The effect of acute ethanol treatment on rates of oxygen uptake, ethanol oxidation and gluconeogenesis in isolated rat hepatocytes

Kathryn M. STOWELL and Kathryn E. CROW

Department of Chemistry and Biochemistry, Massey University, Palmerston North, New Zealand

(Received 2 January 1985/7 May 1985; accepted 17 May 1985)

1. In hepatocytes isolated from fed rats, acute ethanol pretreatment (at a dose of 5.0 g/kg body wt.) did not change rates of O₂ uptake. 2. In cells from starved animals, acute ethanol pretreatment increased O₂ uptake by 17–29%. 3. The increased O₂ uptake in hepatocytes from starved rats was not accompanied by increased rates of ethanol oxidation, but was accompanied by increased rates of gluconeogenesis under some conditions. 4. The provision of ethanol (10 mM) as a substrate to cells from fed or starved rats decreased O₂ uptake in the absence of other substrates or in the presence of lactate, and increased it in the presence of pyruvate or lactate and pyruvate. 5. The results of this study show that the acute effects of ethanol on liver O₂ uptake are dependent on the physiological state of the liver. Previously reported large (2-fold) increases in O₂ uptake after acute ethanol pretreatment may have been an artefact owing to low control uptake rates ($\sim 1.8 \mu\text{mol/min per g wet wt. of cells}$) in the liver preparation used. 6. The ATP contents ($2.4\text{--}2.6 \mu\text{mol/g wet wt. of cells}$) and rates of O₂ uptake ($2.5\text{--}5.0 \mu\text{mol/min per g wet wt. of cells}$) of cells used in the present study were the same as values reported under conditions close to those *in vivo*. Therefore the increase in O₂ uptake in cells from starved rats after acute ethanol pretreatment is likely to be of physiological significance.

Administration of a single large dose (5.0–6.5 g/kg) of ethanol to rats has been reported to increase the rate of ethanol metabolism in intact animals and perfused liver (Wendell & Thurman, 1979; Bleyman & Thurman, 1979; Thurman *et al.*, 1979). The phenomenon has been named the ‘swift increase in alcohol metabolism’ (SIAM). An accompanying increase in liver O₂ uptake has been observed, and it has been assumed that an increase in the rate of NADH reoxidation explains the increased rates of ethanol oxidation (Yuki & Thurman, 1980). This explanation is difficult to reconcile with reports which show that the activity of alcohol dehydrogenase, rather than the rate of NADH reoxidation, is the major factor controlling the rate of ethanol metabolism in rat liver (Crow *et al.*, 1977, 1982, 1983a; Cornell, 1983). In order to clarify the situation, we attempted to reproduce SIAM in intact animals, but could not do so (Braggins & Crow, 1981). Our approach was criticised by the proponents of SIAM (Thurman *et al.*, 1982, 1983), and a considerable amount of new work supporting the occurrence of SIAM has been

published since our first study (Yuki *et al.*, 1982; Thurman *et al.*, 1982, 1983, 1984). The present study was initiated to investigate further the effects of large acute ethanol doses on rates of ethanol metabolism and O₂ uptake in rat liver. We chose to use isolated hepatocytes rather than perfused livers because of the increased versatility in terms of experimental conditions that is allowed by this preparation.

Materials and methods

Rats

Male Sprague–Dawley rats (180–250 g) were obtained from the Massey University Small Animal Production Unit. The animals were maintained on a standard pellet diet, supplied *ad libitum*. Starved animals were deprived of food for 48 h. Ethanol-pretreated rats were given a single dose of 25% (v/v) ethanol via gastric intubation 2.5 h before surgery. The volume of ethanol was adjusted according to the weight of each animal to give a dosage of 5.0 g of ethanol/kg body wt. of rat.

Control rats were untreated unless otherwise specified.

Reagents

Collagenase was obtained from Worthington Biochemical Corp. (Freehold, NJ, U.S.A.). All other enzymes and cofactors were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Ethanol (spectroscopic grade; 96%, v/v) was obtained from Fluka A.G. (Buchs, Switzerland). Other chemicals were reagent-grade products.

