Effect of a Single Oral Dose of Methanol, Ethanol and Propan-2-ol on the Hepatic Microsomal Metabolism of Foreign Compounds in the Rat

By GARTH POWIS

Department of Pharmacology, University of Glasgow, Glasgow G12 8QQ, U.K.

(Received 3 October 1974)

Methanol and ethanol administered to rats as a single oral dose increased aniline hydroxylation by the hepatic microsomal fraction by a maximum of 169 and 66% respectively, whereas aminopyrine demethylation was inhibited by 51 and 61%. The concentration of microsomal cytochrome *P*-450, and the activities of NADPH-cytochrome *c* reductase and NADPH-cytochrome *P*-450 reductase were unchanged. Propan-2-ol, administered as a single oral dose, increased microsomal aniline hydroxylation by 165% and increased aminopyrine demethylation by 83%. The concentration of cytochrome *P*-450 was unchanged whereas NADPH-cytochrome *c* reductase and NADPH-cytochrome *P*-450 reductase were both increased by 38%. Methanol, ethanol and propan-2-ol administration resulted in a decreased type I spectral change but had no effect on the reverse type I spectral change. Methanol administration decreased the type II spectral change whereas ethanol and propan-2-ol had no effect. Cycloheximide blocked the increases in aniline hydroxylation and aminopyrine demethylation but could not completely prevent the decreases in aminopyrine demethylation. The increases in aniline hydroxylation were due to an increase in V , but K_m was unchanged. The ability of acetone to enhance and compound SKF 525A to inhibit microsomal aniline hydroxylation was decreased by the administration of all three alcohols. The decrease in the metabolism of aminopyrine may result from a decrease in the binding to the type I site with a consequent failure of aminopyrine to stimulate the reduction of cytochrome *P*-450. Methanol administration may lead to an increase in aniline hydroxylation because of a failure of aniline to inhibit cytochrome *P*-450 reduction.

The ability of the chronic administration of ethanol to increase the metabolism of foreign compounds by the hepatic microsomal fraction is well documented (Rubin & Lieber, 1968; Ariyoshi *et al.*, 1970; Misra *et al.*, 1971; Nakanishi *et al.*, 1971; Singlevich & Barboriak, 1971; Tobon & Mezey, 1971; Joly *et al.*, 1973). There is an increase in microsomal ethanol oxidation and evidence has been presented favouring either the existence of a unique enzyme system (Lieber & DeCarli, 1973) or a catalase-mediated reaction (Thurman & Scholz, 1973). It is also recognized that ethanol and other alcohols will inhibit the microsomal metabolism of foreign compounds *in vitro* (Rubin *et al.*, 1970; Cinti *et al.*, 1973; Cohen & Mannering, 1973). Much less is known, however, of the effects of the administration of a single dose of ethanol and other alcohols, on the activity of the microsomal mixed-function oxidase. The only reports on the effects of a single dose of ethanol on the microsomal metabolism of foreign compounds (Ariyoshi *et al.*, 1970; Tobon & Mezey, 1971; Mallov & Baesl, 1972) are far from complete. It has been suggested that the ability of certain alcohols, administered as a single dose, to

potentiate the hepatotoxicity of carbon tetrachloride given to animals several hours later may be related to an increase in the hepatic microsomal metabolism of carbon tetrachloride (Sipes *et al.*, 1973). The metabolism of other foreign compounds may also be increased.

The present investigation is a study of the effects of a single oral dose of methanol, ethanol and propan-2-ol, on the activity of the hepatic microsomal mixed-function oxidase in the rat.

