_2 to form an inactive intermediate (catalase-NO) when administered to animals 1–2 hr prior to surgical preparation.

Aminotriazole had very little effect on the rate of ethanol utilization in livers from normal animals (Table 3). However, in livers from ethanol-treated animals, the elevated rate of ethanol utilization (85 ± 5) was diminished (to 66 ± 7) in one experiment (Table 3, experiment 1), whereas in another experimental series aminotriazole had no effect (Table 3, experiment 2). In view of these contradictory results, the participation of catalase in the elevated rate of ethanol utilization due to chronic treatment with ethanol cannot be totally ruled out at this time.

Effects of inhibitors of mitochondrial respiratory chain. Rotenone and antimycin A are well-known inhibitors of the mitochondrial respiratory chain, acting at energy conservation sites I and II, respectively (36, 37). When rotenone and antimycin A were individually added to perfused livers from normal rats, the control rate was diminished from 58 ± 4 to 23 ± 3 with rotenone and to 24 ± 6 with antimycin A. Similarly, the elevated rate of ethanol utilization in the ethanol-treated animals (85 ± 5) was diminished essentially to the same residual value by addition of these inhibitors (rotenone, 25 ± 2; antimycin A, 32 ± 6).

TABLE 3

Effect of aminotriazole treatment on ethanol utilization by perfused livers from normal and ethanol-treated rats

Livers were perfused as described in Table 1. Aminotriazole was administered to animals intraperitoneally (1 g/kg) 60–90 min prior to surgical preparation. Values are means ± standard errors of the *n* values shown in parentheses.

Rats	Ethanol utilization		
	Normal	Ethanol-treated	
		Expt. 1	Expt. 2
	μmoles/g/hr		
Control	58 ± 4 (6)	85 ± 5 (4)	80 ± 6 (7)
Aminotriazole-treated	52 ± 4 (6)	66 ± 7 (9)	80 ± 3 (6)

Effect of atractyloside. Atractyloside, a thistle derivative, is an inhibitor of the adenine nucleotide translocase located in the mitochondrial membrane (38). The addition of atractyloside in these perfusion studies inhibited both the normal and the elevated rate of ethanol utilization due to ethanol treatment, in a manner similar to that observed with inhibitors of the mitochondrial respiratory chain (Table 2).

Effect of ouabain. Ouabain has been demonstrated to be an effective inhibitor of the sodium- plus potassium-activated ATPase of the plasma membrane in a wide variety of tissues (39). When ouabain was added to livers of normal rats, a slight decrease in the rate of ethanol utilization was observed (Table 2); however, ouabain completely abolished the adaptive increase in ethanol utilization resulting from chronic ethanol treatment (Table 2).

Effect of Ethanol, 4-Methylpyrazole, and Aminotriazole on Pyridine Nucleotide and Flavoprotein Fluorescence and on Absorption of Catalase-H_2O_2

In these studies the hemoglobin-free perfused liver was selected because it is possi-

ble to monitor the oxidation-reduction state of pyridine nucleotides, flavoproteins, and the catalase-H_2O_2 complex continuously, thereby gaining additional insight into the participation of the alcohol dehydrogenase and catalase pathways in ethanol metabolism and their response to inhibitors such as 4-methylpyrazole and aminotriazole (see also ref. 19).

Effects of ethanol on pyridine nucleotide and flavoprotein fluorescence and steady-state concentration of catalase-H_2O_2. The addition of ethanol (0.7 mM) to a perfused liver produced a characteristic reduction in pyridine nucleotides as a result of the oxidation of ethanol by alcohol dehydrogenase (Fig. 1). This was followed by a reduction in flavoproteins (see ref. 23) as the hydrogen generated from the first step of ethanol metabolism was transferred into the mitochondrial space by the hydrogen shuttle mechanisms, predominantly the malate-aspartate shuttle (40–42). Simultaneously, a marked decrease in the steady-state concentration of the catalase-H_2O_2 intermediate was observed, reflecting the peroxidation of ethanol via catalase-H_2O_2. Under these conditions ethanol did not cause a perturbation in the steady-state level of cytochrome P-450 (not shown) (19, 43). When ethanol disappeared from the perfusate, all three monitors returned approximately to their baselines. Similar results were obtained with higher ethanol concentrations (19). It is therefore concluded that these direct read-out techniques nondestructively and qualitatively monitor various pathways of alcohol oxidation. For example, fluorescent changes in pyridine nucleotides reflect ethanol oxidation via alcohol dehydrogenase. In addition, flavoprotein fluorescence serves as an index of the hydrogen transfer reactions while the steady-state concentration of catalase-H_2O_2 varies because of its interaction with ethanol. These monitors also allow us to check the specificity of inhibitors of alcohol dehydrogenase and catalase in the intact cell. When 4-methylpyrazole is administered to the liver of an ethanol-treated rat, the characteristic reduction in pyridine nucleotides and flavoproteins due to ethanol is abolished (Fig. 2).

