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REGULATION OF NADPH-DEPENDENT MIXED-FUNCTION OXIDATION IN PERFUSED LIVERS

COMPARATIVE STUDIES WITH SORBITOL AND ETHANOL*

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Abstract—Sorbitol and ethanol were shown to have opposite effects on *p*-nitroanisole *O*-demethylation in perfused livers from fasted, phenobarbital-treated rats. Sorbitol (2 mM) stimulated drug metabolism by 50% while ethanol (20 mM) caused 80% inhibition. Both sorbitol and ethanol infusion decreased the NAD^+/NADH ratio and increased fluorescence of pyridine nucleotides monitored from the liver surface; however, the $NADP^+$/NADPH ratio was decreased by sorbitol but tended to be increased by ethanol. Stimulation of drug metabolism by sorbitol was abolished by pretreatment of fasted rats with 6-aminonicotinamide, an inhibitor of the pentose phosphate shunt, but was not affected by aminooxyacetate, a transaminase inhibitor. These results indicate that sorbitol stimulated *p*-nitroanisole metabolism by providing NADPH via the pentose phosphate shunt. Ethanol and sorbitol caused changes in intracellular concentrations of NADPH in livers from fasted rats which correlated directly with changes in hepatic levels of citrate and aspartate. Furthermore, aspartate infusion reduced the inhibition of *p*-nitroanisole *O*-demethylation by ethanol. This inhibition was also reversed partially by sorbitol in livers from 6-aminonicotinamide-treated rats. It is concluded that ethanol inhibits mixed-function oxidation primarily by decreasing the concentrations of citric acid cycle intermediates which leads to depletion of cytosolic NADPH.

The role of the mixed-function oxidases in the biotransformation of drugs, toxins and carcinogens has stimulated considerable interest in the regulation of this enzyme system. Regulation of mixed-function oxidation in intact cells involves concentrations and catalytic properties of the various components of the microsomal electron transport chain as well as the supply of reduced cofactor [1]. It is well known that chronic exposure of experimental animals to a variety of chemicals [2], as well as changes in hormonal or nutritional states [3, 4] can affect the activity of the mixed-function oxidases and alter the content of cytochrome P-450(s). Further, addition of various carbohydrates to perfused livers from fasted rats affects rates of mixed-function oxidation by changing the intracellular supply of NADPH [1].

The complex relationships between the supply of NADPH and mixed-function oxidation have been studied in intact cells. For example, in the fasted state, ethanol inhibits *p*-nitroanisole *O*-demethylation [5] whereas sorbitol stimulates this reaction [6]. Therefore, studies with sorbitol and ethanol were undertaken to define pathways involved in the supply of reducing equivalents for mixed-function oxidation. The present study compares the effects of sorbitol and ethanol on levels of pyridine nucleotides and intermediates involved in the movement of reducing equivalents from the mitochondrial space into the cytosol. The data indicate that sorbitol increases NADPH supply via the pentose phosphate shunt whereas ethanol inhibits the movement of reducing equivalents of mitochondrial origin into the cytosol.

MATERIALS AND METHODS

Animals. Female Sprague–Dawley rats, 100–200 g, received sodium phenobarbital (1 mg/ml) in drinking water for 2 weeks prior to perfusion experiments to induce the microsomal mixed-function oxidases [7]. Fasted animals were deprived of food for 24 hr prior to use.

Liver perfusion. Details of the perfusion technique have been described eslewhere [8]. Livers were perfused at 37° with Krebs–Henseleit bicarbonate buffer, pH 7.4, saturated with an oxygen–carbon dioxide mixture (95:5) in a non-recirculating system. The fluid was pumped via a cannula placed in the vena cava past a Teflon-shielded oxygen electrode before being discarded. *p*-Nitroanisole (0.2 mM) was dissolved in Krebs–Henseleit bicarbonate buffer and the continuous formation of *p*-nitrophenolate ion was monitored spectrally as described previously [8].

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Under these conditions, the formation of 4-nitrocatechol was minimal. Ethanol, sorbitol and other compounds were dissolved in the buffer and infused into the perfusion fluid entering the liver at final concentrations indicated in the text and figure legends.

Where indicated, conjugates of *p*-nitrophenol were measured by incubating 1.0 ml of perfusate with 25 units of sulfatase and 275 units of β-glucuronidase (Sigma) for 90 min at room temperature. This procedure hydrolyzed greater than 95% of all glucuronide and sulfate conjugates of *p*-nitrophenol.