Preparation and incubation of hepatocytes

Isolated hepatocytes were prepared by the method of Berry & Friend (1969) as modified by Cornell *et al.* (1973). All hepatocyte preparations were washed and incubated as described previously (Crow *et al.*, 1978). Cell viability was tested by measuring Trypan Blue exclusion and ATP content. Preparations showing less than 95% viability and/or ATP content less than $2.0 \mu\text{mol/g}$ wet wt. of cells were discarded.

Analytical

Hepatocytes were incubated in triplicate, and O_2 consumption was monitored as described previously (Crow *et al.*, 1978). The O_2 electrode was calibrated regularly with air-saturated water, by using sodium dithionite to set the zero point. The calibration was checked with xanthine oxidase (Billiar *et al.*, 1970). The two methods agreed to within 6%. Ethanol (Cornell & Veech, 1983), glucose (Bergmeyer *et al.*, 1974) and ATP (Lamprecht & Trautschold, 1974; Lund *et al.*, 1975) were assayed by published procedures.

Results and discussion

ATP content

Hepatocyte ATP content was measured in each experiment, both as an index of cell viability and to

determine the effects of ethanol pretreatment on this parameter. The ATP contents obtained were similar to ATP contents reported for freeze-clamped rat liver (Krebs *et al.*, 1974) and those reported in other studies using isolated hepatocytes (Krebs *et al.*, 1974; Crow *et al.*, 1978). Pretreatment with a large acute ethanol dose did not alter the ATP content in cells from either fed (control, 2.45 ± 0.08 ; ethanol, 2.61 ± 0.09) or starved (control, 2.57 ± 0.07 ; ethanol, 2.54 ± 0.08) animals. The ATP contents are expressed as $\mu\text{mol/g}$ wet wt. of cells, and represent means $\pm$ S.E.M. for 16 determinations on cells from four fed animals or 106 determinations on cells from nine starved animals.

Effect of acute ethanol pretreatment on O_2 uptake in cells from fed rats

In a previous study (Yuki & Thurman, 1980), the greatest effect of acute ethanol pretreatment on O_2 uptake was found in perfused livers from fed rats, where close to a 2-fold increase in rate occurred (control O_2 uptake, $1.87 \mu\text{mol/min per g}$; ethanol-pretreated, $3.2 \mu\text{mol/min per g}$). Accordingly, in initial investigations, hepatocytes from fed animals were used. In contrast with the results of Yuki & Thurman (1980), no significant increase in O_2 uptake was found after acute ethanol pretreatment under any of the incubation conditions used (Table 1). With cells incubated in the absence of substrates, a condition corresponding to that used in the previous perfusion studies (Yuki & Thurman, 1980), acute ethanol pretreatment actually appeared to give a decrease in O_2 uptake (Table 1, top line), but the change was not statistically significant ($P > 0.05$, Student's *t* test).

The rates of O_2 uptake measured by Yuki & Thurman (1980) in livers from untreated rats were very low ($1.87 \mu\text{mol/min per g}$) compared with those from other studies using perfused liver from fed rats [$2.4 \mu\text{mol/min per g}$ (Schaffer *et al.*, 1980;

Table 1. Effect of ethanol pretreatment on rates of O_2 uptake in cells from fed rats

Rates of O_2 uptake were measured as described in the Materials and methods section. Concentrations of substrates were: ethanol, 10 mM; lactate, 10 mM; pyruvate, 5 mM. Results are means $\pm$ S.E.M. for four experiments. Within each experiment, all determinations were carried out in triplicate. Ethanol-pretreated animals received a dose of 5.0 g of ethanol/kg by gastric intubation 2.5 h before surgery.

Substrates added	Rates of O_2 uptake ($\mu\text{mol/min per g}$ wet wt. of cells)	
	Untreated	Ethanol-pretreated
None	3.23 ± 0.29	2.75 ± 0.16
Ethanol	2.81 ± 0.20	2.76 ± 0.11
Lactate	3.86 ± 0.24	3.49 ± 0.16
Lactate, ethanol	3.31 ± 0.22	3.51 ± 0.21
Pyruvate	3.15 ± 0.22	3.17 ± 0.21
Pyruvate, ethanol	3.93 ± 0.39	3.71 ± 0.10
Lactate, pyruvate	3.07 ± 0.17	3.06 ± 0.20
Lactate, pyruvate, ethanol	3.87 ± 0.32	3.80 ± 0.19