Conversely, following treatment with aminotriazole, the addition of ethanol produced a reduction in pyridine nucleotides and flavoproteins (Fig. 3); however, no change in the steady-state concentration of

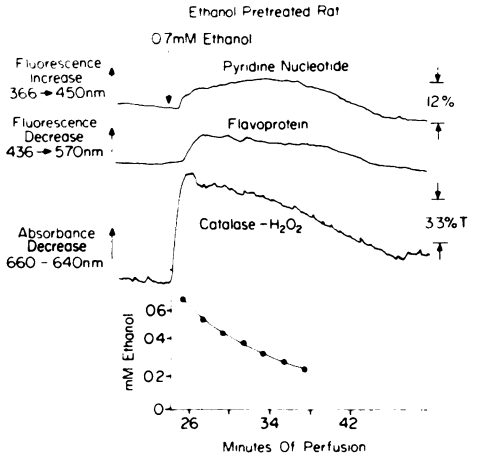

FIG. 1. *Effect of ethanol on fluorescence of pyridine nucleotides and flavoproteins and on steady-state concentration of catalase-H_2O_2 in perfused livers from ethanol-treated rats*

Surface fluorescence of pyridine nucleotides (excited at 366 nm; emitted at 450 nm) and flavoproteins (excited at 436 nm; emitted at 570 nm) was determined as described previously (23). The absorption of catalase-H_2O_2 was measured according to Sies and Chance (27). Ethanol (0.7 mM, final concentration) was added as indicated by the arrow. %T = %Transmission.

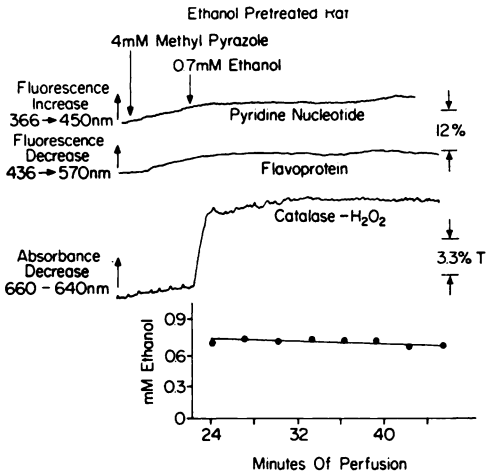

FIG. 2. *Effect of ethanol on pyridine necleotide and flavoprotein fluorescence and on steady-state concentration of catalase-H_2O_2 in perfused liver from ethanol-treated rats in the presence of 4-methylpyrazole*

Conditions were the same as in Fig. 1.

the catalase-H_2O_2 compound could be demonstrated.

Effect of Ethanol Treatment on Oxygen Uptake of Perfused Livers from Normal and Ethanol-Treated Rats

The basal respiration determined in the absence of ethanol in these livers was 127 ± 14 μmoles/g (wet weight) per hour; however, in livers from ethanol-treated animals, basal respiration was nearly doubled, to 205 ± 18 μmoles/g/hr (Table 4). This elevated rate of oxygen uptake was not affected by 4-methylpyrazole, but was markedly diminished to the same residual values in both groups by addition of an inhibitor of the mitochondrial respiratory chain, antimycin A (Table 4).