Surface fluorescence of pyridine nucleotides and flavoproteins. The pyridine and flavin nucleotide redox states of tissues were monitored noninvasively employing surface fluorescence [9]. Certain oxidized flavoproteins fluoresce at 520 nm when excited at 460 nm, while reduced pyridine nucleotide fluorescence is excited at 366 nm and monitored at 450 nm. Changes in flavoprotein fluorescence reflect predominantly changes in the mitochondrial oxidation-reduction state, while pyridine nucleotide fluorescence indicates changes in both mitochondrial and extramitochondrial NAD^+ and $NADP^+$ coenzyme systems [9]. With this technique, the liver is illuminated alternately with pulses of 366 and 460 nm light. The emitted fluorescence is detected by the photomultiplier after passing through secondary filters having transmission maxima at 450 and 520 nm, respectively. The mechanical and electronic details of the double fluorometer are described eslewhere [10].

Metabolite measurement. Perfused livers were freeze-clamped with tongs chilled in liquid nitrogen. Pyridine nucleotides were measured in alkaline extracts of liver by enzymatic cycling techniques [11]. Intermediates of the citric acid cycle were measured in $HClO_4$ extracts of liver by methods described elsewhere [12]. Lactate and glucose in perfusate were measured enzymatically [13].

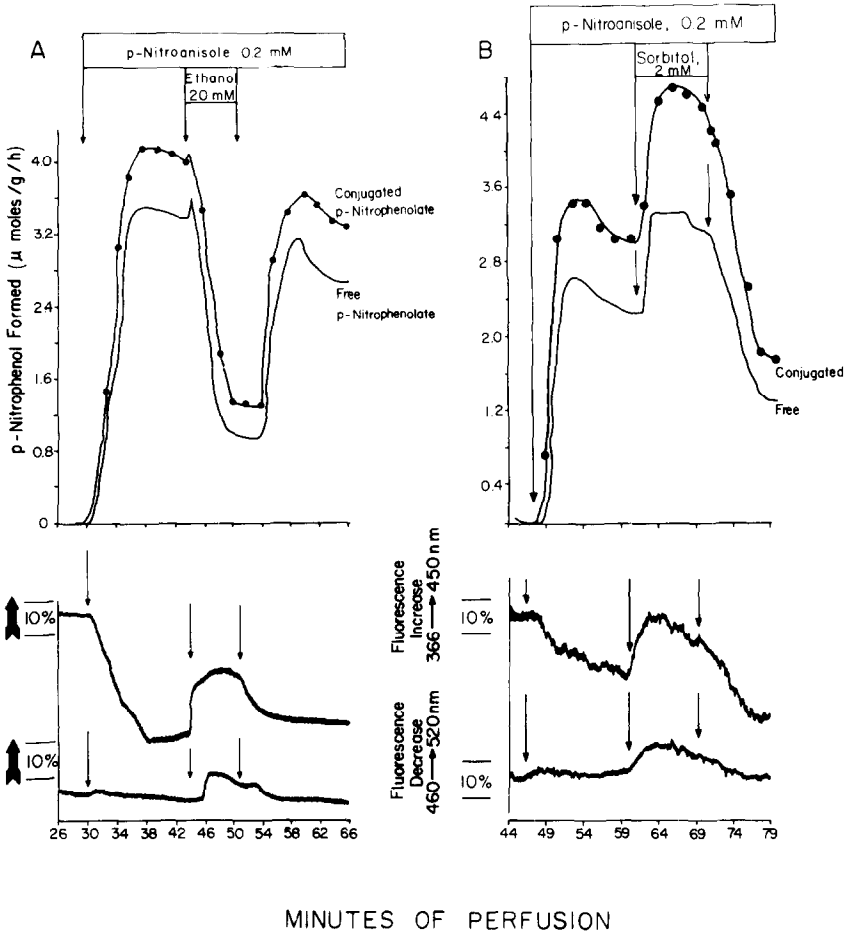

Fig. 1. Effects of ethanol (A) and sorbitol (B) on *p*-nitrophenolate production from *p*-nitroanisole and on pyridine nucleotide and flavoprotein fluorescence of perfused livers from fasted, phenobarbital-treated rats. Conjugates of *p*-nitrophenol were hydrolyzed as described in Materials and Methods. Introduction of *p*-nitroanisole (0.2 mM), ethanol (20 mM) or sorbitol (2 mM) is designated by the horizontal bars and vertical arrows. Fluorescence increase (366 → 450 nm) represents reduction in pyridine nucleotide oxidation–reduction state, whereas fluorescence decrease (460 → 520 nm) represents flavoprotein reduction.

RESULTS

Effects of ethanol and sorbitol on p-*nitroanisole* O-*demethylation and surface fluorescence in perfused rat livers.* The effects of ethanol and sorbitol on the rates of *p*-nitroanisole *O*-demethylation and surface fluorescence of pyridine nucleotides and flavoproteins in perfused livers from fasted, phenobarbital-treated rats are compared in Fig. 1. Following a transient stimulation of *p*-nitroanisole metabolism, ethanol (20 mM) inhibited mixed-function oxidation by 70–80% (Fig. 1A). In contrast, sorbitol (2 mM) increased the rate of *p*-nitrophenol formation from *p*-nitroanisole by 50%, from 3.0 to 4.6 μmoles$\cdot g^{-1}\cdot hr^{-1}$ (Fig. 1B; Table 1). Although ethanol and sorbitol had opposite actions on *p*-nitroanisole *O*-demethylation, both agents increased pyridine nucleotide and flavoprotein fluorescence measured from the liver surface (Fig. 1A and 1B). When sorbitol and ethanol infusions were discontinued, all variables returned to their respective baselines. The decrease in pyridine nucleotide fluorescence which occurred when *p*-nitroanisole infusion was initiated (Fig. 1) was due to quenching of fluorescence by *p*-nitroanisole and *p*-nitrophenol and does not reflect oxidation of NADPH [14].