Damgaard *et al.*, 1974); $2.8 \mu\text{mol/min per g}$ (Kondrup *et al.*, 1979)] or with rates obtained with isolated hepatocytes in the present study ($3.2 \mu\text{mol/min per g}$). It is therefore possible that the observation of a swift increase in O_2 consumption may depend on having a low initial O_2 consumption in the liver preparation used. Even the higher O_2 uptakes observed in other perfusion studies and in our isolated hepatocytes from fed rats are probably less than the rate of liver O_2 consumption *in vivo* (Folke, 1974). Thus the effect observed by Yuki & Thurman (1980) in livers from fed rats is probably not relevant to physiological conditions.

Effect of ethanol as a substrate in cells from fed rats

The data in Table 1 also illustrate the effects of ethanol as a substrate on rates of O_2 uptake by hepatocytes from fed rats. In cells from untreated animals ('Untreated' column, Table 1), with no added substrates, ethanol decreased O_2 uptake. This contrasts with the increase in O_2 uptake observed in other studies with ethanol addition to liver from fed rats perfused with basal medium (Scholz *et al.*, 1974; Thurman & Scholz, 1977; Yuki & Thurman, 1980; Yuki *et al.*, 1980, 1982). Again, these studies all had low basal rates of liver O_2 consumption. In another study showing higher basal rates of O_2 uptake by perfused liver from fed rats (Damgaard *et al.*, 1974), addition of ethanol as a substrate caused a decrease in O_2 consumption similar to that seen in the present experiments.

In cells from untreated animals, ethanol addition decreased O_2 uptake in the presence of lactate, but gave marked stimulation in the presence of pyruvate or lactate and pyruvate (Table 1). The changes in O_2 uptake produced by ethanol addition were all statistically significant ($P < 0.05$, paired *t* test).

Decreases in O_2 uptake with ethanol as a substrate were not observed in the absence of other substrates or in the presence of lactate after acute ethanol pretreatment. However, acute ethanol pretreatment did not affect the increase in O_2 uptake occurring with ethanol addition in the presence of pyruvate ($P < 0.005$) or lactate and pyruvate ($P < 0.0005$, paired *t* test).

From their observation of an increase in O_2 uptake with ethanol as a substrate, Thurman & Scholz (1977) proposed that the effects of ethanol on O_2 uptake in livers from fed rats arose from the inhibition of glycolysis. The present results show, however, that the effects of ethanol as a substrate on O_2 uptake in hepatocytes from fed rats depend on the other substrates present, and cannot be explained simply by inhibition of glycolysis. Inhibition of glycolysis should produce an increase in O_2 consumption, whereas in the present study ethanol decreased O_2 consumption both in the

absence of other substrates and in the presence of lactate.

To assess the net effect of ethanol on O_2 consumption under physiological conditions, a medium containing metabolite concentrations as close as possible to those *in vivo* would be needed. However, since the main purpose of this study was to attempt to reproduce the increase in O_2 uptake seen with large acute ethanol doses, the experiments with cells from fed animals were not pursued further.

Effect of ethanol pretreatment on O_2 uptake in cells from starved rats

In hepatocytes isolated from starved animals, pretreatment with a large acute ethanol dose led to an increase in O_2 uptake under all incubation conditions (Table 2a). The increase was small (17–29%), but highly significant ($P < 0.0005$; paired *t* test). In the experiments giving the results in Table 2(a), control animals received no pretreatment at all. The stress of the ethanol-intubation process or the ether anaesthetic, rather than the ethanol, may have produced the increased O_2 uptake. However, in a further set of experiments, O_2 uptakes were compared in untreated controls and animals anaesthetized and intubated with an equivalent volume of water. The results of these experiments are shown in Table 2(b). Anaesthesia followed by water intubation as a pretreatment resulted in the same or slightly lower O_2 uptakes as those observed in untreated controls. Again, ethanol pretreatment resulted in an increase in O_2 uptake of a similar order to that seen in the first experiment.