Experimental

Methods

Administration of drugs and chemicals. Alcohols were administered by stomach tube to unanaesthetized male Wistar albino rats weighing between 200 and 250 g. Those alcohols that were sufficiently miscible with water were administered as a 25% (v/v) solution in water, otherwise they were administered undiluted. Control animals were fed with 10 ml of water/kg by stomach tube. The administration of sucrose solutions, isocaloric with the larger doses of alcohols used, produced results no different from the water-fed

controls. Cycloheximide (1mg/kg) dissolved in 0.9% NaCl was administered by intraperitoneal injection 5min before the administration of the alcohols. Phenobarbitone (100mg/kg) dissolved in 0.9% NaCl and 20-methylcholanthrene (80mg/kg) dissolved in corn oil were administered daily by intraperitoneal injection for 4 days and the rats killed 24h after the last injection. Corn oil administered by itself had no effect on the metabolism of foreign compounds by the microsomal fraction. The rats were at all times allowed free access to food and water.

Preparation of the microsomal fraction. The hepatic microsomal subcellular fraction was prepared by the method of Ernster *et al.* (1962) in 0.25M-sucrose containing 0.05M-Tris-HCl buffer, pH7.4, and adjusted to give a protein concentration of 10mg/ml. Protein was determined by the method of Lowry *et al.* (1951) with crystalline bovine serum albumin as a standard.

Assays. The metabolism of foreign compounds by the microsomal fraction was measured over 30min at 37°C with a reaction mixture containing 4mg of microsomal protein, Tris-HCl buffer, pH7.4 (112 μ mol), $MgCl_2$ (10 μ mol), nicotinamide (50 μ mol), glucose 6-phosphate (25 μ mol), glucose 6-phosphate-NADP⁺ oxidoreductase (EC 1.1.1.49) (2units), NADP⁺ (1 μ mol) and either aminopyrine (4-dimethylaminoantipyrine) (10 μ mol), hexobarbitone (0.6 μ mol) or aniline (5 μ mol), all in a final incubation volume of 2ml. Formaldehyde formed from aminopyrine was trapped with semicarbazide hydrochloride (10 μ mol) and measured by the method of Nash (1953). Hexobarbitone disappearance was followed by the method of Remmer (1959) and the formation of *p*-aminophenol from aniline was measured by the method of Schenkman *et al.* (1967). The cytochrome *b*₅ and cytochrome *P*-450 content of the microsomal fraction was determined by the method of Dallner (1963). The molar extinction coefficient of cytochrome *b*₅ was taken as 171 cm⁻¹·mm⁻¹ and cytochrome *P*-450 as 91 cm⁻¹·mm⁻¹ (Mazel, 1971). NADPH-cytochrome *c* reductase was measured by the method of Mazel (1971). NADPH-cytochrome *P*-450 reductase was measured by a modification of the method of Gigon *et al.* (1969). Duplicate spectrophotometer cuvettes containing 15mg of microsomal protein, Tris-HCl buffer, pH7.4 (200 μ mol), $MgCl_2$ (10 μ mol), DL-isocitrate (25 μ mol) and isocitrate-NADP⁺ oxidoreductase (EC 1.1.1.42) (1 unit), in a total volume of 3ml were overlaid with a 1cm layer of light liquid paraffin (sp. gr. 0.85) which effectively sealed the cell contents from the atmosphere. Both cuvettes were gassed for 5min with O₂-free CO through a fine tube which passed through the liquid paraffin. The cuvettes were placed in a Unicam SP.8000 split-beam recording spectrophotometer at 37°C for 5min and the reaction was started by adding NADP⁺ (2 μ mol in 10 μ l of water) to the test cuvette. The rate

of reduction of cytochrome *P*-450 was measured over the first 10s and expressed as nmol of cytochrome *P*-450 reduced/min per mg of microsomal protein. Aminopyrine (3mM) and 12mM-aniline were added to modify the rate of reduction of cytochrome *P*-450. The spectral changes produced by the interaction of foreign compounds with the microsomal fraction were measured by the method of Schenkman *et al.* (1967) by using 5mM-aminopyrine (type I compound), 5mM-aniline (type II compound) and 0.7M-acetone (reverse type I compound).

Statistical treatment of results. Groups of data were subjected to a Student's *t* test (Snedecor & Cochran, 1967) to determine if a significant difference existed between the means of the groups of data.

