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The Swift Increase in Alcohol Metabolism

TIME COURSE FOR THE INCREASE IN HEPATIC OXYGEN UPTAKE AND THE INVOLVEMENT OF GLYCOLYSIS

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Gastric intubation of female Sprague–Dawley rats with 5g of ethanol/kg body wt. nearly doubled oxygen uptake by the isolated perfused rat liver maximally after only 2.5h of treatment (Swift Increase in Alcohol Metabolism). Inhibition of enhanced oxygen uptake by KCN (2mM) and 4-methylpyrazole (0.8mM) suggested the involvement of the mitochondrial respiratory chain and alcohol dehydrogenase in this phenomenon. Glycolysis was depressed after ethanol treatment. Diminished ATP generation via glycolysis accounts for a portion (23–50%) of the increased oxygen uptake, assuming that other rates of biosynthesis remain constant. Injection of adrenaline (2mg/kg) 1h before perfusion mimicked partially the action of ethanol on hepatic oxygen uptake. The increases produced by ethanol and adrenaline were not additive, suggesting that adrenaline is involved in the action of ethanol. Moreover, the increase in hepatic oxygen uptake produced by 2.5h of ethanol treatment could be blocked by either α- (phenoxybenzamine; 40mg/kg) or β- (propranolol; 40mg/kg) adrenergic blocking agents. Blood glucose increased after ethanol treatment, supporting the involvement of glycogenolytic hormones in this effect. These data indicate that at least part of the stimulated oxygen uptake after treatment with ethanol is a result of lower rates of glycolytic ATP generation resulting from hormone (e.g. adrenaline etc.) action. The ADP not phosphorylated in the cytosol enters the mitochondria, where it stimulates oxygen uptake.

An adaptive increase in ethanol metabolism after chronic treatment with ethanol involving the alcohol dehydrogenase pathway is well documented (Hawkins & Khanna, 1966; Mendelson & Mello, 1966; DeCarli & Lieber, 1967; Porta *et al.*, 1968; Toban & Mezey, 1971; Thurman *et al.*, 1975). Evidence supporting this includes the observation that the adaptive increase is sensitive to an inhibitor of alcohol dehydrogenase, 4-methylpyrazole (Thurman *et al.*, 1975), as well as the fact that it is observed at low ethanol concentrations (<1mM), where non-alcohol dehydrogenase-dependent pathways are minimal (Thurman & Scholz, 1976). Furthermore, ethanol treatment increased both oxygen uptake and ethanol metabolism in liver slices (Bernstein *et al.*, 1973) and perfused rat liver (Thurman *et al.*, 1975). This increase in oxygen uptake is responsible for stimulation of ethanol metabolism by enhancing reoxidation of NADH, thereby allowing a more rapid dissociation of the binary alcohol dehydrogenase–NADH complex to occur.

In contrast, ethanol produced only a small stimulation (5–10%) of hepatic oxygen uptake when added directly to the perfused liver (Thurman & Scholz, 1977). This effect has been explained by the following sequence of events: ethanol increases the NADH redox state, which inhibits glycolysis. Since the glycolytic pathway is an ATP-generating enzyme sequence, inhibition makes more ADP available to stimulate mitochondrial electron transport and increase oxygen uptake (Thurman & Scholz, 1977).

Wendell & Thurman (1979) have shown that one single large dose of ethanol (5.0g/kg) increased the rate of ethanol elimination about 60% in the rat *in vivo* in only a few hours. This increase in ethanol elimination was blocked by 4-methylpyrazole. Taken together, these observations predict that one dose of ethanol may stimulate oxygen uptake in the liver in a short period of time.

The purpose of the present experiments was therefore to establish the minimum time after ethanol treatment *in vivo* that would cause an increase in oxygen uptake of the perfused rat liver. The data in the present paper indicate that oxygen uptake and ethanol uptake were nearly doubled 2.5h after one large dose (5.0g/kg) of ethanol to the rat. Diminished rates of ATP production from glycolysis are partially responsible for this rapid increase in oxygen uptake. Rapid enhanced oxygen uptake probably explains the Swift Increase in Alcohol Metabolism (Thurman

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et al., 1979; Yuki & Thurman, 1978). Preliminary accounts of this work have appeared elsewhere (Thurman *et al.*, 1979; Yuki & Thurman, 1978).

Methods and Materials

Animals

Female albino rats (Sprague–Dawley strain; 80–150g) received laboratory chow and water *ad libitum*. When indicated, some rats were starved for 24h before surgical preparation. Animals were either treated with ethanol via gastric intubation with three fractional doses for 24h or with one single dose (5.0g/kg) 2.5h before surgery. Controls were intubated with 0.9% NaCl.

