Factors that Modify the Metabolism of Ethanol in Rat Liver and Adaptive Changes Produced by its Chronic Administration

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1. 2,4-Dinitrophenol (0.1 mM) increases by 100–160% the rate of ethanol metabolism by rat liver slices incubated in a medium saturated with a gas mixture containing $O_2 + CO_2 + N_2$ (18:5:77). Similar effects are produced by relatively low concentrations of arsenate (10 mM). At higher concentrations (37.5 and 50 mM) arsenate inhibits the rate of ethanol metabolism. 2. When liver slices are incubated under an atmosphere containing $O_2 + CO_2$ (95:5) the metabolism of ethanol increases by about 100% over that obtained with $O_2 + CO_2 + N_2$ (18:5:77). However, under these conditions the activating effect of dinitrophenol is no longer observed. 3. Chronic administration of ethanol to rats for 3–4 weeks, in doses from 3 to 8 g/kg per day, increases by 70–90% the ability of the liver to metabolize ethanol. In the liver slices of these rats, although an $O_2 + CO_2 + N_2$ (18:5:77) mixture was used, dinitrophenol does not further increase the metabolism of ethanol. If the chronic administration of ethanol is discontinued for two weeks, the rate of ethanol metabolism is lowered to control values and the activating effect of dinitrophenol is recovered. 4. No change in alcohol dehydrogenase activity was found in the liver of the rats in which the metabolism of ethanol had been increased as a result of the chronic ethanol treatment; a 40% increase in the activity of succinate dehydrogenase was observed.

Ethanol is known to be oxidized to acetaldehyde by alcohol dehydrogenase (alcohol-NAD oxidoreductase, EC 1.1.1.1). In the liver cell, the metabolism of ethanol leads to an increase in the NADH/NAD ratio (Smith & Newman, 1959; Reboucas & Isselbacher, 1961; Rih & Oura, 1962; Cherrick & Leevy, 1965). Ethanol has also been found to increase the lactate/pyruvate ratio in the liver (Forsander, 1966; Kreisberg, 1967; Krebs, Freedland, Hems & Stubbs, 1969). This redox pair is in equilibrium with the NADH/NAD⁺ pair in the cell extramitochondrial compartment (Schimassek, 1963). The NADH formed by the action of alcohol dehydrogenase, although in the cytosol, is known to increase the cell β -hydroxybutyrate/acetoacetate ratio (Forsander, Menp & Salaspuro, 1965; Rawat, 1968; Lindros & Aro, 1969), a redox couple in equilibrium with the intramitochondrial NADH/NAD⁺ pair (Krebs, 1967).

At least three limiting steps in the metabolism of ethanol in the liver cell are conceivable: (a) the amount (maximum activity) of alcohol dehydrogenase; (b) the transfer of reducing equivalents into the mitochondria; (c) the rate of NADH reoxidation by the mitochondria.

Theorell & Chance (1951) have shown, by using a purified liver alcohol dehydrogenase in an isolated system, that the limiting step for the oxidation of ethanol is the dissociation of the enzyme-NADH complex. This information has been interpreted by most investigators as an indication that the limiting factor in the metabolism of ethanol in the liver cell is the amount of alcohol dehydrogenase and there have been several studies in which the amount of enzyme (maximum activity of alcohol dehydrogenase assayed with saturating concentrations of NAD⁺) has been determined as an indication of the ability of the liver to metabolize ethanol (see Hawkins, Kalant & Khanna, 1966). Smith & Newman (1959) have shown that the rate of ethanol oxidation is slower in starved than fed animals, despite the fact that ethanol increases the cell NADH/NAD ratio to a greater extent in the starved animals. These results indicate that the oxidation of NADH, rather than the amount of enzyme, could be an important factor in determining the rate of ethanol oxidation in the liver cell. Further, the more reduced state of the intramitochondrial redox couples in the presence of ethanol (Forsander *et al.* 1965; Rawat, 1968; Lindros & Aro, 1969) may be taken as an indication that the mitochondrial oxidation of NADH is the rate-limiting step in the metabolism of ethanol.