Materials

Compound SKF 525A (2-diethylaminoethyl 2,2-diphenylvalerate hydrochloride) was supplied by Smith, Kline and French Laboratories, Welwyn Garden City, Herts., U.K. Glucose 6-phosphate (disodium salt), glucose 6-phosphate-NADP⁺ oxidoreductase, NADP⁺ and cytochrome *c* were purchased from the Boehringer Corp. (London) Ltd., Ealing, London W5 2TZ, U.K. DL-Isocitrate (trisodium salt), isocitrate-NADP⁺ oxidoreductase and crystalline bovine serum albumin were from the Sigma Chemical Co., Kingston-upon-Thames, Surrey, U.K. Aminopyrine was from Ralph N. Emanuel Ltd., Wembley, Middx., U.K., hexobarbitone sodium was from May and Baker, Dagenham, Essex, U.K., phenobarbitone sodium was from Cockburn and Co., Glasgow, U.K. and 20-methylcholanthrene was from Calbiochem Ltd., London W1H 1AS, U.K. Acetone, aniline hydrochloride, methanol, ethanol, propan-2-ol and other alcohols were obtained from BDH Chemicals, Poole, Dorset, U.K., and were the purest grades available.

Results

Effect of some alcohols on hepatic microsomal metabolism

The effect of the lower-saturated monohydric alcohols administered to rats by stomach tube, at a dose of half the reported LD₅₀ (Spector, 1956), on the ability of the hepatic microsomal fraction prepared 24h later, to metabolize aminopyrine and aniline is shown in Table 1. The alcohols may be divided into three groups: those that had no effect; those that produced an increase in aniline hydroxylation and a decrease in aminopyrine demethylation, namely methanol, ethanol and 2-methylpropan-2-ol; and those that produced an increase in aniline hydroxylation and an increase (although not significant) in aminopyrine demethylation, namely propan-2-ol, butan-2-ol and pentan-2-ol. The alcohols chosen for further study were methanol and ethanol,

Table 1. *Effect of some alcohols on the hepatic microsomal metabolism of aniline and aminopyrine*

Alcohols were fed to unanaesthetized male rats by stomach tube at the doses indicated, which were half the reported LD₅₀ (Spector, 1956), and the hepatic microsomal fraction was prepared 24 h later. Metabolizing activity was determined as described in the text. Values represent means $\pm$ S.E.M. and are expressed per mg of microsomal protein. The number of animals is given in parentheses.

	Dose (mmol/kg)	Aniline hydroxylation (nmol/30min per mg)	Aminopyrine demethylation (nmol/30min per mg)
Control	— (8)	12.1 $\pm$ 0.7	119.1 $\pm$ 5.7
Methanol	200 (4)	16.1 $\pm$ 1.6*	78.1 $\pm$ 12.1†
Ethanol	150 (4)	18.7 $\pm$ 2.9*	52.4 $\pm$ 10.1†
Propan-1-ol	16 (4)	14.3 $\pm$ 1.0	118.0 $\pm$ 8.1
Propan-2-ol	49 (4)	21.8 $\pm$ 0.9†	126.9 $\pm$ 11.3
Butan-1-ol	30 (4)	14.4 $\pm$ 0.8	118.0 $\pm$ 8.3
Butan-2-ol	20 (4)	15.6 $\pm$ 0.5†	131.4 $\pm$ 3.6
2-Methylpropan-1-ol	16 (4)	13.5 $\pm$ 0.4	110.2 $\pm$ 5.1
2-Methylpropan-2-ol	24 (4)	20.9 $\pm$ 1.1†	80.2 $\pm$ 4.5†
Pentan-1-ol	1.4 (4)	12.9 $\pm$ 0.3	98.9 $\pm$ 10.1
Pentan-2-ol	1.6 (4)	18.8 $\pm$ 0.9†	127.7 $\pm$ 9.0
3-Methylbutan-1-ol	2.3 (4)	14.3 $\pm$ 0.6	110.5 $\pm$ 4.7
2-Methylbutan-2-ol	6.8 (4)	12.7 $\pm$ 0.3	104.1 $\pm$ 8.2
Hexan-2-ol	24 (4)	10.5 $\pm$ 0.6	109.5 $\pm$ 6.3

* Value significantly different from control, $P < 0.05$.