Non-recirculating haemoglobin-free liver perfusion

The perfusion technique has been described elsewhere (Scholz, 1968; Scholz *et al.*, 1973). With this technique, the liver is isolated surgically under pentobarbital (50mg/kg) anaesthesia. The perfusion fluid was Krebs–Henseleit bicarbonate buffer (Krebs & Henseleit, 1932), pH7.4, saturated with O_2/CO_2 (19:1). The fluid was pumped into the liver via a cannula inserted in the portal vein. The effluent perfusate was collected with a cannula placed in the vena cava and flowed past an oxygen electrode before it was discarded. Ethanol or drugs were infused into the perfusion fluid before it entered the liver with precision infusion pumps.

The oxygen concentration in the effluent perfusate was monitored continuously with a Teflon-shielded platinum electrode. The influent oxygen concentration was maintained constant by the oxygenator and was measured before and after each experiment. Influent ethanol concentrations were determined under identical perfusion conditions in the absence of the liver. Metabolic rates were calculated from influent concentration differences and the constant flow rate and expressed per g wet wt. of liver per h.

Subcellular fractionation

Mitochondrial and microsomal fractions were isolated by standard techniques of differential centrifugation (Hogeboom *et al.*, 1948; Hildebrandt *et al.*, 1968). Mitochondrial respiration was monitored continuously in a temperature-regulated chamber (1.0ml) polarographically. Ethanol oxidation by the microsomal fractions was determined by the method of Lieber & DeCarli (1970).

Blood-sampling technique

After anaesthesia of the rat with pentobarbital (50mg/kg), the femoral artery was ligated, and blood samples (50μl) were collected every 10–30min. Blood was deproteinized with $HClO_4$ (1.5M; 300μl), centrifuged, and neutralized with NaOH (2.5M; 200μl). Glucose was determined in the supernatant by standard enzymic procedures (Bergmeyer, 1970).

Analytical

Samples of the effluent perfusate were collected every 2min and analysed for glucose, lactate, pyruvate, β-hydroxybutyrate, acetoacetate and ethanol by standard enzymic procedures (Bergmeyer, 1970). Liver glycogen contents were determined by the method of Fong *et al.* (1953). Protein was determined by the method of Gornall *et al.* (1949).

Alcohol dehydrogenase activity of liver was determined at 25°C in the direction ethanol→acetaldehyde on the 100000*g* supernatant as described by Crow *et al.* (1977).

4-Methylpyrazole was obtained from Research Plus Laboratories (Dennville, NJ, U.S.A.). Enzymes, adrenaline and propranolol were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Phenoxybenzamine was the kind gift of Smith, Kline and French (Philadelphia, PA, U.S.A.). All other chemicals were reagent grade from standard sources.

Statistical comparisons were performed with Student's *t* test (Goulden, 1939).

Results

Effect of ethanol treatment on oxygen uptake in livers perfused with ethanol-free buffer

To determine the minimal time necessary for ethanol to increase hepatic oxygen uptake, rats were given ethanol via gastric intubation (5.0g/kg) at zero time, and 8 and 14h later (Fig. 1). Basal rates of oxygen uptake by the ethanol-free perfused livers were between 100 and 110μmol/h per g and were unchanged by gastric intubation with saline (0.9% NaCl). The oxygen uptake increased swiftly in livers from rats previously treated with ethanol, and was nearly doubled 2.5h after the initial dose (Fig. 1; Table 1). The rate subsequently declined nearly to basal values at 5h and slowly increased to a new maximum at 16h before slowly returning again toward basal values (Fig. 1). Because the maximum increase in respiration after treatment with ethanol was observed 2.5h after the initial dose of ethanol, all subsequent experiments were performed at that time.

A possible involvement of the mitochondrial respiratory chain in the increase in oxygen uptake was examined by using KCN, an inhibitor of cytochrome oxidase. Cyanide infusion diminished oxygen uptake in livers from both control and ethanol-treated (2.5h) rats approx. 75–80% (Fig. 2). Carboxyatractyloside (0.1mM), an inhibitor of the adenine nucleotide translocase, also decreased respiration in alcohol-treated livers by 35–40%.