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The information above prompted us to study the effect of uncoupling agents, substances that are known to increase the rate of mitochondrial oxidation, in the liver cell. We have also studied the effect of the chronic administration of ethanol on its catabolism.

Most studies in which the metabolism of ethanol has been measured *in vitro* have been done under conditions of oxygenation that are not physiological (95–100% O₂). Work by Gordon (1966, 1968) has shown the difficulties arising from the extrapolation of data obtained under these conditions to the intact animal breathing air. We have, therefore, also studied the effect of different conditions of oxygenation on the rate of ethanol metabolism in rat liver slices.

MATERIALS AND METHODS

Determination of ethanol metabolism in liver slices. Female albino rats of the University of Chile inbred strain weighing 180–250 g and male Wistar rats of 300–400 g (Canadian Breeding Laboratories, Lapraire, Que., Canada) were used. All the animals had free access to rat Purina chow and water until the time of the experiments. The animals were killed by decapitation and bled. When animals chronically treated with ethanol were used, they were killed 24–30 h after the last administration of ethanol. The livers were rapidly removed and placed on an inverted Petri-dish containing ice. Blocks (0.7 cm × 0.7 cm) were prepared and slices (0.35–0.40 mm thick) were cut with a modified Steady-Riggs slicer. About 300 mg of slices (fresh wt.) were placed in a 25 ml Erlenmeyer flask containing 3 ml of a Ringer bicarbonate buffer, pH 7.4, to which glucose was added to a final concentration of 10 mM (Umbreit, Burris & Stauffer, 1959). The buffer was saturated with either a mixture of O₂ + CO₂ + N₂ (18:5:77) or one containing O₂ + CO₂ (95:5) (referred to below simply as 18% O₂ and 95% O₂ respectively). The flasks were placed in a Dubnoff bath at 37°C and shaken at 80 strokes/min. After a preincubation period of 30 min, ethanol was added to the flasks to a final concentration of 10 mM and the slices were incubated for a further 32 min period. During this period a small sample of medium was withdrawn for ethanol determination every 8 min. The gas mixture employed was constantly bubbled through the medium during the whole incubation period. This was done by introducing a thin polyethylene tube (0.13 mm internal diam.) into the medium and bubbling at 35–40 ml of gas/min. This procedure was important since otherwise the diffusion of oxygen into the medium was insufficient. In one experimental series the saturation of the incubation medium was measured with a Clark oxygen electrode. By this procedure the oxygen saturation of the flasks containing tissue was found to be virtually identical with those in which no tissue had been added (control flasks). In every experiment two control flasks were carried to correct for the small amount of ethanol that evaporated. This correction did not exceed 5% of the ethanol metabolized in the presence of tissue. The different substances that were studied were added to the medium at the beginning of the preincubation period to allow for

equilibration into the cells. The rate of ethanol metabolism was calculated from its disappearance from the incubation medium. Ethanol was determined enzymically as described by Hawkins *et al.* (1966). The results are expressed as μmol of ethanol metabolized/h per g of fresh liver.

Chronic ethanol treatment. The rats were separated in two groups. One group received ethanol for 21–26 days. Ethanol was given by stomach tube in increasing doses: 3 g/kg body wt. for 4 days [as a 20% (w/v) soln.]; 4 g/kg for 4 days [25% (w/v) soln.]; 5 g/kg for 4 days [30% (w/v) soln.]; 6 g/kg for 3 days [35% (w/v) soln.]; 7 g/kg for 3 days [40% (w/v) soln.] and 8 g/kg for 3–8 days [40% (w/v) soln.]. Another group was used as control and received isocaloric amounts of glucose according to the same schedule. Both the ethanol-treated and the control animals were fed *ad libitum* with rat Purina chow enriched with casein to a final protein content of 28%. This diet and the intubation of animals early in the morning (8–9 a.m.) seemed to be important in preventing triglyceride accumulation in the liver. In a first study in which the protein content of the diet was 19% and in which ethanol was administered in the afternoon or evening, 60% of the rats had died by the 20th day and the liver triglyceride content of the survivors was found to have increased fivefold. The initial weight of the animals (female rats on high protein diets) was 198 ± 4.0 g (controls) and 188 ± 3.7 g (ethanol-treated). At the end of the treatment the weights were 189 ± 5.5 g (controls) and 165 ± 4.5 g (ethanol-treated). No differences were found in the liver triglyceride content between the glucose group and the ethanol-treated animals. The values determined were 6.36 ± 0.28 mg/g of liver (controls) and 6.37 ± 0.32 mg/g of liver (ethanol-treated). At the end of the treatment the liver weight was 3.16 ± 0.12 g/100 g body wt. for the control animals and of 3.73 ± 0.09 g/100 g body wt. for the ethanol-treated rats ($P < 0.01$). Liver protein content was not significantly altered: 273 ± 16.0 mg/g of liver (controls) and 202 ± 2.03 mg/g of liver (ethanol-treated).