Effect of 6-*aminonicotinamide on sorbitol-stimulated* p-*nitroanisole* O-*demethylation.* Pretreatment of rats with 6-aminonicotinamide has been shown to drastically inhibit the activity of the pentose phosphate shunt in the perfused liver [15] due to inhibition of 6-phosphogluconate dehydrogenase by the antimetabolite 6-amino-$NADP^+$ [16, 17]. Surprisingly, *p*-nitroanisole *O*-demethylation was not affected by 6-aminonicotinamide pretreatment in livers from fed rats [18] even though carbon flux in the pentose phosphate shunt was diminished by 88–93% [15]. Since sorbitol, but not ethanol, furnishes glucose-6-phosphate for the pentose pathway, it was of interest to determine whether the stimulation of mixed-function oxidation by sorbitol was sensitive to 6-aminonicotinamide pretreatment. In a liver from a fasted, phenobarbital-treated rat, sorbitol (2 mM) and fructose (2 mM) stimulated *p*-nitroanisole metabolism (Fig. 2A). Pretreatment of rats with 6-aminonicotinamide blocked completely the effect of sorbitol and fructose on *p*-nitroanisole *O*-demethylation (Fig. 2B). However, the conversion of sorbitol to lactate and glucose was unaffected by 6-aminonicotinamide treatment (Fig. 2).

Effects of ethanol and sorbitol on intracellular pyridine nucleotide contents and metabolites. Under conditions similar to those shown in Fig. 1, ethanol and sorbitol both significantly decreased the hepatic $NAD^+/NADH$ ratios in livers from fasted rats (Table 1). This reduction in NAD^+ was anticipated since sorbitol and ethanol are metabolized predominantly by the NAD^+-requiring enzymes alcohol dehydrogenase [19] and sorbitol dehydrogenase [20], respec-

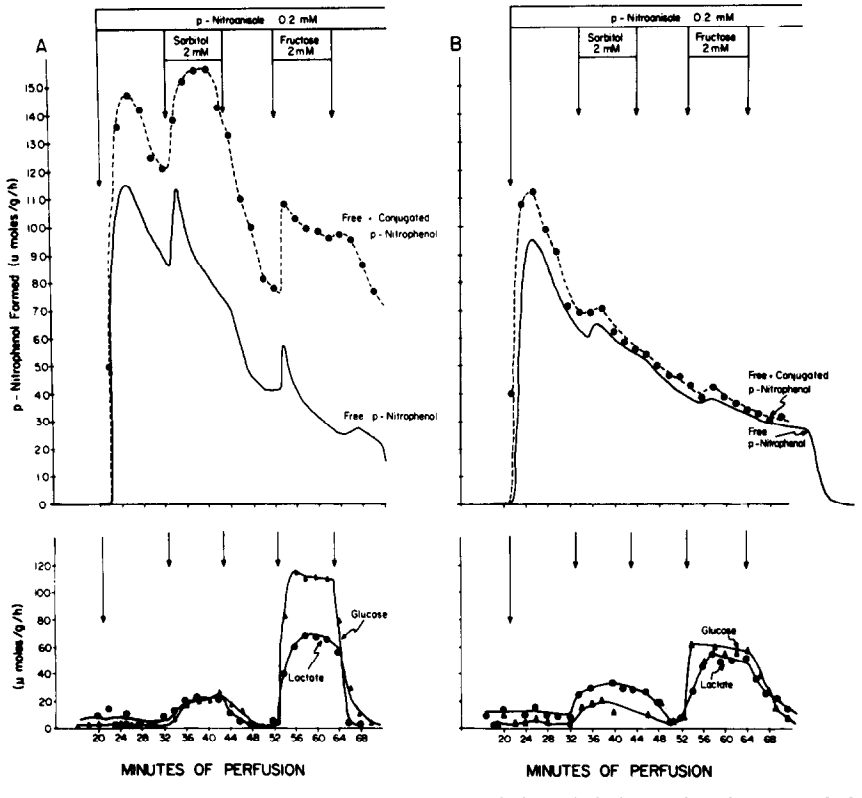

Fig. 2. Effects of sorbitol and fructose on *p*-nitroanisole *O*-demethylation and on lactate and glucose production in perfused livers from fasted rats. Glucose (▲—▲) and lactate (●—●) were measured enzymatically [13] in 1.0 ml samples of perfusate. Other conditions are as in Fig. 1. (A) Fasted, phenobarbital-treated rat. (B) Fasted, phenobarbital-treated rat 6 hr after 6-aminonicotinamide (70 mg/kg, i.p.).