In experiments using perfused liver from starved animals, Yuki & Thurman (1980) found a 38% increase in O_2 uptake after acute ethanol pretreatment. The O_2 uptakes in livers from untreated animals in the perfusion study were lower than those in other studies using perfused livers from starved rats (Krebs *et al.*, 1974) and lower than those in hepatocytes from the present study. The greater percentage increase in O_2 uptake after ethanol pretreatment in the study by Yuki & Thurman (1980) could perhaps be due to the low O_2 uptakes in their control preparations. However, the results of the present study confirm that a swift increase in O_2 consumption does still occur in cells with high control rates of O_2 consumption, and this indicates that the effect may be of significance *in vivo*.

The O_2 uptakes measured in cells from starved rats in this study agree well with those reported in several other studies (Table 3). The rates obtained in the presence of substrates are similar to those obtained in perfusions closely simulating physiological conditions (Folke, 1974). Therefore, the

Table 2. *Effect of ethanol pretreatment (a) or water or ethanol pretreatment (b) on rates of O₂ uptake in cells from starved rats*
 Rats were starved for 48 h before experiments. Rates of O₂ uptake were determined as described in the Materials and methods section. Concentrations of substrates were: ethanol, 10 mM; lactate, 10 mM; pyruvate, 5 mM. Results are means $\pm$ S.E.M. for four experiments. Within each experiment all determinations were carried out in triplicate. Ethanol-pretreated animals received a dose of 5.0 g of ethanol/kg by gastric intubation 2.5 h before surgery. In (b) water-pretreated animals were anaesthetized and intubated with water 2.5 h before surgery. In (a), the differences between O₂ uptakes in cells from pretreated and untreated rats with each individual substrate combination were significant at a low level ($P < 0.05$, Student's *t* test). The overall difference between pretreated and untreated rats was highly significant ($P < 0.0005$) by a paired *t* test. In (b), the differences between O₂ uptakes in cells from ethanol-pretreated animals and either untreated or water-pretreated controls with each individual substrate combination were significant as follows: * $P < 0.01$; † $P < 0.05$; ‡ $P < 0.1$; § not significant. The overall differences in rates of O₂ uptake between untreated and ethanol-pretreated ($P < 0.005$) or water-pretreated and ethanol-pretreated ($P < 0.0005$) rats were highly significant by a paired *t* test.

Substrates added	Rates of O ₂ uptake (μ mol/min per g wet wt. of cells)				
	(a)		(b)		
	Untreated	Ethanol-pretreated	Untreated	Water-pretreated	Ethanol-pretreated
None	2.75 $\pm$ 0.24	3.34 $\pm$ 0.15	2.96 $\pm$ 0.14†	2.51 $\pm$ 0.04†	3.55 $\pm$ 0.18
Ethanol	2.57 $\pm$ 0.16	3.02 $\pm$ 0.15	2.77 $\pm$ 0.33‡	2.41 $\pm$ 0.05†	3.30 $\pm$ 0.16
Lactate	4.24 $\pm$ 0.25	5.42 $\pm$ 0.45	4.25 $\pm$ 0.45†	4.15 $\pm$ 0.15†	5.70 $\pm$ 0.47
Lactate, ethanol	3.80 $\pm$ 0.25	4.89 $\pm$ 0.41	3.74 $\pm$ 0.32‡	3.38 $\pm$ 0.11†	4.45 $\pm$ 0.30
Pyruvate	3.78 $\pm$ 0.24	4.57 $\pm$ 0.16	3.62 $\pm$ 0.31‡	3.53 $\pm$ 0.10‡	4.33 $\pm$ 0.38
Pyruvate, ethanol	4.98 $\pm$ 0.28	6.00 $\pm$ 0.35	4.30 $\pm$ 0.36†	4.40 $\pm$ 0.24‡	5.63 $\pm$ 0.43
Lactate, pyruvate	3.86 $\pm$ 0.28	4.97 $\pm$ 0.33	4.13 $\pm$ 0.24§	3.64 $\pm$ 0.12‡	4.46 $\pm$ 0.42
Lactate, pyruvate, ethanol	5.04 $\pm$ 0.15	6.00 $\pm$ 0.36	4.84 $\pm$ 0.27*	4.30 $\pm$ 0.40*	6.20 $\pm$ 0.15