† Value significantly different from control, $P < 0.01$.

Fig. 1. *Effect of pretreatment of rats with alcohols on the hepatic microsomal metabolism of foreign compounds*

□, Methanol (250 mmol/kg), ○, ethanol (170 mmol/kg) and △, propan-2-ol (65 mmol/kg) were administered to rats by stomach tube and the hepatic microsomal fraction was prepared at various times thereafter. The values are the means from eight animals and bars represent S.E.M. Protein refers to microsomal protein.

taken as representative of the second group of alcohols, and propan-2-ol, taken as representative of the third group of alcohols.

Time-course of the response

Preliminary investigation revealed that the doses producing the maximum increase in microsomal aniline hydroxylation 24 h after administration were 250 mmol of methanol/kg, 170 mmol of ethanol/kg and 65 mmol of propan-2-ol/kg. The time-course of the response to these doses of alcohols is shown in Fig. 1. The maximum increase in aniline hydroxylation of 66% ($P < 0.05$) after the administration of ethanol occurred at 24 h, whereas the maximum increase of 169% ($P < 0.001$) after methanol and 165% ($P < 0.001$) after propan-2-ol both occurred at 36 h. Both methanol and ethanol administration inhibited aminopyrine demethylation, methanol by a maximum of 51% ($P < 0.05$) at 36 h and ethanol by 61% ($P < 0.001$) at 24 h. In contrast, propan-2-ol administration produced an increase in aminopyrine demethylation of 83% ($P < 0.001$) at 36 h. The oxidation of hexobarbitone, classified as type I substrate the same as aminopyrine (Remmer *et al.*, 1966), was affected in a similar manner to aminopyrine demethylation (results not shown).

Components of the microsomal mixed-function oxidase

None of the alcohols had any effect on the concentrations of cytochrome P-450 (Table 2),

Table 2. Effect of methanol, ethanol and propan-2-ol on the components of the hepatic microsomal mixed-function oxidase and on spectral changes

Rats were fed by stomach tube with ethanol (170mmol/kg) 24h, and methanol (250mmol/kg) and propan-2-ol (65mmol/kg) 36h before being killed. Values represent means $\pm$ s.e.m. and are expressed per mg of microsomal protein. The spectral changes represent the peak-to-trough changes caused by the addition of 5mm-aminopyrine (type I), 5mm-aniline (type II) or 0.7M-acetone (reverse type I) to a cuvette containing the microsomal fraction (2mg of protein/ml). For convenience these results are expressed per g of microsomal protein. Values in parentheses are the numbers of animals.

	Cytochrome b_5 (nmol/mg) (4)	Cytochrome P-450 (nmol/mg) (4)	NADPH-cytochrome <i>c</i> reductase (nmol/min per mg) (4)	NADPH-cytochrome P-450 reductase (nmol/min per mg) (12)	Spectral changes		
					Type I ($E_{385-416}$ /g) (4)	Type II ($E_{429-395}$ /g) (4)	Reverse type I ($E_{418-390}$ /g) (4)
Control	0.26 $\pm$ 0.02	0.45 $\pm$ 0.02	94.4 $\pm$ 5.0	1.50 $\pm$ 0.07	15.5 $\pm$ 0.6	38.9 $\pm$ 1.7	4.5 $\pm$ 0.5
Methanol	0.13 $\pm$ 0.01*	0.47 $\pm$ 0.04	90.8 $\pm$ 8.2	1.66 $\pm$ 0.11	4.5 $\pm$ 0.5*	20.2 $\pm$ 0.8*	5.3 $\pm$ 0.6
Ethanol	0.22 $\pm$ 0.01	0.41 $\pm$ 0.03	97.7 $\pm$ 9.4	1.29 $\pm$ 0.10	11.0 $\pm$ 1.0*	35.1 $\pm$ 1.3	3.6 $\pm$ 0.4
Propan-2-ol	0.30 $\pm$ 0.02	0.47 $\pm$ 0.03	130.0 $\pm$ 7.6*	2.07 $\pm$ 0.15*	4.7 $\pm$ 0.7*	33.0 $\pm$ 4.7	3.4 $\pm$ 0.3