Effect of Ethanol Treatment on Isolated Rat Liver Mitochondria

Mitochondria were isolated from control and ethanol-treated animals (44) and oxygen uptakes were compared (Fig. 4). The state 3 respiratory rate was somewhat slower in rat liver mitochondria isolated from ethanol-treated animals; however, there was no qualitative difference in respiratory control in liver mitochondria from normal and ethanol-treated rats. These data confirm the findings of Israel *et al.* (18) but are in contrast to reports by Cederbaum *et al.* (46), stating that there is a major defect in respiratory site I in mitochondria following treatment with ethanol. However, in the latter studies a different diet with higher fat content was employed.

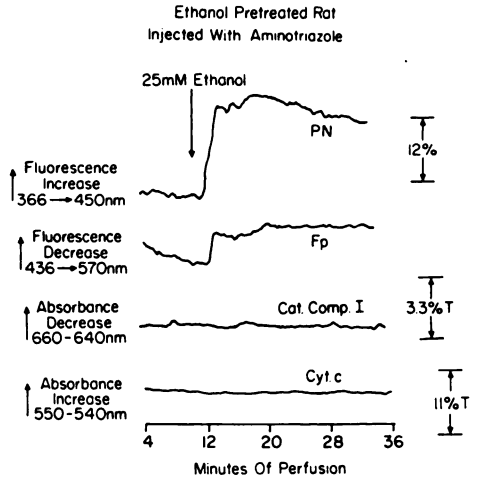

FIG. 3. *Effect of ethanol on fluorescence of pyridine nucleotides (PN) and flavoproteins (Fp) and on steady-state concentration of catalase-H_2O_2 (Cat. Comp. I) in perfused liver of ethanol-treated rat injected with aminotriazole*

Conditions were the same as in Figs. 1 and 2. The rat received aminotriazole (1 g/kg) intraperitoneally 60 min prior to surgical preparation. Cytochrome *c* was determined with the wavelength pairs indicated.

TABLE 4

Basal, antimycin A-insensitive, and 4-methylpyrazole-dependent rates of oxygen uptake by perfused livers from normal and ethanol-treated rats

Perfusion conditions were the same as in Table 1. Oxygen concentrations were determined polarographically (23), and rates of respiration were calculated from arteriovenous concentration differences, the flow rate, and the liver wet weight. Values are means ± standard errors (n = 5–8).

Rats	Oxygen uptake		
	Basal	Antimycin A	4-Methylpyrazole
	μmoles/g/hr		
Normal	127 ± 14	73 ± 16	125 ± 12
Ethanol-treated	205 ± 18	74 ± 17	198 ± 18

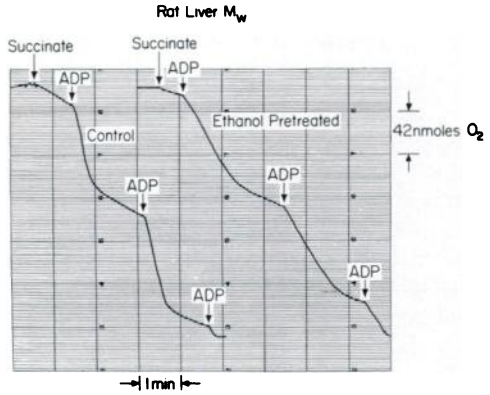

FIG. 4. *Respiratory control of isolated liver mitochondria from control and ethanol-treated rats*

Mitochondria were incubated in a temperature-regulated (37°) chamber (2.0-ml volume) in a buffer containing 100 M KCl, 50 M sucrose, 20 M Tris-Cl, 5 M Tris-phosphate, and 10 M rotenone, pH 7.2. Succinate (5 M) and ADP (0.5 M) were added as indicated by arrows. Protein concentrations (45) were 1.01 mg/ml and 1.03 mg/ml in mitochondria from control and ethanol-treated rats, respectively.

Relationship between Ethanol Utilization and Respiration

Results obtained under various perfusion conditions were compared. They establish a relationship between the rate of ethanol utilization and respiration. For example, the ethanol-treated animals had the highest rate of respiration (200 μmoles/g/hr). On the other hand, when livers were perfused with a sodium-free buffer, respiration of less than 50 μmoles/g/hr and ethanol utilization of less than 20 μmoles/g/hr were observed. These data demonstrate a direct relationship between the rate of oxygen uptake and the rate of ethanol utilization (see Fig. 5).