Table 1. Effects of sorbitol and ethanol infusion on pyridine nucleotide ratios in perfused livers from fasted, phenobarbital-treated rats*

	NAD^+/NADH	$NADP^+$/NADPH
Controls (8)	5.75 ± 1.06	2.75 ± 0.29
Controls + 20 mM ethanol (4)	1.42 ± 0.05†	3.88 ± 0.73
Controls + 5 mM sorbitol (4)	0.97 ± 0.12‡	0.45 ± 0.12§

* Livers were perfused with *p*-nitroanisole (0.2 mM) after 20 min of pre-perfusion with Krebs–Henseleit bicarbonate buffer. Ethanol and sorbitol were introduced after 20 min of *p*-nitroanisole infusion, and all livers were freeze-clamped 8 min later. Pyridine nucleotides were measured in alkaline extracts of tissue as described in Materials and Methods. Values are means ± S.E.M. for the number of livers shown in parentheses.
† $P < 0.05$ with respect to controls.
‡ $P < 0.01$ with respect to controls.
§ $P < 0.001$ with respect to controls.

tively. In contrast, the effects of ethanol and sorbitol on the $NADP^+$ oxidation:reduction state differed remarkably. Sorbitol infusion caused a large decrease in the $NADP^+$/NADPH ratio whereas ethanol tended to increase it (Table 2). Thus, sorbitol infusion caused a reduction in $NADP^+$ while ethanol tended to cause oxidation of NADPH. Sorbitol increased the hepatic concentrations of citrate, glutamate and aspartate whereas ethanol tended to decrease malate (Table 2).

Effect of aminooxyacetate. Aminooxyacetate is a well-documented transaminase inhibitor which diminishes the transfer of reducing equivalents between the cytosol and mitochondria [21]. In the liver from a fasted, phenobarbital-treated rat, sorbitol increased the rate of *p*-nitrophenol production from *p*-nitroanisole by about 50% in both the presence and absence of aminooxyacetate (Fig. 3).

Influence of ethanol on p-*nitroanisole* O-*demethylation in the presence of aspartate.* Ethanol (20 mM) characteristically caused 80% inhibition of *p*-nitroanisole *O*-demethylation in perfused livers from fasted, phenobarbital-treated rats (Fig. 1; Table 3). However, when ethanol was infused in the presence of aspartate (5 mM), an amino acid which is converted into oxaloacetate, *p*-nitroanisole metabolism was inhibited only 38% (Table 3). Aspartate alone did not affect *p*-nitroanisole metabolism (data not shown).

Reversal of ethanol-mediated inhibition of p-*nitroanisole* O-*demethylation by sorbitol.* The addition of ethanol to a liver from a fasted, 6-aminonicotinamide-pretreated animal resulted in the characteristic inhibition of *p*-nitroanisole metabolism (Fig. 4). However, the subsequent infusion of sorbitol partially reversed (50%) this inhibition (Fig. 4).

DISCUSSION

Role of NADPH in the differential effect of sorbitol and ethanol on mixed-function oxidation. At the concentrations employed in this study, neither ethanol [5] nor sorbitol [6] affected the oxidation of *p*-nitroanisole in isolated microsomes supplemented with excess NADPH. Thus, the differential effect of these alcohols on mixed-function oxidation in the whole cell cannot be explained by a direct action on the microsomal membrane. Both sorbitol and ethanol increased hepatic concentrations of NADH significantly (Table 1). Moreover, both compounds increased pyridine nucleotide and flavoprotein fluorescence from the surface of the perfused liver (Fig. 1) and have also been shown to elevate both mitochondrial and cytosolic NADH as indexed by substrate couples [22, 23]. Thus, ethanol and sorbitol have similar effects on the intracellular NADH redox state. In contrast, these agents had different effects

Table 2. Effect of sorbitol and ethanol infusion on cellular intermediates in perfused livers from fasted, phenobarbital-treated rats*

	Cellular intermediates (μmoles/kg wet liver weight)		
	Control	Ethanol (20 mM)	Sorbitol (5 mM)
Citrate	16.0 ± 2.6	11.6 ± 3.2	22.4 ± 1.9†
α-Ketoglutarate	97.0 ± 8.6	102.7 ± 16.2	97.2 ± 16.8
Malate	32.8 ± 3.9	53.8 ± 12.3	30.6 ± 5.9
Glutamate	681.3 ± 47.2	836.9 ± 107.7	906.6 ± 85.6†
Aspartate	303.3 ± 21.6	264.1 ± 25.2	503.8 ± 79.9†

* Conditions were as in Table 1. Values are averages ± S.E.M. of four to nine livers per group.
† $P < 0.05$ with respect to control values.