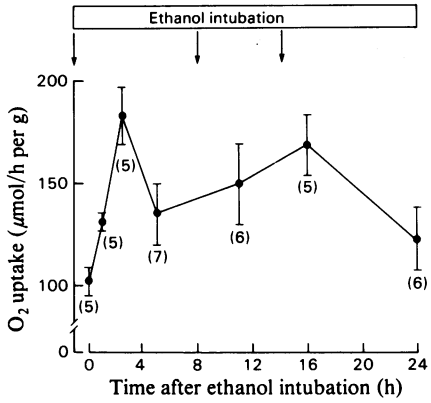


Fig. 1. *Time course of the effect of ethanol treatment in vivo on oxygen uptake by the perfused liver*

Ethanol (5.0g/kg) was administered to rats via gastric intubation at times 0, 8h and 14h (arrows). Thus animals at longer time points received multiple doses of ethanol. Untreated rats were intubated with 0.9% NaCl. At times indicated by the filled circles, livers were perfused with ethanol-free Krebs–Henseleit bicarbonate buffer, pH7.4. After 20–30min of pre-perfusion, steady-state rates of oxygen uptake were calculated from the influent-effluent oxygen-concentration difference, the flow rate and the liver weight. Results are means ± S.E.M. for the numbers of experiments in parentheses.

State-3 and state-4 respiration (Chance & Williams, 1955) were identical (statistically not significantly different by Student's *t* test) in mitochondria isolated from control and ethanol-treated rats (2.5h) with succinate as the substrate ($n = 5$; results not shown).

Rates of oxygen uptake and glycolysis in livers from control and ethanol-treated rats

Rates of glycolysis were estimated by the production of lactate+pyruvate by the perfused liver. This index is reliable because concentrations of pyruvate in these experiments were far below the K_m of pyruvate dehydrogenase for pyruvate (Scholz *et al.*, 1976).

In livers from control rats, rates of lactate+pyruvate production and oxygen uptake were both between 100 and 120μmol/h per g (Fig. 3*a*); when ethanol (1.5mM) was infused, a small increase in respiration of about 20μmol/h per g was observed. This increase was not statistically significant. Concomitantly, lactate+pyruvate production decreased to about 40μmol/h per g (Fig. 3*a*). In contrast, average rates of oxygen uptake were initially much higher (200–220μmol/h per g) in livers from ethanol-treated rats (Fig. 3*a*). In livers from ethanol-treated rats, rates of lactate+pyruvate production were much lower (approx. 30μmol/h per g) (Table 2), and ethanol infusion had no effect on either oxygen uptake or lactate+pyruvate production (Fig. 3*b*). Rates of glucose production were also decreased after ethanol pretreatment, whereas rates of ketogenesis (β-hydroxybutyrate+acetoacetate production) were increased 2–3-fold (Table 2).

When glucose (20mM) was infused into livers from ethanol-treated rats, rates of lactate+pyruvate production were increased from 30 to 60μmol/h per g (results not shown). Simultaneously, rates of oxygen uptake were decreased by 20μmol/h per g.

Table 1. *Effect of ethanol, adrenaline and adrenergic-blocking-agent treatment on oxygen uptake by perfused rat livers*

Rats were treated with ethanol 2.5h before surgical preparation. Oxygen uptake by the perfused liver was determined polarographically as described in the Methods and Materials section. Adrenaline and adrenergic blocking agents were injected intraperitoneally 1 and 3h before liver perfusion respectively: adrenaline, 2mg/kg; phenoxybenzamine, 40mg/kg; propranolol, 40mg/kg. Results are means ± S.E.M. for the numbers of experiments in parentheses. Starved rats were deprived of food 24h before surgical preparation. Phenoxybenzamine and propranolol had no significant effect on oxygen uptake in the control rat. Each group was compared with its own non-treated control by Student's *t* test (column *P*): n.s., not significant ($P > 0.05$).

Treatment	Oxygen uptake (μmol/h per g wet wt. of liver)	*P*
From fed rats		
None (8)	107 ± 8	—
Ethanol (10) (2.5h)	192 ± 16	<0.001
Adrenaline (9)	150 ± 5	<0.001
Ethanol+ adrenaline (7)	150 ± 18	<0.001
Ethanol+ phenoxy-benzamine (5)	116 ± 9	n.s.
Ethanol+ propranolol (5)	112 ± 11	n.s.
From starved rats		
None (8)	113 ± 19	n.s.
Ethanol (8)	157 ± 16	n.s.
Adrenaline (8)	141 ± 18	n.s.