* Value significantly different from control, $P < 0.01$.

although with methanol and propan-2-ol there was a shift in the peak absorbance of the reduced cytochrome P-450-CO complex to 451nm. Ethanol and propan-2-ol had no effect on the concentrations of cytochrome b_5 although methanol decreased the concentrations by 50%. The significance of this finding is not clear as the participation of cytochrome b_5 in the hepatic microsomal mixed-function oxidase is still subject to some debate (Jansson & Schenkman, 1973; Sasame *et al.*, 1973). Neither methanol nor ethanol pretreatment had any effect on the activity of the microsomal NADPH-cytochrome *c* reductase or NADPH-cytochrome P-450 reductase. Propan-2-ol pretreatment, however, increased the activity of both NADPH-cytochrome *c* reductase and NADPH-cytochrome P-450 reductase by 38%. The effect of pretreating rats with alcohols on microsomal NADPH-cytochrome P-450 reductase activity, as modified by the presence of aminopyrine or aniline, is shown in Fig. 2. Pretreatment of animals with

Fig. 2. Effect of pretreatment of rats with alcohols on hepatic microsomal NADPH-cytochrome P-450 reductase

The superimposed recordings obtained with pooled microsomal fractions from four rats: (a) control animals; (b) animals fed with ethanol (170mmol/kg) 24h previously; (c) animals fed with methanol (250mmol/kg) 36h previously; (d) animals fed with propan-2-ol (65mmol/kg) 36h previously. The incubation conditions are described in the text. Each panel shows the reduction of cytochrome P-450 by NADPH: 0, in the absence of added substrate; I, in the presence of a type I substrate (3mm-aminopyrine) and, II, in the presence of a type II substrate (12mm-aniline). The initial peak is a recording artifact.

Fig. 3. Effect of pretreatment of rats with alcohols on the kinetics of hepatic microsomal aniline hydroxylation

●, Control rats; ○, rats fed with ethanol (170mmol/kg) 24 h previously; □, rats fed with methanol (250mmol/kg) 36 h previously; △, rats fed with propan-2-ol (65 mmol/kg) 36 h previously. The microsomal fractions from four rats were pooled.

ethanol had no marked effect on either the increased rate of reduction of cytochrome *P*-450 caused by aminopyrine or on the decreased rate of reduction caused by aniline. Neither aminopyrine nor aniline had any effect on the rate of cytochrome *P*-450 reduction in the microsomal fraction from methanol-pretreated animals. Propan-2-ol pretreatment abolished the stimulation caused by aminopyrine but had no effect on the inhibition caused by aniline.

The spectral change produced by aminopyrine, a type I substrate (Remmer *et al.*, 1966), was decreased over 70% in rats pretreated with either methanol or propan-2-ol and decreased by 29% in rats pretreated with ethanol. The spectral change produced by aniline, a type II substrate (Remmer *et al.*, 1966) was unaffected by pretreatment with ethanol or propan-2-ol but was decreased by 48% by pretreatment of rats with methanol. The reverse type I spectral change produced by acetone (Imai & Sato, 1967) was unaffected by pretreatment with methanol, ethanol or propan-2-ol.

Kinetics of aniline hydroxylation

All of the alcohols resulted in an increase in V , whereas K_m was unaltered (Fig. 3).

Enhancement and inhibition of microsomal hydroxylation

The enhancement of aniline hydroxylation by acetone (Anders, 1968) and the inhibition by compound SKF 525A (Cooper *et al.*, 1954) were both decreased by alcohol pretreatment (Figs. 4 and 5).