Alcohol dehydrogenase activity. Alcohol dehydrogenase activity was determined at 25°C as described by Bonnichsen & Brink (1955) in supernatants after centrifugation at 5000 g for 10 min of 1:10 liver homogenates in 0.25 M-sucrose containing 1% Triton (Räihä & Koskinen, 1964).

Succinate dehydrogenase activity. Succinate dehydrogenase activity was determined in 1:50 liver homogenates in water by measuring the reduction of cytochrome *c* at 540 nm as described by Feldman, Wende & Kessler (1961).

Determination of pyruvate and lactate. These compounds were determined enzymically as described by Bücher, Czok, Lamprecht & Latzko (1965) and Hohorst (1965) respectively, in a sample of incubation medium containing the liver slices after the 32 min incubation period.

Protein determination. Proteins were determined by the biuret method (Gornall, Bardawill & David, 1949).

RESULTS

The effect of two different conditions of oxygenation on the rate of ethanol metabolism by liver slices is shown in Table 1. In the presence of 18% O₂, the

Table 1. *Effects of different oxygen tensions, cyanide and dinitrophenol on the rate of ethanol metabolism by liver slices from female rats*Results are means $\pm$ s.e.m. N.S., Not significantly different.

Incubation conditions	Ethanol metabolism (μ mol disappeared/h per g)	Difference from control
O ₂ +CO ₂ +N ₂ (18:5:77)		
Control	16.1 $\pm$ 2.9 (4)	—
Cyanide (3mm)	1.6 $\pm$ 0.2 (3)	— 90% ($P < 0.001$)
Dinitrophenol (0.1 mm)	42.6 $\pm$ 5.8 (4)	+160% ($P < 0.01$)
O ₂ +CO ₂ (95:5)		
Control	37.5 $\pm$ 2.7 (7)	—
Cyanide (3mm)	10.9 $\pm$ 3.0 (3)	— 70% ($P < 0.05$)
Dinitrophenol (0.1 mm)	33.0 $\pm$ 4.0 (5)	— 12% (N.S.)

rate of ethanol disappearance was (female rats) $16.1 \pm 2.9 \mu\text{mol/h}$ per g of tissue whereas with 95% O₂ this value was $37.5 \pm 2.7 \mu\text{mol/h}$ per g of tissue. Under the conditions used, the rate of ethanol metabolism was found to be constant with either 18% or 95% O₂. Cyanide strongly inhibited the metabolism of ethanol but its effect was more marked at the lower oxygen partial pressure. The effect of 2,4-dinitrophenol was completely different at the two oxygen partial pressures. When 18% O₂ was used to saturate the medium, 0.1 mm-2,4-dinitrophenol produced a 160% increase in the rate of ethanol metabolism. This concentration produced the largest increase; due to the relative insolubility of dinitrophenol, higher concentrations were not tried. Dinitrophenol had no effect on the metabolism of ethanol under 95% O₂ (Table 1). Preliminary experiments had shown that dinitrophenol has no effect on liver alcohol dehydrogenase activity assayed spectrophotometrically.