Effect of adrenaline treatment on oxygen uptake in the perfused liver

It has previously been shown that adrenaline treatment increases the oxygen consumption of liver slices (Bernstein *et al.*, 1974). In fed rats, injection of adrenaline (2mg/kg intraperitoneally 1h before perfusion) produced a significant increase in oxygen uptake by the perfused liver (Table 1). The effects of adrenaline and ethanol on oxygen uptake were not additive. No explanation for why the oxygen

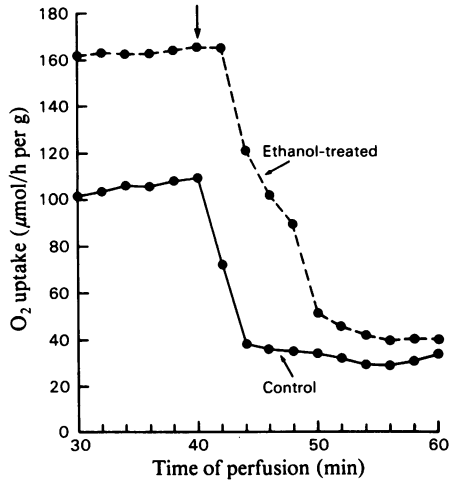


Fig. 2. *Effect of KCN on oxygen uptake in perfused livers from control and ethanol-treated rats*
Rats were treated with ethanol (●----●) or saline (control; ●——●) 2.5h before surgical preparation. Livers were then perfused for 20min before the experiment was initiated. Livers were perfused and oxygen uptake was determined as in Fig. 1. At 40min of perfusion, KCN (2mM) was infused as indicated by the arrow. These are typical representative experiments, which have been repeated at least three times in each group.

uptake with ethanol+adrenaline was only 150μmol/h per g in these experiments is presently available. Both phenoxybenzamine, an α-adrenergic blocking agent, and propranolol, a β-adrenergic blocker, prevented the increase in oxygen uptake caused by ethanol treatment (Table 1).

Table 2. *Rates of glycolysis, ketone-body production and glucose production in perfused livers from normal and ethanol-treated rats*
Livers were perfused as described in the Methods and Materials section. Lactate, pyruvate, β-hydroxybutyrate, acetoacetate and glucose were measured in samples of effluent perfusate by standard enzymic techniques (see the Methods and Materials section). Rates of production of lactate+pyruvate, β-hydroxybutyrate+acetoacetate and glucose were calculated from the influent−effluent concentration differences, the flow rate and the liver wet weight. Results are means ± S.E.M. for the numbers of experiments in parentheses. Abbreviation: n.s., not significant ($P > 0.05$).

	Rate of production (μmol/h per g)		
	Control	Ethanol-treated	*P*
Lactate+pyruvate (7)	94±11	29±11	<0.01
β-Hydroxybutyrate+acetoacetate (5)	13±2	33±10	<0.05
Glucose (8)	67±12	52±16	n.s.

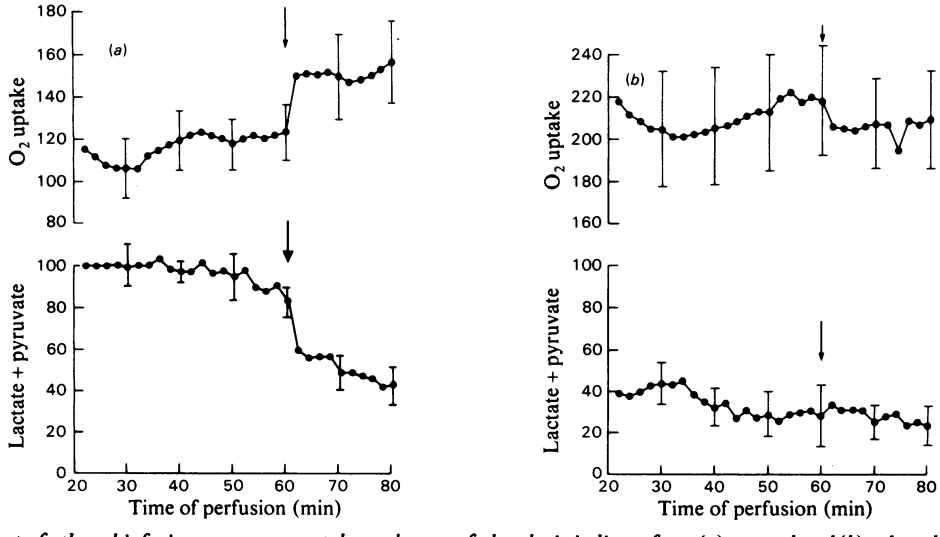


Fig. 3. *Effect of ethanol infusion on oxygen uptake and rates of glycolysis in livers from (a) control and (b) ethanol-treated rats*
Rates of oxygen uptake (upper panel) and glycolysis (lactate+pyruvate production; lower panel) were determined as described previously (Table 2). Ethanol (1.5mM) was infused as indicated by the arrow. Values are means ± S.E.M. ($n = 5$). In (*b*) rats had been pretreated with ethanol (5.0g/kg) 2.5h before perfusion. All rates are expressed as μmol/h per g.