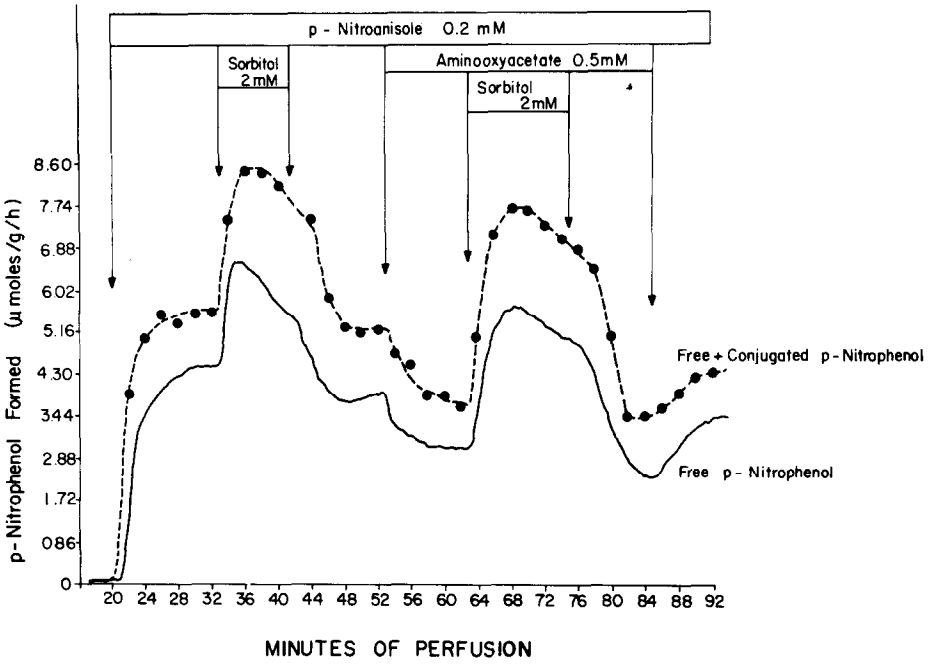

Fig. 3. Influence of sorbitol (2 mM) on *p*-nitroanisole *O*-demethylation in perfused rat livers in the absence or presence of aminooxyacetate (0.5 mM). Conditions as in Fig. 1.

on the $NADP^+$ redox state. Sorbitol decreased the $NADP^+$/NADPH ratio while ethanol tended to cause this ratio to increase (Table 1). These changes reflect an oxidation of intracellular NADPH with ethanol and a reduction of $NADP^+$ with sorbitol. Thus, the contrasting effects of these two alcohols on mixed-function oxidation are most likely due to opposite actions on the NADPH redox state.

Mechanism whereby sorbitol increases intracellular NADPH. In liver, sorbitol is metabolized primarily to fructose by an NAD^+-requiring sorbitol dehydrogenase (EC 1.1.1.14) [20]. It can also be metabolized by the $NADP^+$-requiring aldose reductase [24]. One of the first questions that was addressed in this study was which of these pathways predominates. Both sorbitol and fructose provide substrate for NADPH generation via the pentose phosphate shunt; however, only sorbitol can generate NADPH directly via aldose reductase. Since both sorbitol and fructose stimulate mixed-function oxidation to about the same magnitude (Fig. 2A), formation of NADPH from sorbitol via aldose reductase for this stimulation seems unlikely. This conclusion is supported by the observation that fructose and sorbitol failed to stimulate *p*-nitroanisole *O*-demethylation in livers from rats pretreated with 6-aminonicotinamide (Fig. 2B).

6-Aminonicotinamide is converted by a variety of tissues into 6-amino-$NADP^+$, a potent inhibitor of 6-phosphogluconate dehydrogenase [16, 17]. Pretreatment of rats with 6-aminonicotinamide, as well as fasting, has been shown to diminish the flux of carbon through the pentose phosphate shunt more than 80% [15]. Thus, 6-aminonicotinamide was employed to evaluate whether or not sorbitol could provide reducing equivalents for mixed-function oxidation via the pentose phosphate shunt in livers from fasted animals. The data indicate that both sorbitol and fructose failed to stimulate *p*-nitroanisole *O*-demethylation after 6-aminonicotinamide pretreatment. This effect cannot be explained by

Table 3. Effects of sorbitol and ethanol on *p*-nitrophenol production in perfused livers from fasted, phenobarbital-treated rats*

Addition	*p*-Nitrophenol production [μmoles·g^{-1}·hr^{-1}]	% of Controls
None	6.0 ± 0.5	
+ Ethanol (20 mM)	1.2 ± 0.8†	20
+ Sorbitol (2 mM)	9.0 ± 0.5‡	150
+ Ethanol (20 mM) and aspartate (5 mM)	3.7 ± 0.6‡	62

* Values represent total rates of *p*-nitrophenol production following hydrolysis of conjugates (see Fig. 1). N = 4 for each group.