A different set of experiments was performed with arsenate, a compound also known to be an uncoupler of oxidative phosphorylation (Ernster, Lee & Janda, 1967). The experiments in which the effect of arsenate was studied, and all subsequent experiments, were done with 18% O₂. Arsenate at a concentration of 10 mm increased the metabolism of ethanol by 80%, although at 37.5 and 50 mm, it inhibited (Table 2).

The effect of 10 mm-fructose in the incubation medium was studied in a separate series of experiments. The rate of ethanol metabolism for the controls (male rats) was 28.1 ± 2.1 (5) $\mu\text{mol/h}$ per g and 46.2 ± 2.2 (5) $\mu\text{mol/h}$ per g in the presence of fructose.

The effect of chronic administration of ethanol on the rate of metabolism of this compound is shown in Table 3. Chronic administration markedly increased the rate of ethanol metabolism by 67% with respect to the metabolism in slices from isocaloric-glucose-control rats and by 85% with respect to the untreated controls. The activating

Table 2. *Effect of different concentrations of arsenate on the rate of ethanol metabolism by liver slices from male rats*

The values given are means $\pm$ s.e.m. The gas mixture was: O₂+CO₂+N₂ (18:5:77). (Control versus 10 mm arsenate: $P < 0.001$.)

Concn. of arsenate (mm)	Alcohol metabolism (μ mol/h per g)
None	28.0 $\pm$ 1.2 (6)
5	35.4 $\pm$ 3.1 (3)
10	50.3 $\pm$ 2.0 (4)
25	35.4 $\pm$ 2.7 (3)
37.5	20.4 (18.6; 22.2) (2)
50.0	1.5 (0.9; 2.1) (2)

effect of dinitrophenol was observed in the liver slices from the glucose-control rats but not in the slices from the ethanol-treated rats, in which the rate of ethanol metabolism was already elevated. There was no significant difference between the rate of ethanol disappearance from slices from glucose-control rats incubated in the presence of dinitrophenol and those from the ethanol-treated rats in the absence of the uncoupler. In a group of rats, the chronic ethanol treatment was discontinued after the 26th day and the metabolism of ethanol was measured 15–17 days later. Under these conditions the rate of ethanol metabolism was decreased to values that were similar to those obtained in glucose-control and untreated rats and the stimulating effect of dinitrophenol was again observed.

Since the study of the adaptive changes produced by the chronic administration of ethanol was done with female rats of the University of Chile strain (Table 3), it was desirable to repeat the experiments with male rats, preferably of a commercially available strain. The effect of chronic administration of ethanol was therefore studied on male Wistar rats

Table 3. *Rate of ethanol metabolism by liver slices of female rats chronically treated with ethanol and of control rats in the presence and absence of dinitrophenol*

Dinitrophenol was added to the incubation medium to a final concentration of 0.1 mM. The gas mixture was: $O_2 + CO_2 + N_2$ (18:5:77). The ethanol-treated rats were given ethanol in increasing doses for 21–26 days. The glucose controls were given isocaloric amounts of glucose (see the text). The treatment was discontinued at day 26 and the metabolism was studied 15–17 days later ('ethanol withdrawn' group). Untreated controls A and D are from Table 1 and are included for comparison. The values shown represent the means $\pm$ S.E.M. Significance studies (*t* test): group A versus B (not significantly different: N.S.); A versus C and B versus C ($P < 0.05$); A versus D ($P < 0.01$); B versus E ($P < 0.05$); C versus F (N.S.); G versus H ($P < 0.01$); all combinations of C, D, E, F, and H (N.S.).

Animals	Dinitrophenol in medium	Ethanol metabolism (μ mol/h per g of tissue)
A. Untreated controls	—	16.1 ± 2.9 (4)
B. Glucose controls	—	18.4 ± 2.2 (7)
C. Ethanol-treated	—	29.1 ± 3.9 (7)
D. Untreated controls	+	42.6 ± 5.8 (4)
E. Glucose controls	+	34.5 ± 5.2 (8)
F. Ethanol-treated	+	28.1 ± 5.5 (8)
G. Ethanol withdrawn	—	16.9 ± 2.1 (5)
H. Ethanol withdrawn	+	40.8 ± 5.3 (5)

Table 4. *Rate of ethanol metabolism by liver slices of male rats chronically treated with ethanol in the presence and absence of dinitrophenol*

The values given correspond to the means $\pm$ S.E.M. *a* versus *b* ($P < 0.05$); *a* versus *c* ($P < 0.05$).