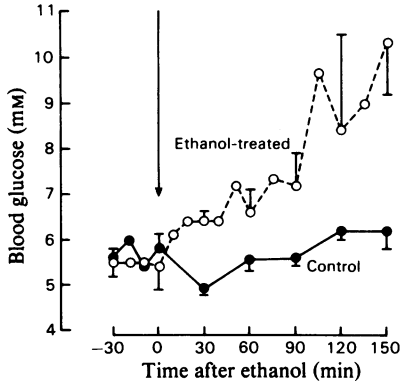


Fig. 4. *Effect of ethanol treatment on blood glucose concentrations in fed rats*

Rats were anaesthetized lightly with pentobarbital (20mg/kg) and secured on an animal board. An incision was made in the skin covering the right thigh area, and the femoral artery was exposed. Two surgical ligatures were placed around the femoral artery to secure a heparinized catheter. Blood samples (50μl) were withdrawn from control (●) and ethanol-treated (○) rats. Ethanol (5g/kg) was given to one group as indicated by the arrow. Blood glucose was measured enzymically in deproteinized blood samples (see the Methods and Materials section). Results are means ± S.E.M. ($n = 6$–8).

Control rates of oxygen uptake were unchanged after 24h of starvation, confirming earlier studies (Thurman & Scholz, 1969). Treatment with ethanol and adrenaline increased oxygen uptake by 44 and 28μmol/h per g respectively (Table 1). These differences were, however, not statistically significantly different from the controls.

Effect of ethanol treatment on blood glucose concentrations in the rat in vivo

Blood glucose concentrations in the rat were constant between 5 and 6mM over the 3h period of the experiment in control rats (Fig. 4). After addition of ethanol (5.0g/kg), blood glucose concentrations increased to between 9 and 10mM within 2.5h. After 2.5h of treatment with ethanol, hepatic glycogen contents and rates of glucose production by the liver were also decreased by approx. 40% and 20% respectively (Table 2).

Effect of ethanol treatment on ethanol utilization, alcohol dehydrogenase activity and microsomal ethanol oxidation

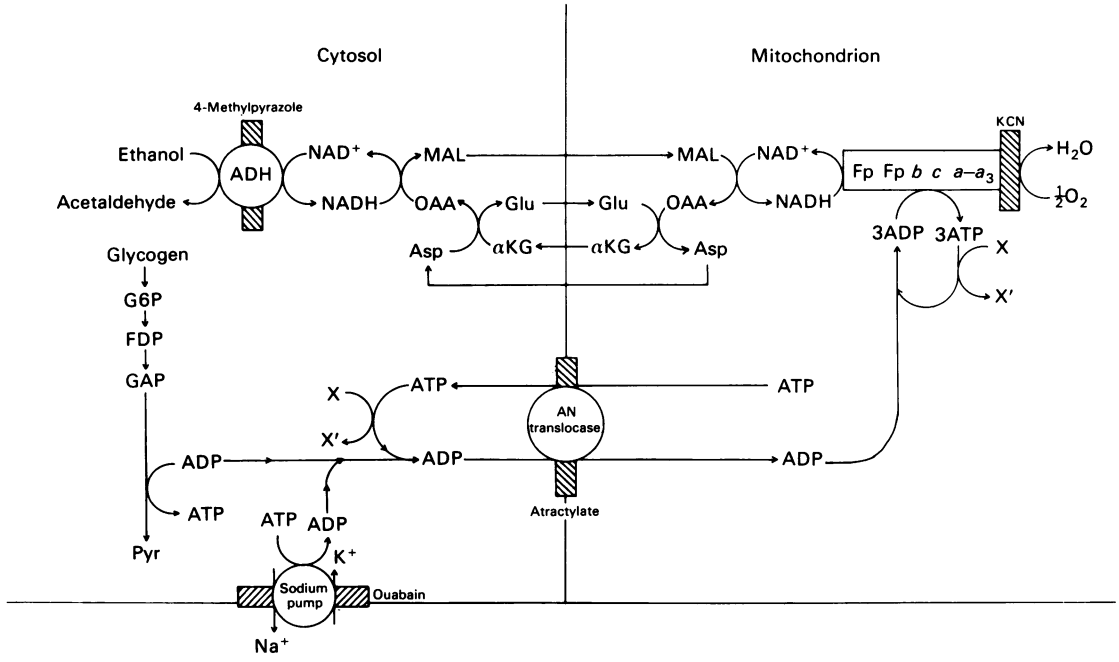
A clear correlation between oxygen uptake and ethanol metabolism has been established previously (Thurman *et al.*, 1975). In these studies, the rate of ethanol uptake was nearly doubled 2.5h after treatment with ethanol [control (8), 68 ± 8μmol/h per g; ethanol-treated (4), 137 ± 54; ethanol-treated +4-methylpyrazole (4), 32 ± 8]. This increase in rate could be inhibited by approx. 80% by an inhibitor of alcohol dehydrogenase, 4-methylpyrazole, without altering hepatic oxygen uptake. However, ethanol treatment for 2.5h did not affect the activity of hepatic alcohol dehydrogenase assayed at 25°C [control (4), 1.4 ± 0.2μmol/h per g; ethanol-treated (4), 1.6 ± 0.1] or ethanol oxidation by the microsomal fraction [control (4), 4.7 ± 0.3nmol of acetaldehyde formed/min per mg of protein; ethanol-treated (4), 3.9 ± 0.3].