DISCUSSION

Rate-Controlling Factors for Alcohol Dehydrogenase Reaction in Normal and Ethanol-Treated Animals

L-Alanine stimulated ethanol metabolism in livers from both normal and ethanol-treated animals (Table 1), although the rate of ethanol oxidation was nearly 60% higher in the latter group. This suggests that the rate of reoxidation of NADH is the rate-limiting step for ethanol metabolism in both cases. Alanine is metabolized in two different ways, both of which lead to an accelerated rate of NADH oxidation. First, it generates pyruvate via lactate dehydrogenase; second, it is a substrate for gluconeogenesis. This latter process requires ATP, generates ADP, and thereby stimulates electron flux in the respiratory chain. In addition, it has been demonstrated that dinitrophenol, an uncoupler of oxidative phosphorylation, stimulates ethanol oxidation in the perfused rat liver (23, 47) and in liver slices from normal and ethanol-treated animals, although the effect was considerably smaller following ethanol treatment (18). Thus these data support the conclusion that the alcohol dehydrogenase pathway is limited by the rate of reoxidation of NADH in both ethanol-treated and control animals.

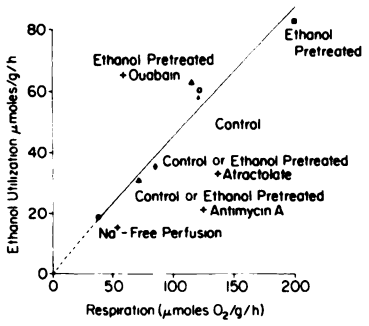

FIG. 5. *Relationship between hepatic ethanol utilization and oxygen uptake*

Data were taken from Table 1. Sodium-free perfusate was prepared by substituting choline for sodium chloride in Krebs-Henseleit buffer (24). In the latter experiments $n = 5$.

A number of reports have appeared suggesting that alcohol dehydrogenase is decreased by ethanol treatment (48, 49). However, those experiments have not been universally reproducible (11). In fact, since the alcohol dehydrogenase reaction is controlled by the supply of the oxidized cofactor NAD^+, it is scarcely relevant whether the enzyme is slightly induced or reduced by ethanol treatment.

Mechanism of Adaptive Increase in Ethanol Metabolism Due to Chronic Treatment with Ethanol

Microsomal ethanol-oxidizing system. Following the initial observations of Orme-Johnson and Ziegler (50) and Schüppel (51) that NADPH-dependent oxidation of ethanol occurs in the microsomal subfraction, Lieber and his co-workers (8) postulated a unique ethanol-oxidizing system. In addition, Lieber suggested that the adaptive increase in ethanol utilization due to chronic treatment with ethanol is due to an induction of the endoplasmic reticulum, which produces an increase in the activity of the microsomal ethanol-oxidizing system. However, evidence against this viewpoint was presented by Mezey (9), who observed that the decrease in the enhanced ethanol utilization due to chronic treatment with ethanol following removal of ethanol from the diet was very rapid (less than 48 hr) whereas the decrease in the endoplasmic reticulum was much slower (weeks). Thus, Mezey concluded that the adaptive increase is not due to the microsomal ethanol-oxidizing system and does not involve the endoplasmic reticulum.

Moreover, a specific inhibitor of alcohol dehydrogenase (4-methylpyrazole, Table 2) abolished the adaptive increase due to chronic treatment with ethanol without affecting the oxidation-reduction state of cytochrome P-450, a component of the microsomal system (8). Thus it is safe to conclude that the microsomal ethanol-oxidizing system is not involved in the adaptive increase in ethanol metabolism due to chronic treatment with ethanol.

Furthermore, the microsomal ethanol-oxidizing system hypothesis itself is still highly controversial and remains to be proven. In fact, several laboratories have suggested that the microsomal system is due to NADPH-dependent H_2O_2 production and catalase (52–55). A review of that literature, however, is beyond the scope of this paper (see ref. 56).

Involvement of alcohol dehydrogenase. 4-Methylpyrazole inhibited the adaptive increase and was shown to be an effective inhibitor of the characteristic reduction in pyridine nucleotides following the addition of ethanol (Fig. 2). Thus it is concluded that alcohol dehydrogenase is involved in the adaptive increase due to chronic treatment with ethanol, either directly or indirectly (see below).