Table 3. *O₂ uptakes in isolated hepatocytes from starved rats*

Reference	Conditions	Substrates	O ₂ uptake (μ mol/min per g wet wt.)	Lactate stimulation (%)
Krebs <i>et al.</i> (1974)	48 h starved	None	2.44	51
		Lactate	3.70	
Crow <i>et al.</i> (1978)	48 h starved	None	2.3–2.5	47
		Lactate	3.38–3.67	
Van Der Meer <i>et al.</i> (1976)	24 h starved (perifusion)	None	3.35	38
		Lactate	4.62	
Berry <i>et al.</i> (1983)	24 h starved	None	2.62	37
		Lactate	3.59	
		Ethanol	2.46	
		Lactate, ethanol	3.92	
This study (Table 2a)	48 h starved	None	2.75	54
		Lactate	4.24	
		Ethanol	2.57	
		Lactate, ethanol	3.80	

increase in O₂ uptake observed in the present study in cells from starved animals after acute ethanol pretreatment is not likely to be merely an artefact of the liver cell preparation. Table 3 indicates not only similarity in absolute rates of O₂ uptake in hepatocyte studies from a range of other laboratories, but also a remarkable consistency in the degree of stimulation of O₂ uptake caused by the addition of 10 mM-lactate. This consistency suggests that isolated hepatocytes are a good prepara-

tion in which to study factors affecting rates of O₂ uptake.

Effect of ethanol as a substrate on O₂ uptake in cells from starved rats

Addition of ethanol as a substrate to perfused liver from starved animals has been claimed to decrease the rate of O₂ uptake (Scholz & Nohl, 1976; Thurman & Scholz, 1977). However, the results in Table 2 show that in hepatocytes ethanol

can either increase or decrease rates of O_2 uptake, depending on the accompanying substrates. All the changes in O_2 uptake produced by ethanol were statistically significant ($P < 0.025$, paired t test), except for that with ethanol alone. The effects observed correlate with the effects of ethanol on gluconeogenesis (Crow *et al.*, 1978): an inhibition in the presence of lactate and stimulation in the presence of pyruvate. The present results differ from a report by Berry *et al.* (1983) in which ethanol added to hepatocytes in the presence of lactate gave a slight increase in O_2 consumption. The reason for this difference is not apparent, although the cells used by Berry *et al.* (1983) were isolated from animals starved for only 24 h. This factor may be significant, since a difference in the degree of lactate stimulation of O_2 uptake in 24-h and 48 h-starved animals is indicated by the results in Table 3. Acute ethanol pretreatment did not alter the pattern of response to ethanol as a substrate (Table 2).

Effect of acute ethanol pretreatment on rates of ethanol oxidation

Since O_2 uptake was increased by acute ethanol pretreatment in cells from starved animals, the possibility of an accompanying increase in the rate of ethanol metabolism was investigated. Rates of ethanol metabolism were measured in unsupplemented cells or in the presence of lactate and/or pyruvate. As previously demonstrated (Crow *et al.*, 1978; Cornell *et al.*, 1979; Crow *et al.*, 1983b), the addition of these substrates increased rates of ethanol metabolism by hepatocytes to rates similar to those observed in intact rats.

The results in Fig. 1 show that rates of ethanol metabolism were not increased by ethanol pretreatment. Thus the increase in O_2 uptake with ethanol pretreatment did not lead to increased rates of ethanol metabolism. This finding does not support the conclusion that rates of ethanol metabolism are closely linked to the rates of respiration, a conclusion based on results of studies in which close correlations between O_2 uptakes and rates of ethanol metabolism were observed (Yuki & Thurman, 1980; Thurman *et al.*, 1976).

The reason for the difference between results of the present study and those of others (Bleyman & Thurman, 1979; Thurman *et al.*, 1979; Wendell & Thurman, 1979), in which increased rates of ethanol oxidation have been observed after acute ethanol pretreatment, is not apparent. The present results agree with our previous findings in live animals (Braggins & Crow, 1981), but these findings have been criticised on the basis that we did not consider the need to select SIAM-type rats (Thurman *et al.*, 1982, 1983). It is possible that the use of genetically different animals incapable of

Fig. 1. Effect of acute ethanol pretreatment on rates of ethanol oxidation

Rats were starved for 48 h before experiments. Hepatocytes were prepared and incubated, and ethanol was assayed, as described in the Materials and methods section. Animals pretreated with ethanol received a dose of 5.0 g/kg by stomach tube 2.5 h before cell preparation. All incubations contained 10 mM-ethanol and other substrates as indicated by the labels on the Figure. Results are means of five (control, ●, ▲) or six (ethanol-pretreated, ○, △) experiments, with bars indicating S.E.M.