Discussion

Rapid activation of hepatic oxygen uptake by ethanol

Previously it has been assumed that the activation of ethanol metabolism by ethanol treatment requires considerable time. For example, 3–5 weeks of treatment with diets containing ethanol have been routine (Porta *et al.*, 1968; DeCarli & Lieber, 1967). The data in the present paper indicate clearly, however, that the oxygen uptake by the ethanol-free perfused liver and subsequent ethanol metabolism can be nearly doubled only 2–3h after one large dose of ethanol (5.0g/kg) to the rat (Fig. 1). Because this activation of oxygen uptake occurs in the absence of ethanol, it can be concluded that this response is 'remembered' by the liver (i.e. it is not a direct response to ethanol which is readily reversible). On the other hand, the activation of oxygen uptake was swift (Fig. 1) and tended to return to the basal value at 5h after the dose, indicating that the effect of ethanol was transient (Fig. 1).

Several studies have shown a direct relationship between oxygen uptake and ethanol uptake (Thurman *et al.*, 1975; Israel *et al.*, 1973). Enhanced oxygen uptake activated NADH reoxidation, thereby facilitating the rate-limiting step in ethanol metabolism, the dissociation of the alcohol dehydrogenase–NADH complex. This allows ethanol to be oxidized more rapidly when the liver is respiring at a more rapid rate. This observation that ethanol uptake was greater in the perfused liver 2.5h after ethanol treatment than in livers from untreated rats is consistent with this hypothesis (see the Results section). It is also likely that the rapid increase in oxygen uptake caused by ethanol treatment is responsible for the 'concentration effect' observed *in vivo* (Wendell & Thurman, 1979), since that process is also 'remembered' (i.e. the accelerated rate of ethanol elimination is linear with time) and occurs as soon as 2h after the administration of ethanol (Wendell & Thurman, 1979). Thus, taken together, these experiments suggest strongly that a repeated acute rather than a chronic action of ethanol is responsible for the 'adaptive increase' in ethanol



Scheme 1. *Scheme depicting rapid increase in hepatic oxygen uptake caused by ethanol treatment*
Abbreviations used: ADH, alcohol dehydrogenase; MAL, malate; OAA, oxaloacetate; Glu, glutamate; αKG, α-oxoglutarate; Asp, aspartate; G6P, glucose 6-phosphate; FDP, fructose 1,6-bisphosphate; GAP, glyceraldehyde 3-phosphate; Pyr, pyruvate; AN, adenine nucleotide; X, unknown ATPase; Fp, flavoprotein; *b*, *c* etc., cytochromes *b*, *c* etc.

metabolism caused by chronic prior treatment with ethanol.

Physiological trigger for the increase in oxygen uptake caused by ethanol administration

Because the activation of oxygen uptake was rapid (2–3h) in these experiments, involvement of hormonal factors in the mechanism is predicted. This hypothesis is supported by several observations. First, adrenaline injected into the rat partially mimicked the increase observed with ethanol (Table 1), confirming previous observations with liver slices by Bernstein *et al.* (1974). Second, the increase in hepatic respiration observed 2.5h after treatment with ethanol could be blocked by both α- and β-adrenergic blocking agents (Table 1). Third, the actions of ethanol and adrenaline were not additive, indicating that the rapid increase in hepatic oxygen uptake is triggered, at least in part, via an ethanol-mediated release of adrenaline. The observation that blood glucose concentrations increased (Fig. 4) and hepatic glycogen concentrations declined with a time course similar to the increase in oxygen uptake observed in these studies is consistent with this hypothesis, since the stimulation of glycogenolysis by adrenaline is well documented (Sutherland *et al.*, 1965).