† $P < 0.01$ with respect to control values.

‡ $P < 0.05$ with respect to control values.

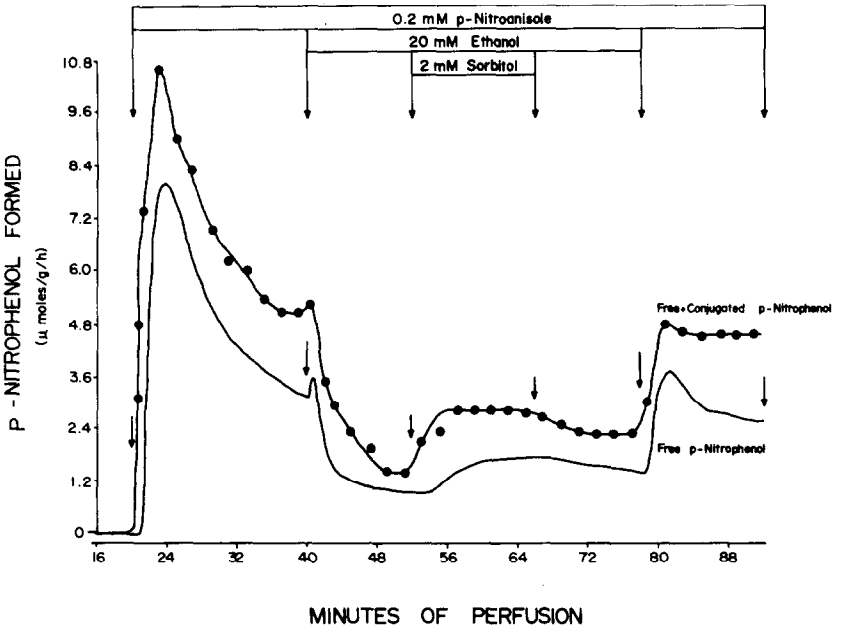

Fig. 4. Partial reversal of ethanol inhibition of *p*-nitroanisole *O*-demethylation by sorbitol. A fasted, phenobarbital-treated rat received 6-aminonicotinamide (70 mg/kg, i.p.) 6 hr prior to the perfusion experiment. Other conditions as in Fig. 1.

decreased metabolism of carbohydrate since 6-aminonicotinamide did not prevent the metabolism of sorbitol to lactate and glucose (Fig. 2). Thus, the pentose phosphate shunt is involved in the stimulation of mixed-function oxidation by sorbitol. We have concluded that sorbitol stimulates mixed-function oxidation by the following sequence of events (see Fig. 5). First, sorbitol is converted into fructose by the NAD^+-dependent sorbitol dehydrogenase; second, fructose is phosphorylated and converted into glucose-6-phosphate; and third, NADPH is generated from the metabolism of glucose-6-phosphate and 6-phosphogluconate via the pentose phosphate shunt providing NADPH for mixed-function oxidation. This conclusion is supported by the observation that aminooxyacetate, an inhibitor of transaminase-dependent shuttle mechanisms, did not affect the stimulation of mixed-function oxidation by sorbitol (Fig. 3).

Sequence of events leading to inhibition of drug metabolism by ethanol. Ethanol is metabolized to acetaldehyde predominantly in the cytosol by an NAD^+-dependent alcohol dehydrogenase (EC 1.1.1.1). Acetaldehyde is subsequently metabolized to acetate, primarily in the mitochondria, by a low K_m aldehyde dehydrogenase (EC 1.2.1.3). The net oxidation of 1 mole of ethanol leads to the synthesis of 2 moles of NADH. Thus, ethanol and acetaldehyde oxidations cause a reduction in intracellular NAD^+ as reflected by a decrease in the NAD^+/NADH ratio (Table 1) and an increase in surface fluorescence of pyridine nucleotides (Fig. 1). This elevation in intracellular NADH causes a redox inhibition of the citric acid cycle [25] leading to decreased intracellular concentrations of citrate and aspartate (Table 2).

Do the well-documented effects of ethanol on the NAD^+ redox state play a role in its ability to tend to oxidize intracellular NADPH and inhibit drug metabolism? In the fasted state where pentose shunt activity is minimal [15], the liver is dependent on mitochondrial oxidations to provide substrate for cytosolic NADPH generation, primarily via isocitrate dehydrogenase [26]. Thus, the citric acid cycle must provide not only the immediate substrate for this enzyme but also other intermediates required for the tricarboxylate shuttle mechanism that transfers mitochondrial NADPH into the cytosol (Fig. 5) [26]. Ethanol inhibits the citric acid cycle [25] and tends to decrease hepatic levels of citrate and aspartate (Table 2). Ethanol has also been shown to decrease isocitrate levels in perfused rat liver [5]. Thus, the tendency toward oxidation of NADPH observed in the presence of ethanol (Table 1) is most likely explained by diminished NADPH-dependent isocitrate dehydrogenase activity subsequent to inhibition of the citric acid cycle. This interpretation of the effect of ethanol is supported by the observation that aspartate, which provides oxaloacetate to the citric acid cycle, partially reversed the inhibition of mixed-function oxidation by ethanol (Table 3). Thus, ethanol inhibits the metabolism of *p*-nitroanisole by diminishing the flow of mitochondrial NADPH into the cytosol.