Animals	Dinitrophenol (0.1 mM)	Ethanol metabolism (μ mol/h per g)
I. Isocaloric glucose control rats	—	(a) 24.0 ± 3.1 (5)
	+	(b) 44.0 ± 7.0 (5)
II. Ethanol-treated rats	—	(c) 43.3 ± 12.4 (6)
	+	(d) 46.8 ± 12.8 (6)

Table 5. *Alcohol dehydrogenase activity in the liver of rats chronically treated with ethanol*

The values given are the means $\pm$ S.E.M. (N.S. = $P > 0.05$). Rats were administered either ethanol or isocaloric glucose for 21–26 days. (See the Materials and Methods section for schedule of treatment.)

	Alcohol dehydrogenase activity (nmol/min per mg of protein)	
Female rats	pH 7.4	pH 9.2
	Glucose controls	3.61 ± 0.33 (9)
	(N.S.)	(N.S.)
Ethanol-treated	4.30 ± 0.44 (9)	11.1 ± 0.50 (8)
Male rats		
	Glucose controls	2.95 ± 0.60 (7)
	(N.S.)	(N.S.)
Ethanol-treated	3.01 ± 0.56 (7)	7.48 ± 0.99 (7)

(Canadian Breeding Laboratories). Essentially the same results were obtained; the metabolism of ethanol was significantly increased (by 80%) in the ethanol-treated male rats. As had been found for the female rats, dinitrophenol increased the rate of ethanol metabolism in slices from control male

rats but did not in those from the ethanol-treated rats (Table 4).

Alcohol dehydrogenase activity was measured in 50 000g-min supernatants from liver homogenates of ethanol-treated female and male rats. No significant increase was found as a result of the

Table 6. *Effect of chronic ethanol treatment and dinitrophenol on the production of lactate and pyruvate by liver slices from female rats*

The values given represent the mean $\pm$ S.E.M. Dinitrophenol was added to the incubation medium to a final concentration of 0.1 mM. Ethanol was always present in the medium at an initial concentration of 10 mM. The lactate/pyruvate ratio was calculated for each animal and then averaged (see the Materials and Methods section).

	Dinitrophenol in medium	Lactate (μ mol/h per g)	Pyruvate (μ mol/h per g)	Lactate/pyruvate ratio
Glucose controls	—	11.8 $\pm$ 1.4 (7) ($P < 0.01$)	0.26 $\pm$ 0.05 (6) ($P < 0.05$)	49.1 $\pm$ 9.0 (6) (N.S.)
	+	18.9 $\pm$ 1.6 (7)	0.49 $\pm$ 0.07 (6)	50.4 $\pm$ 9.2 (6)
Ethanol-treated	—	13.1 $\pm$ 1.4 (7) ($P < 0.01$)	0.28 $\pm$ 0.04 (7) (N.S.)	54.1 $\pm$ 8.6 (7) (N.S.)
	+	21.2 $\pm$ 2.0 (7)	0.35 $\pm$ 0.03 (7)	65.1 $\pm$ 9.4 (7)

Table 7. *Succinate dehydrogenase activity in liver homogenates from rats chronically treated with ethanol and control rats*

The values given are means $\pm$ S.E.M. Male rats were used for the experiments.

	Succinate dehydrogenase activity [k (s^{-1})/mg of protein]
Glucose control rats	840 $\pm$ 67 (7)
Ethanol-treated rats	1,163 $\pm$ 97 (7) ($P < 0.02$)

chronic ethanol treatment in rats from either sex, at the two pH values studied (7.4 and 9.2) (Table 5).