Mechanism of increase in hepatic oxygen uptake caused by treatment with ethanol

What is responsible for the increase in hepatic respiration after acute treatment with ethanol? Several possibilities seem unlikely. First, induction of ethanol-metabolizing enzymes probably does not occur within the short time frame of these experiments (2.5h). This hypothesis is supported by the observations that activities of alcohol dehydrogenase (see the Results section) and ethanol oxidation by the microsomal fraction were unaltered 2.5h after ethanol treatment. It is also unlikely that catalase activity or H_2O_2-generating flavoproteins are induced in only 2.5h.

Second, an induction or activation at the Na^+-plus-K^+-activated ATPase has been postulated to be a primary event in the mechanisms of the 'adaptive increase' in ethanol metabolism caused by prior pretreatment with ethanol in liver slices (Israel *et al.*, 1973) and perfused liver (Thurman *et al.*, 1975). This conclusion rests primarily on the observation that oxygen and ethanol uptake by slices or perfused livers was diminished to a larger degree by ouabain in tissues from alcohol-treated than from control rats (Israel *et al.*, 1973; Thurman *et al.*, 1975). This postulate has, however, subsequently been challenged (Thurman *et al.*, 1979). It was

demonstrated that the inhibition by ouabain was a slow process most likely resulting from the accumulation of ions in the liver as a consequence of inhibition of the Na^+-plus-K^+-activated ATPase (Thurman *et al.*, 1979). Inhibition of ethanol and oxygen uptake did not correlate with the inhibition of the sodium pump as judged kinetically from the rapid change in ionic composition of the perfusion fluid. Therefore, it is unlikely that an induction of the sodium pump is involved in the mechanism of the 'adaptive increase' (Thurman *et al.*, 1979). Furthermore, it is also improbable that it could adapt in only 2.5h.

Enhanced ethanol uptake could be largely blocked by an alcohol dehydrogease inhibitor, 4-methylpyrazole (see the Results section). This shows clearly that alcohol dehydrogenase is involved in the increased rate of ethanol elimination observed after 2.5h of treatment with ethanol in spite of the observation that the activity of alcohol dehydrogenase was not changed by ethanol treatment.

The mitochondrial respiratory chain is also implicated in this increase in oxygen uptake, on the basis of sensitivity to an inhibitor of cytochrome oxidase, KCN (Fig. 2). It is well established that the rate of electron flux in the mitochondrial respiratory chain is controlled by the phosphate potential or, simply, by the supply of ADP (Chance & Williams, 1955). Partial inhibition of the increased oxygen uptake by atractyloside, an inhibitor of the adenine nucleotide translocase, suggests that an ATPase of cytosolic origin is responsible for increasing ADP and thereby stimulating oxygen uptake after treatment with ethanol.

Role of glycolysis in the mechanism of increased oxygen uptake caused by treatment with ethanol

Acute treatment with ethanol has been shown to increase hepatic triacylglycerols (Abrams & Cooper, 1976); however, fatty acid oxidation inhibits rather than stimulates ethanol uptake (T. Yuki & R. G. Thurman, unpublished work). Thus it is unlikely that fatty acid oxidation plays a significant role in the mechanism of this phenomenon.

It is well known that ethanol inhibits glycolysis through redox inhibition of glyceraldehyde 3-phosphate dehydrogenase (Fig. 3*a*; Rawat & Lundquist, 1968). Thurman & Scholz (1977) demonstrated that the diminished rate of ATP generation after inhibition of glycolysis by ethanol is responsible for the small (5–10%) increase in hepatic oxygen uptake observed when ethanol is infused into the liver (Fig. 3*a*). However, this phenomenon, unlike the processes described in the present paper, has an absolute requirement for the presence of ethanol in the liver.

In the present studies, livers from animals treated with ethanol were characterized by low glycogen contents and low rates of glycolysis (Fig. 3*b*). Also, the activation of oxygen uptake by ethanol was considerably less (about 50% as large) in livers from starved rats, where glycolysis was absent. The increase in oxygen uptake also was partially reversed by infusion of glucose. Since glycolysis is an ATP-production reaction, the inhibition of glycolysis is equivalent to the stimulation of an ATPase. These data are consistent with the hypothesis that a major factor responsible for the elevation of oxygen uptake after ethanol treatment is diminished rates of glycolysis. However, one important question arises: how much of the stimulation of oxygen uptake can the ethanol-mediated inhibition of glycolysis account for quantitatively? If one calculates the difference in oxygen uptake between control (107 μmol/h per g) and ethanol-treated (192 μmol/h per g) livers by assuming a P/O ratio of 2.5, the ATP produced from glycolysis can account for 20 μmol of oxygen/h per g, or about 23% of the observed increase (85 μmol/h per g). On the other hand, this calculation does not take 'futile cycling' in glycolysis or the small energy demands for enhanced ketogenesis (about 10% of the increase; Table 2) into account. In livers from starved rats, however, the increase in oxygen uptake caused by ethanol treatment was only about 50% as great as in livers from fed rats. Thus the contribution of inhibition of glycolysis to the stimulation of oxygen uptake is between 23 and 50% of the total increase. One must therefore postulate another ATP-ase activity of unknown nature (Scheme 1; X) to account for the rest of the increase in oxygen uptake after treatment with ethanol.