Sorbitol–ethanol interactions. NADH-mediated inhibition of the citric acid cycle is intimately involved in the mechanism of inhibition of drug metabolism by ethanol. Since sorbitol causes the same elevation in pyridine nucleotide fluorescence (Fig. 1) and a similar change in the NAD^+:NADH ratio (Table 1) as ethanol, it is surprising that sorbitol does not also inhibit drug metabolism. A possible

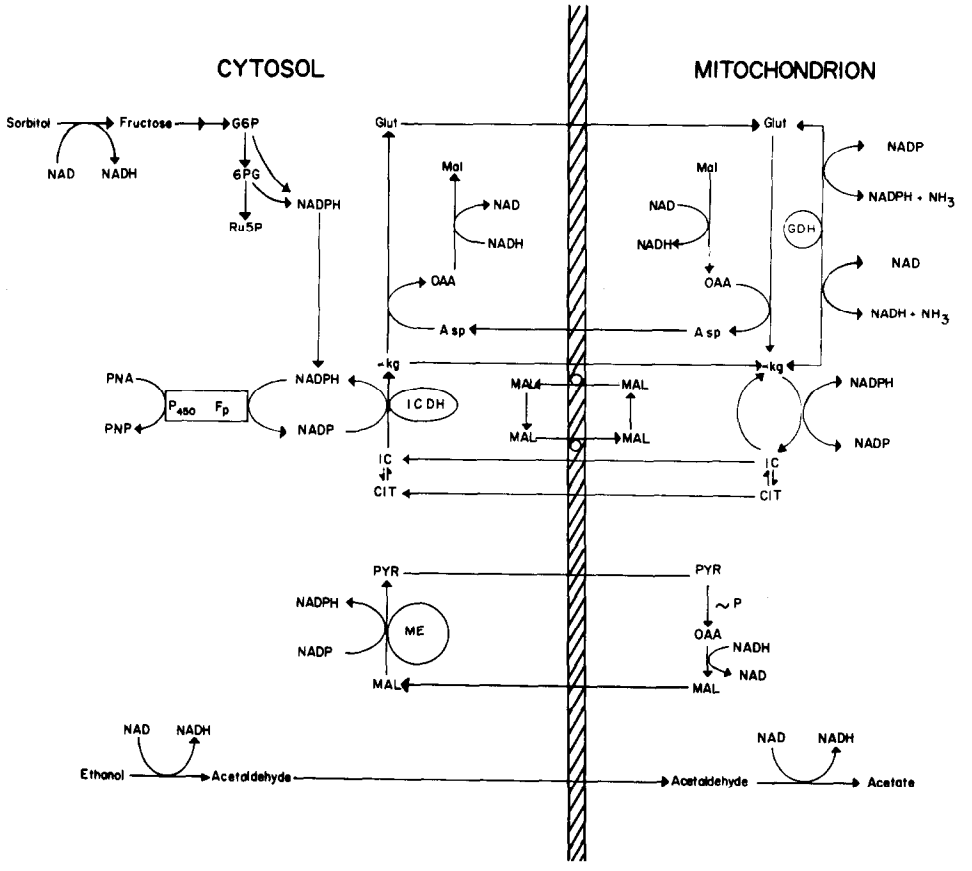

Fig. 5. Scheme depicting proposed mechanisms for generation of cytosolic NADPH. The pentose phosphate shunt generates NADPH via glucose-6-phosphate (G6P) dehydrogenase and 6-phosphogluconate (6PG) dehydrogenase. Cytosolic NADPH may also be generated by a malate shuttle which involves the carboxylation of pyruvate (PYR) via pyruvate carboxylase to form oxaloacetate (OAA), reduction to malate (MAL), egress of MAL into the cytosol and NADPH production via malic enzyme (ME). Alternatively, α-ketoglutarate (αkg) may be reductively carboxylated in the mitochondria to isocitrate (IC) via an NADPH-specific isocitrate dehydrogenase (ICDH). IC is then converted to citrate (CIT) through the aconitase reaction and CIT and IC are transported into the cytosol in which NADPH may be generated by the reverse reactions. Then αkg is moved into the mitochondria, either directly or as glutamate (GLUT) after transamination. Other abbreviations used are: Asp (aspartate), ~P (high energy phosphates), F_p (specific flavoprotein reductases for cytochromes P-450 and b_5), and GDH (glutamate dehydrogenase).