producing a SIAM response explains our failure to observe the effect. However, we were using the same strain of animals as those specified by Yuki &

Thurman (1980), and those authors used no apparent selection of rats in their studies on O_2 consumption in which a mechanism to explain the SIAM response was proposed. Control rates of ethanol oxidation in perfusion studies by Thurman and others (Yuki *et al.*, 1982; Yuki & Thurman, 1980) have been much lower (1.1 – $1.4 \mu\text{mol/min per g}$) than physiological rates (2.5 – $4.0 \mu\text{mol/min per g}$; Cornell, 1983; Braggins & Crow, 1981), and it is possible that the SIAM effect in perfused livers could be an artefact owing to these low control rates. This possibility is not supported, however, by the observation that low rates of ethanol metabolism in unsupplemented cells in the present study were not affected by ethanol pretreatment.

Effects of acute ethanol pretreatment on rates of gluconeogenesis

Since rates of O_2 uptake paralleled previously reported changes in rates of gluconeogenesis in cells from starved rats, the possibility that increased O_2 uptake with ethanol pretreatment was due to increased rates of gluconeogenesis was examined. The rates of glucose synthesis observed in the present study were similar to other published values for both isolated hepatocytes (Krebs *et al.*, 1974; Crow *et al.*, 1978) and perfused liver (Krebs *et al.*, 1974). Rates calculated over a 60 min interval in control cells were: lactate + ethanol, 0.50 ± 0.07 ; pyruvate + ethanol, 0.93 ± 0.05 ; lactate + pyruvate + ethanol, 1.11 ± 0.06 (results are expressed as $\mu\text{mol/min per g wet wt. of cells}$, and are means $\pm$ S.E.M. for five experiments.)

Fig. 2 shows that glucose synthesis was increased under some conditions in cells from ethanol-pretreated animals. The time course of the increase suggests that this is an effect, rather than a cause, of the increased O_2 uptake. O_2 uptakes were measured after 12 min of incubation, whereas glucose measurements were made after 10, 20, 40 and 60 min incubation. At 10 and 20 min, no significant increase in glucose synthesis was found under any conditions in cells from ethanol-pretreated animals. A significant increase in the amount of glucose formed occurred after incubation for 40 and 60 min in the presence of lactate and pyruvate. This suggests that the increase in O_2 uptake, observable at 12–15 min, is the primary event, and may lead to increased rates of gluconeogenesis in the presence of suitable substrates. In cells incubated in the absence of substrates other than ethanol, rates of glucose synthesis were very low and identical in ethanol-treated and -untreated animals, whereas O_2 uptakes were higher in cells from the ethanol-pretreated animals. This also suggests that increased O_2 consumption is the primary event.

Other published evidence supports the sugges-

Fig. 2. *Effect of acute ethanol pretreatment on rates of gluconeogenesis*

Rats were starved 48 h before experiments. Hepatocytes were prepared and incubated, and glucose was assayed, as described in the Materials and methods section. Animals pretreated with ethanol received a dose of 5.0 g/kg by stomach tube 2.5 h before cell preparation. All incubations contained 10 mM-ethanol and other substrates as indicated by the labels on the Figure. Results are means of five (control, ●, ▲) or six (ethanol-pretreated, ○, △) experiments, with bars indicating S.E.M.

tion that changes in the rate of respiration as measured by O_2 uptake can lead to changes in the amount of glucose produced in hepatocytes from starved rats incubated with lactate and pyruvate. Groen *et al.* (1982) have demonstrated that small decreases in O_2 uptake caused by inhibition of mitochondrial respiration by atractyloside are closely paralleled by decreases in glucose production.

Conclusion

Pretreatment of rats with a large acute dose of ethanol increases the rate of O_2 consumption in hepatocytes isolated from starved, but not from fed, animals. The increased O_2 uptake does not

lead to increased rates of ethanol oxidation, but may be the cause of increased rates of gluconeogenesis. Considering the apparent complexity of control of mitochondrial respiration (Tager *et al.*, 1983; Wanders *et al.*, 1984), a detailed analysis of which potential control steps are affected by acute ethanol pretreatment should probably be left until the analysis of control of respiration in untreated liver has progressed further.