It is known that the lactate/pyruvate ratio in the blood or in the liver extracellular fluid is an indicator of the redox state in the cytoplasm of the liver cell (Schimassek, 1963). We therefore measured the concentration of lactate and pyruvate in the incubation medium containing the slices, at the end of the incubation period. Ethanol was always present in the medium at an initial concentration of 10 mM. Table 6 shows that dinitrophenol significantly increased the production of lactate and pyruvate. This effect of dinitrophenol was similar in the slices from the ethanol-treated and the control rats. No significant changes were obtained in the lactate/pyruvate ratio in either group in the presence or absence of dinitrophenol.

Since it was conceivable that an increased number of mitochondria or an enlargement of these organelles could be in part responsible for the increased rates of ethanol metabolism in the livers of the ethanol-treated rats, we measured the activity of succinate dehydrogenase as an index of mitochondrial activity. Table 7 shows that succinate dehydrogenase activity was significantly increased (by 40%) in the ethanol-treated rats with respect to the controls.

DISCUSSION

The results presented show that dinitrophenol and arsenate, two uncoupling agents, markedly increase the rate of ethanol metabolism by rat liver slices. Since these compounds increase the ability of mitochondria to oxidize NADH, it is likely that, in normal rats, the reoxidation of this coenzyme is the limiting step in the metabolism of ethanol. Uncoupling agents are known to inhibit the translocation of malate into rat liver mitochondria (Haslam & Griffiths, 1968). Since the malate-aspartate shuttle appears to be the mechanism by which the cytoplasmic NADH reducing equivalents are oxidized by the mitochondria (Hassinen, 1967; Greville, 1969), the activation of the metabolism of ethanol by the uncouplers would suggest that the shuttle mechanism is not the rate-limiting step in the oxidation of alcohol. Newman (1941) (see Kalant, 1970) has reported that dinitrophenol increases the rate of ethanol disappearance *in vivo* in several species by up to 100%. It was suggested, however, that this effect was due to an increased hyperventilation in the presence of dinitrophenol.

The higher rate of ethanol metabolism obtained when the liver slices are incubated under 95% O₂ over that under 18% O₂ is in agreement with results reported by Gordon (1966, 1968) in the perfused rat liver. Since it is known that some flavoproteins can be oxidized directly by molecular oxygen, it is not unreasonable to think that, at high oxygen partial pressures, the reducing equivalents could be transferred from NADH to flavoproteins and then directly oxidized by O₂ (Mahler & Cordes, 1966). It is also conceivable that under these conditions some of the cytoplasmic NADH could be oxidized through the peroxisomal lactate oxidase electron shuttle proposed by de Duve & Baudhuin (1966). An increased non-mitochondrial oxidation of NADH under high oxygen partial pressures

would explain the lack of effect of dinitrophenol on the oxidation of ethanol by liver slices under 95% O₂.

Chronic administration of ethanol to rats resulted in a 70–90% increase in the ability of the liver to metabolize the ethanol. This was found to occur in male and female rats of two different strains. It was noteworthy that dinitrophenol did not produce any further increase in the rate of ethanol metabolism in the livers of the ethanol-treated rats. This would suggest that in these rats the mitochondrial oxidation is no longer the limiting step. This might conceivably occur if the number of mitochondria per cell, or any other mechanism to oxidize cytoplasmic NADH, is increased.

The increase in succinate dehydrogenase activity found in the liver of rats chronically treated with ethanol may be relevant to the higher rate of ethanol metabolism in these livers. If succinate dehydrogenase activity is taken as an indication of the amount of 'mitochondrial activity'/g of tissue, and thus of the capacity of the tissue to reoxidize cytoplasmic NADH, an increased rate of regeneration of NAD⁺ would explain the higher rate of ethanol metabolism.