explanation is that stimulation of the pentose phosphate shunt by sorbitol would offset any inhibition of the generation of mitochondrial NADPH. However, since sorbitol did not inhibit drug metabolism in the 6-aminonicotinamide-treated animal, this possibly can be excluded (Fig. 2B). This lack of inhibition stems from the fact that sorbitol increases the supply to hexose in the fasted state whereas ethanol does not. Thus, like aspartate, sorbitol could increase flux in the citric acid cycle providing intermediates both for NADPH synthesis and substrate shuttle mechanisms. This hypothesis is supported by the observation that citrate and aspartate were elevated by the infusion of sorbitol (Table 2) and by the capacity of sorbitol to partially reverse the inhibition of mixed-function oxidation by ethanol in a 6-aminonicotinamide-treated animal (Fig. 4). This reversal could be due either to an inhibition of ethanol metabolism by sorbitol [22] or to provision of substrate for the citric acid cycle. Although these alternatives cannot presently be differentiated, the fact that sorbitol did not inhibit *p*-nitroanisole *O*-demethylation in the presence of 6-aminonicotinamide supports the latter possibility.

Taken together, these data indicate that mitochondrial citric acid cycle activity may be required for extramitochondrial mixed-function oxidation in a variety of metabolic states.

REFERENCES

1. R. G. Thurman and F. C. Kauffman, *Pharmac. Rev.* **31**, 229 (1979).
2. R. W. Eastabrook and E. Lindenlaub, (Eds.), *The*

Induction of Drug Metabolism, F. K. Schattauer, Stuttgart (1979).
3. R. Kato, *Xenobiotica* **7**, 25 (1977).
4. J. C. Campbell and J. R. Hayes, *Pharmac. Rev.* **26**, 171 (1974).
5. L. A. Reinke, F. C. Kauffman, S. A. Belinsky and R. G. Thurman, *J. Pharmac. exp. Ther.* **213**, 70 (1980).
6. L. A. Reinke, F. C. Kauffman and R. G. Thurman, *Biochem. Pharmac.* **29**, 813 (1980).
7. W. J. Marshall and A. E. M. McLean, *Biochem. Pharmac.* **18**, 153 (1969).
8. R. G. Thurman, D. P. Marazzo, L. S. Jones and F. C. Kauffman, *J. Pharmac. exp. Ther.* **201**, 498 (1977).
9. R. Scholz, R. G. Thurman, J. R. Williamson, B. Chance and T. Bucher, *J. biol. Chem.* **244**, 2317 (1969).
10. B. Chance, V. Legallis, J. Sorge and N. Graham, *Analyt. Biochem.* **66**, 498 (1975).
11. O. H. Lowry and J. V. Passonneau, *A Flexible System of Enzymatic Analysis*, p. 129. Academic Press, New York (1972).
12. N. D. Goldberg, J. V. Passonneau and O. H. Lowry, *J. biol. Chem.* **241**, 3997 (1966).
13. H. U. Bergmeyer, *Methods of Enzymatic Analysis*, 2nd English Edn. Chemie, Weinheim (1970).
14. L. A. Reinke, R. G. Thurman and F. C. Kauffman, *Biochem. Pharmac.* **28**, 2381 (1979).
15. S. A. Belinsky, L. A. Reinke, F. C. Kauffman, R. Scholz and R. G. Thurman, *Fedn Proc.* **40**, 2874 (1981).
16. E. Kohler, H-J. Barrach and D. Neubert, *Fedn Eur. Biochem. Soc. Lett.* **6**, 225 (1970).
17. R. G. Thurman, M. Lurquin, R. Evans and F. C. Kauffman, in *Microsomes and Drug Oxidations* (Ed. V. Ullrich), p. 315. Pergamon Press, Oxford (1977).
18. S. A. Belinsky, L. A. Reinke, F. C. Kauffman and R. G. Thurman, *Archs Biochem. Biophys.* **204**, 207 (1980).
19. H. Theorell and B. Chance, *Acta chem. scand.* **5**, 1127 (1951).
20. N. Leissing and E. T. McGuinness, *Biochim. biophys. Acta* **524**, 251 (1978).
21. S. Hopper and H. L. Segall, *J. biol. Chem.* **237**, 3189 (1962).
22. M. E. Hillbom and K. O. Lindros, *Metabolism* **20**, 843 (1971).
23. M. N. Berry, E. Kun and H. V. Werner, *Eur. J. Biochem.* **33**, 407 (1973).
24. H. G. Hers, *Biochim. biophys. Acta* **37**, 120 (1960).
25. R. Blomstrand and L. Kager, in *Alcohol and Aldehyde Metabolizing Systems* (Eds. R. G. Thurman, T. Yonetani, J. R. Williamson and L. Chance), p. 339. Academic Press, New York (1974).
26. H. Sies, K. Weigl and C. Waydes, in *The Induction of Drug Metabolism* (Eds. R. W. Estabrook and E. Lindenlaub), p. 383. F. K. Schattauer, Stuttgart (1979).