The results of this study make it apparent that the effects of ethanol on O_2 uptake by liver depend entirely on the initial state of the liver. Therefore, caution should be exercised in extrapolating results of experiments in perfused liver or isolated hepatocytes to the situation *in vivo*. In using perfused livers with initial O_2 uptakes lower than *in vivo*, Thurman and others (Thurman & Scholz, 1977; Yuki & Thurman, 1980; Yuki *et al.*, 1980) were probably studying a respiration pathway with different rate-determining steps from those operating *in vivo*. The lower than normal minimal O_2 uptakes observed in the latter studies could be due to poor quality of the liver preparations, since it appears that ATP contents were not measured to give an indication of liver viability during perfusion. Models for quantitative analysis of control of metabolic pathways make it clear that large changes in flux are usually accompanied by changes in the relative degree of control exerted by individual steps in the pathway (Kacser & Burns, 1979). The higher O_2 uptakes observed in the present study, particularly in the presence of added substrates, make it more likely that the rate-determining steps for the respiratory chain are the same as those *in vivo*, and therefore that the effects of ethanol on rates of O_2 uptake represent effects likely to occur *in vivo*.

Yuki & Thurman (1980) have suggested that the increased oxygen uptake observed with acute ethanol pretreatment occurs by the same mechanism as that which causes increased O_2 uptake after chronic ethanol pretreatment. However, the observation of increased O_2 uptake after chronic ethanol pretreatment has not been consistent (Schaffer *et al.*, 1980; Kondrup *et al.*, 1979). In view of this, and of the results of the present study, the effects of chronic ethanol pretreatment on O_2 uptake should be re-examined.

This work was supported by the Medical Research Council of New Zealand. We thank Dr. M. J. Hardman for helpful discussion and Dr. R. J. Brook for assistance with statistical analyses.

References

Bergmeyer, H. U., Bernt, E., Schmidt, F. & Stork, H. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U. & Gawehn, K., eds.), vol. 3, pp. 1196–1201, Academic Press, New York and London

- Berry, M. N. & Friend, D. S. (1969) *J. Cell Biol.* **43**, 506–520
- Berry, M. N., Clark, D. G., Grivell, A. R. & Wallace, P. G. (1983) *Eur. J. Biochem.* **131**, 205–214
- Billiar, R. B., Knappenberger, M. & Little, B. (1970) *Anal. Biochem.* **36**, 101–104
- Bleyman, M. A. & Thurman, R. G. (1979) *Biochem. Pharmacol.* **28**, 2027–2030
- Braggins, T. J. & Crow, K. E. (1981) *Eur. J. Biochem.* **119**, 633–640
- Cornell, N. W. (1983) *Pharmacol. Biochem. Behav.* **18**, Suppl. 1, 215–221
- Cornell, N. W. & Veech, R. L. (1983) *Anal. Biochem.* **132**, 418–423
- Cornell, N. W., Lund, P., Hems, R. & Krebs, H. A. (1973) *Biochem. J.* **134**, 671–672
- Cornell, N. W., Crow, K. E., Leadbetter, M. G. & Veech, R. L. (1979) in *Alcohol and Nutrition* (Li, T.-K., Schenker, S. & Lumeng, L., eds.), pp. 315–330, U.S. Government Printing Office, Washington DC
- Crow, K. E., Cornell, N. W. & Veech, R. L. (1977) in *Alcohol and Aldehyde Metabolising Systems* (Thurman, R. G., Williamson, J. R., Drott, H. & Chance, B., eds.), vol. 3, pp. 335–342, Academic Press, New York
- Crow, K. E., Cornell, N. W. & Veech, R. L. (1978) *Biochem. J.* **172**, 29–36
- Crow, K. E., Braggins, T. J., Batt, R. D. & Harman, M. J. (1982) *J. Biol. Chem.* **257**, 14217–14225
- Crow, K. E., Braggins, T. J., Batt, R. D. & Hardman, M. J. (1983a) *Pharmacol. Biochem. Behav.* **18**, Suppl. 1, 233–236
- Crow, K. E., Newland, K. M. & Batt, R. D. (1983b) *Pharmacol. Biochem. Behav.* **18**, Suppl. 1, 237–240
- Damgaard, S. E., Sestoft, L. & Lundquist, F. (1974) in *Regulation of Hepatic Metabolism* (Lundquist, F. & Tygstrup, N., eds.), pp. 530–540, Academic Press, New York
- Folke, M. (1974) in *Regulation of Hepatic Metabolism* (Lundquist, F. & Tygstrup, N., eds.), pp. 596–606, Academic Press, New York
- Groen, A. K., Van der Meer, R., Westerhoff, H. V., Wanders, R. J. A., Akerboom, T. P. M. & Tager, J. M. (1982) in *Metabolic Compartmentation* (Sies, H., ed.), pp. 9–37, Academic Press, London
- Kacser, H. & Burns, J. A. (1979) *Biochem. Soc. Trans.* **7**, 1149–1160
- Kondrup, J., Lundquist, F. & Damgaard, S. E. (1979) *Biochem. J.* **184**, 89–95
- Krebs, H. A., Cornell, N. W., Lund, P. & Hems, R. (1974) in *Regulation of Hepatic Metabolism* (Lundquist, F. & Tygstrup, N., eds.), pp. 726–750, Academic Press, New York
- Lamprecht, W. & Trautschold, I. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U. & Gawehn, K., eds.), vol. 4, 2101–2110, Academic Press, New York and London
- Lund, P., Cornell, N. W. & Krebs, H. A. (1975) *Biochem. J.* **152**, 593–599
- Schaffer, W. T., Denckla, W. D. & Veech, R. L. (1980) in *Alcohol and Aldehyde Metabolising Systems* (Thurman, R. G., ed.), vol. 4, pp. 587–593, Plenum Press, New York and London
- Scholz, R. & Nohl, H. (1976) *Eur. J. Biochem.* **63**, 449–458