The liver alcohol dehydrogenase activity remained unchanged after chronic ethanol administration. This is in agreement with results reported by other authors who have found no correlation between the alcohol dehydrogenase activity measured in homogenates and the rate of ethanol metabolism *in vivo* or in incubated slices (Smith & Newman, 1959; Wilson, 1967; Ylikahri & Mäenpää, 1968). However, Hawkins *et al.* (1966) found that chronic ethanol administration to rats led to an increase in both alcohol dehydrogenase activity and ethanol metabolism. Although in the first weeks of treatment there was a good correlation between alcohol dehydrogenase activity and ethanol metabolism, Hawkins *et al.* (1966) found that with longer treatment (up to 12 weeks) the rate of ethanol disappearance *in vivo* increased substantially more than could be explained by the increase in alcohol dehydrogenase activity. Several authors have described an increased alcohol dehydrogenase activity after chronic ethanol treatment in rats and mice (Dajani, Danielski & Orten, 1963; McClearn, Bennett, Herbert, Kakihana & Schlesinger, 1964; Mendelson, Mello, Corbett & Ballard, 1965; Mistilis & Birchall, 1969); unfortunately in most of these studies no correlation was made between alcohol dehydrogenase activity and the rate of ethanol disappearance *in vivo* or *in vitro*. A large number of authors have, conversely, found no change in the liver alcohol dehydrogenase after chronic administration of ethanol (Aebi & von Wartburg, 1960; von Wartburg & Rothlisberger, 1961; Figueroa & Klotz, 1964; Greenberger, Cohen &

Isselbacher, 1965; Ugarte, Pino & Insunza, 1967). The possible explanation of these contradictory results may be found in the differences between the schedules and the amount of ethanol administered per day. These differences have been thoroughly reviewed by Hawkins *et al.* (1966). Nevertheless, our results show that the rate of ethanol metabolism can increase without a concomitant increase in alcohol dehydrogenase activity.

It is known that fructose increases the rate of ethanol metabolism, both *in vivo* and *in vitro* (Lundquist & Walther, 1958; Pawan, 1968; Thieden & Lundquist, 1967). We have confirmed these results with liver slices. Addition of 10 mM-fructose to the incubation medium increased the rate of ethanol metabolism by 65%. This increase is higher than that of 20% reported by Thieden & Lundquist (1967), also with rat liver slices. This difference might be due to the fact that these authors used 100% O₂ to saturate their incubation media whereas our experiments were done under 18% O₂. Thieden & Lundquist (1967) have suggested that the effect of fructose is mediated by glyceraldehyde produced from fructose. It was suggested that glyceraldehyde would directly oxidize the alcohol dehydrogenase–NADH complex (Holzer & Schneider, 1955) and would thus increase the rate of alcohol oxidation. These authors have demonstrated that glyceraldehyde produces similar effects to those of fructose on the rate of ethanol metabolism. These results have been interpreted as an indication that the rate-limiting step for the oxidation of ethanol is the dissociation of the alcohol dehydrogenase–NADH complex; however, if the availability of NAD⁺ to alcohol dehydrogenase is the limiting factor in the metabolism of ethanol, the conversion of glyceraldehyde into glycerol by any NAD-linked system might increase the rate of ethanol metabolism. Thus, in this respect, the effect of glyceraldehyde may be similar to that of pyruvate, a compound which has also been shown to increase the rate of ethanol metabolism in liver slices (Leloir & Muñoz, 1938; Smith & Newman, 1959).

Dinitrophenol was found to increase the synthesis of lactate and pyruvate by the liver slices metabolizing ethanol but it did not change the lactate/pyruvate ratio. The increase in lactate and pyruvate produced by dinitrophenol is likely to be due to an increase in anaerobic glycolysis by removal of feedback inhibition of phosphofructokinase (Atkinson, 1966). The synthesis of lactate and pyruvate was also increased by dinitrophenol in the livers of the ethanol-treated rats, thus suggesting that the mitochondria in the liver of these rats are not in an uncoupled state and that this is not likely to be the cause of the increased rate of ethanol metabolism. The inability of dinitrophenol to alter the NADH/NAD⁺ ratio in the cytosol (meas-

ured indirectly as the lactate/pyruvate ratio) might be explained if the excess of NADH oxidized to NAD⁺ by the mitochondria in the presence of dinitrophenol was promptly reduced in the cytoplasm by ethanol. Since the existence of this mechanism would necessarily result in an increased oxidation of ethanol, it cannot be invoked for the ethanol-treated rats in which this increase does not occur.

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