Involvement of mitochondrial respiratory chain. Israel and his co-workers (15–18) suggested that an increase in the oxidative capacity of the liver cell occurs following ethanol treatment, which presumably increases the supply of the oxidized cofactor for the alcohol dehydrogenase reaction.

The basal oxygen uptake was markedly elevated in perfused livers (Table 4) and liver slices (18) as a result of ethanol treatment. Thus an increase in the oxidative capacity of the liver cell occurs following chronic exposure to ethanol, confirming Israel and his co-workers (18). The involvement of the mitochondrial respiratory chain in this increased oxidative capacity is demonstrated by the observation that antimycin A and rotenone, both specific inhibitors of the mitochondrial respiratory chain, abolished the enhanced oxygen uptake due to ethanol treatment. Moreover, the observation that these inhibitors also abolished the adaptive increase in ethanol metabolism due to ethanol treatment allows the conclusion that the enhanced oxygen uptake following ethanol feeding may be responsible for the increase in ethanol oxidation (18). These contentions are also supported by the observation that a direct relationship exists between the rate of respiration and the rate of ethanol utilization in the perfused liver (Fig. 5).

On the other hand, isolated mitochondria from ethanol-treated animals had either identical or slightly depressed rates of oxygen uptake when compared with controls, depending on whether NADH-linked (46) or flavin-linked substrates were employed. Therefore it is concluded that the increased rate of oxygen uptake by the whole cell following ethanol treatment is not due to a mitochondrial event per se, although the inhibitor data (rotenone, antimycin A) show that the mitochondria are involved. One must postulate, therefore, that an extramitochondrial process influences mitochondrial oxygen uptake and ethanol oxidation as a result of ethanol treatment (see below).

Involvement of ATPase. Work with isolated mitochondria has clearly established that electron flux in the mitochondrial electron transport chain is controlled by ADP (57). Most of the ADP generated in the cell comes from both biosynthetic and catabolic ATPase activities in the extracellular space. The ADP is transferred into the mitochondria by the adenine nucleotide translocase located within the mitochondrial membrane (58). This translocase is sensitive to inhibition by atractyloside. The observation that atractyloside inhibited ethanol metabolism to the same degree as mitochondrial respiratory inhibitors in ethanol-treated livers indicates that an extramitochondrial ATPase activity is responsible for this process, assuming that inhibition is complete under both conditions. Also, since mitochondria isolated from ethanol-treated animals are not different from those isolated from untreated animals (Fig. 4), one may infer that an extramitochondrial process is responsible for the adaptive increase. Israel's observations that the adaptive increase in both oxygen uptake and ethanol

metabolism in isolated liver slices was inhibited by ouabain, an inhibitor of the sodium-plus potassium-activated ATPase, has been confirmed here (Table 2). Ouabain completely abolished the adaptive increase, suggesting that the initial event which sets off a chain of other events is the induction of or increase in activity of the sodium- plus potassium-activated ATPase. In addition, increased ATPase activity of liver homogenates from ethanol-treated rats has been observed (18).

These interactions are summarized schematically in Fig. 6. The primary event in enhanced ethanol metabolism following chronic treatment with ethanol is an increased ATPase activity, most likely attributable to the sodium pump. It has been observed that an increase in rubidium transport in liver cells follows chronic treatment with ethanol (18), which supports this hypothesis. The finding that the adaptive increase in ethanol metabolism was inhibited by atractyloside suggests that the adenine nucleotide translocase is necessary to transport the ADP produced from enhanced ion movement into the mitochondrial space. This increases electron transport which accounts for the enhanced oxygen uptake observed both in liver slices and in the perfused liver. As a consequence of enhanced electron transport, a greater rate of NADH reoxidation occurs in the mitochondria and therefore the rate of production of NAD^+ for the alcohol dehydrogenase reaction is increased. This hypothesis is also consistent with the finding that the adaptive increase was abolished by 4-methylpyrazole (Table 2).