References

Abrams, M. & Cooper, C. (1976) *Biochem. J.* **156**, 33–46

Bergmeyer, H. U. (ed.) (1970) *Methoden der Enzymatischen Analyse*, 2nd edn., Verlag Chemie, Weinheim

Bernstein, J., Videla, L. & Israel, Y. (1973) *Biochem. J.* **134**, 515–522

Bernstein, J., Videla, L. & Israel, Y. (1974) *J. Pharmacol. Exp. Ther.* **192**, 583–591

Chance, B. & Williams, G. R. (1955) *J. Biol. Chem.* **217**, 383–393

Crow, K. E., Cornell, N. & Veech, R. L. (1977) *Alcoholism: Clin. Exp. Res.* **1**, 43–47

DeCarli, L. M. & Lieber, C. S. (1967) *J. Nutr.* **91**, 331–336

Fong, J., Schaffer, F. L. & Kirk, P. L. (1953) *Arch. Biochem.* **45**, 319–326

Gornall, A. G., Bardwill, C. J. & David, M. M. (1949) *J. Biol. Chem.* **177**, 751–766

Goulden, C. H. (1939) *Methods of Statistical Analysis*, John Wiley and Sons, New York

Hawkins, R. & Khanna, J. M. (1966) *Can. J. Physiol. Pharmacol.* **44**, 241–257

Hildebrandt, A., Remmer, H. & Estabrook, R. W. (1968) *Biochem. Biophys. Res. Commun.* **30**, 607–612

Hogeboom, G. H., Schneider, W. C. & Palade, G. E. (1948) *J. Biol. Chem.* **172**, 619–627

Israel, Y., Videla, L., MacDonald, A. & Bernstein, J. (1973) *Biochem. J.* **134**, 523–529

Krebs, H. A. & Henseleit, K. (1932) *Hoppe-Seyler's Z. Physiol. Chem.* **210**, 33–66

Lieber, C. S. & DeCarli, L. M. (1970) *J. Biol. Chem.* **245**, 2505–2512

Mendelson, J. H. & Mello, N.K. (1966) *Ann. N.Y. Acad. Sci.* **133**, 828–845

Porta, E. A., Cesar, L. A. & Gomez-Dumm, S. (1968) *Lab. Invest.* **18**, 352–364

Rawat, A. K. & Lundquist, F. (1968) *Eur. J. Biochem.* **5**, 13–17

Scholz, R. (1968) in *Stoffwechsel der Perfundierten Leber* (Staib, W. & Scholz, R., eds.), pp. 25–47, Springer-Verlag, Berlin, Heidelberg, New York

Scholz, R., Hansen, W. & Thurman, R. G. (1973) *Eur. J. Biochem.* **38**, 64–72

Scholz, R., Braun, W., Kuhn, A. & Zimmer, P. (1976) *Hoppe-Seyler's Z. Physiol. Chem.* **357**, 279–280

Sutherland, E. W., Oye, I. & Butcher, R. W. (1965) *Recent Prog. Horm. Res.* **21**, 623–646

Thurman, R. G. & Scholz, R. (1969) *Eur. J. Biochem.* **10**, 459–467

Thurman, R. G. & Scholz, R. (1976) *Hoppe-Seyler's Z. Physiol. Chem.* **357**, 1443–1446

Thurman, R. G. & Scholz, R. (1977) *Eur. J. Biochem.* **75**, 13–21

Thurman, R. G., McKenna, W. R. & McCaffrey, T. B. (1975) *Mol. Pharmacol.* **12**, 156–166

Thurman, R. G., Yuki, T., Bleyman, M. A. & Wendell, G. (1979) *Drug Alcohol Depend.* **4**, 119–129

Toban, F. & Mezey, E. (1971) *J. Lab. Clin. Med.* **77**, 110–121

Wendell, G. D. & Thurman, R. G. (1979) *Biochem. Pharmacol.* **28**, 273–279

Yuki, T. & Thurman, R. G. (1978) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **37**, 1351