- Scholz, R., Kalstein, A., Schwabe, U. & Thurman, R. G. (1974) in *Alcohol and Aldehyde Metabolising Systems* (Thurman, R. G., Yonetani, T., Williamson, J. R. & Chance, B., eds.), pp. 315–328, Academic Press, New York and London
- Tager, J. M., Groen, A. K., Wanders, R. J. A., Duszynski, J., Westerhoff, H. V. & Vervoorn, R. G. (1983) *Biochem. Soc. Trans.* **11**, 40–43
- Thurman, R. G. & Scholz, R. (1977) *Eur. J. Biochem.* **75**, 13–21
- Thurman, R. G., McKenna, W. R. & McCaffrey, T. B. (1976) *Mol. Pharmacol.* **12**, 156–166
- Thurman, R. G., Yuki, T., Bleyman, M. A. & Wendell, G. (1979) *Drug Alcohol Depend.* **4**, 119–129
- Thurman, R. G., Paschal, D., Abu-Murad, C., Pekkanen, L., Bradford, B. U., Bullock, K. & Glassman, E. (1982) *J. Pharmacol. Exp. Ther.* **223**, 45–49
- Thurman, R. G., Bradford, B. U. & Glassman, E. (1983) *Pharmacol. Biochem. Behav.* **18**, Suppl. 1, 171–175
- Thurman, R. G., McLaughlin, M. G., Glassman, E. & Felder, M. (1984) *Alcohol. Clin. Exp. Res.* **8**, 124
- Van Der Meer, R., De Haan, E. J. & Tager, J. M. (1976) in *Use of Isolated Liver Cells and Kidney Tubules in Metabolic Studies* (Tager, J. M., Söling, H. D. & Williamson, J. R., eds.), pp. 159–164, North-Holland Publishing Co., Amsterdam
- Wanders, R. J. A., Groen, A. K., Van Roermund, C. W. T. & Tager, J. M. (1984) *Eur. J. Biochem.* **142**, 417–424
- Wendell, G. D. & Thurman, R. G. (1979) *Biochem. Pharmacol.* **28**, 273–279
- Yuki, T. & Thurman, R. G. (1980) *Biochem. J.* **186**, 119–126
- Yuki, T., Thurman, R. G., Schwabe, U. & Scholz, R. (1980) *Biochem. J.* **186**, 997–1000
- Yuki, T., Israel, Y. & Thurman, R. G. (1982) *Biochem. Pharmacol.* **31**, 2403–2407