Recently, Israel *et al.* (59) utilized these interrelationships to develop a model for alcohol-induced cirrhosis. They showed that ethanol-treated but not control animals exhibited centrilobular damage (focal to massive necrosis, some inflammatory reactions) when exposed periodically to hypoxia. Moreover, both the elevated rate of oxygen uptake and the centrilobular damage could be prevented with the antithyroid drug 6-propyl-2-thiouracil, suggesting

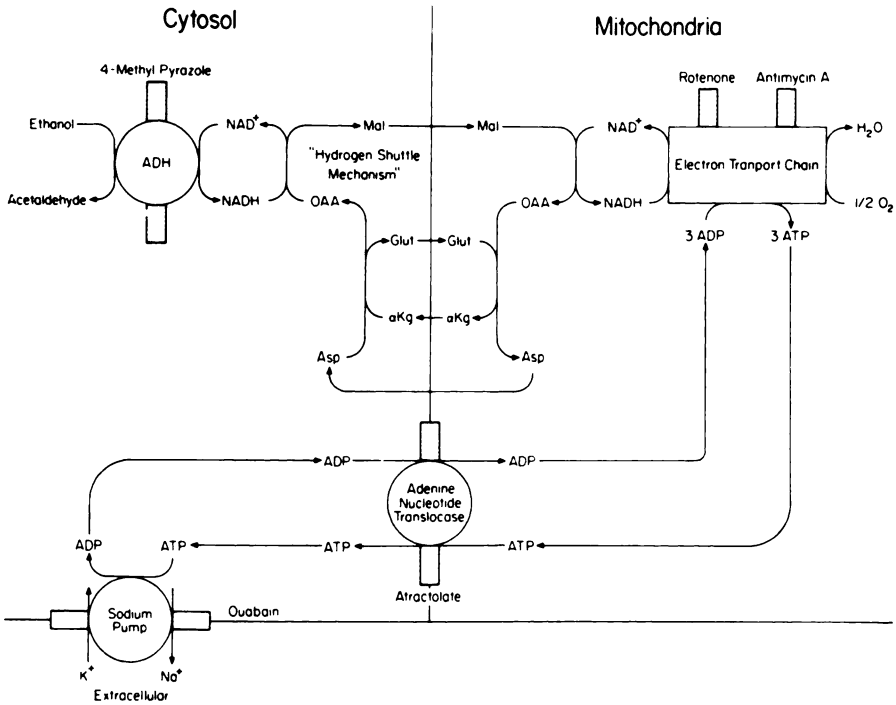

FIG. 6. *Scheme depicting mechanism of adaptive increase in ethanol utilization due to chronic treatment with ethanol*

ADH, alcohol dehydrogenase; Mal, malate; OAA, oxalacetate; Glut, glutamate; αKg, α-ketoglutarate; Asp, aspartate.

that the latter may prove useful in treating cirrhosis in man.

Involvement of catalase in adaptive increase. The data presented in this paper clearly link alcohol dehydrogenase, the electron transport chain, adenine nucleotide translocase, and the sodium pump with the elevated rate of ethanol metabolism resulting from chronic treatment with ethanol. However, data suggesting the involvement of catalase in the adaptive increase have emerged from these studies as well as others. Isselbacher and Carter (60) demonstrated that catalase activity increases approximately 25% as a result of chronic ethanol treatment. On the other hand, considerable evidence has accumulated recently indicating that the rate-limiting step of the catalase reaction in isolated subfractions (53, 61), in the perfused liver (62), and *in situ* (63) is the rate of hydrogen peroxide generation. Thus the observation that catalase activity increases following ethanol treatment may have little consequence. On the other hand, the observation that hydrogen peroxide production by the endoplasmic reticulum was increased after treatment with ethanol (64, 65) suggests that catalase-H_2O_2 could participate in the adaptive increase in ethanol metabolism.

Furthermore, aminotriazole, a catalase inhibitor, abolished about half the adaptive increase in both liver slices (10) and perfused liver (Table 3, experiment 1). However, this finding was not reproducible (Table 3, experiment 2). Moreover, Israel observed that aminotriazole also diminished the oxidative capacity of the cell,[2] and oxygen uptake was depressed by aminotriazole in experiment 1 but unaffected in experiment 2 of Table 3 (oxygen data not shown), suggesting that participation of catalase varies in different animal groups. Although these experiments indicate that catalase may not be involved in the adaptive increase, other data cannot eliminate the possibility at this time.

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