Fig. 4. Effect of pretreatment of rats with alcohols on the enhancement of hepatic microsomal aniline hydroxylation by acetone

●, Control rats; ○, rats fed with ethanol (170mmol/kg) 24 h previously; □, rats fed with methanol (250mmol/kg) 36 h previously; △, rats fed with propan-2-ol (65 mmol/kg) 36 h previously. The microsomal fractions from four rats were pooled. Acetone was added directly to the reaction mixture.

Fig. 5. Effect of pretreatment of rats with alcohols on the inhibition of hepatic microsomal aniline hydroxylation by compound SKF 525A

●, Control rats; ○, rats fed with ethanol (170mmol/kg) 24 h previously; □, rats fed with methanol (250mmol/kg) 36 h previously; △, rats fed with propan-2-ol (65 mmol/kg) 36 h previously. The microsomal fractions from four rats were pooled. Compound SKF 525A was added to the reaction mixture at the start of the reaction.

Table 3. *Effect of cycloheximide on the changes in the metabolism of aniline and aminopyrine produced by methanol, ethanol and propan-2-ol*

Cycloheximide (1mg/kg) was administered to unanaesthetized male rats by intraperitoneal injection 5min before the administration of methanol (250mmol/kg), ethanol (170mmol/kg) and propan-2-ol (65mmol/kg) by stomach tube. The hepatic microsomal fraction was prepared 36h later and metabolizing activity determined as described in the text. Values represent means $\pm$ S.E.M. of six animals and are expressed per mg of microsomal protein.

	Aniline hydroxylation (nmol/30min per mg)		Aminopyrine demethylation (nmol/30min per mg)	
	No drug	Cycloheximide	No drug	Cycloheximide
Control	12.2 $\pm$ 0.3	12.0 $\pm$ 0.5	129.0 $\pm$ 5.5	84.8 $\pm$ 11.0**
Methanol	34.3 $\pm$ 2.4	13.1 $\pm$ 1.6**	65.1 $\pm$ 6.1	53.6 $\pm$ 8.7†
Ethanol	15.6 $\pm$ 0.7	11.4 $\pm$ 0.3**	65.9 $\pm$ 6.7	44.5 $\pm$ 4.2*†
Propan-2-ol	32.2 $\pm$ 0.4	12.8 $\pm$ 1.3**	198.2 $\pm$ 9.4	65.1 $\pm$ 4.1**

* Value significantly different from those obtained in the absence of cycloheximide, $P < 0.05$.

** Value significantly different from those obtained in the absence of cycloheximide, $P < 0.01$.

† Value significantly different from the control value obtained in the presence of cycloheximide, $P < 0.05$.

Table 4. *Effect of phenobarbitone and 20-methylcholanthrene pretreatment on the changes in metabolism of aniline and aminopyrine produced by methanol, ethanol and propan-2-ol*

Phenobarbitone (100mg/kg) and 20-methylcholanthrene (80mg/kg) were administered to rats as daily intraperitoneal injections, for 4 days. The last injection was 24h before death and the preparation of the hepatic microsomal fraction. Methanol (250mmol/kg), ethanol (170mmol/kg) and propan-2-ol (65mmol/kg) were administered by stomach tube 36h before death. Metabolizing activity was determined as described in the text. Values represent means $\pm$ S.E.M. of eight animals and are expressed per mg of microsomal protein.

	Aniline hydroxylation (nmol/30min per mg)	Aminopyrine demethylation (nmol/30min per mg)
Control	11.5 $\pm$ 0.5	111.1 $\pm$ 5.6
Phenobarbitone	21.4 $\pm$ 0.8	205.8 $\pm$ 14.5
20-Methylcholanthrene	19.8 $\pm$ 0.9	37.9 $\pm$ 7.7
Methanol	32.0 $\pm$ 2.6	60.7 $\pm$ 24.4
Ethanol	17.2 $\pm$ 1.9	51.0 $\pm$ 18.7
Propan-2-ol	31.6 $\pm$ 1.9	227.4 $\pm$ 20.4
Phenobarbitone+methanol	27.7 $\pm$ 1.5	227.2 $\pm$ 22.0
Phenobarbitone+ethanol	18.2 $\pm$ 1.4	184.2 $\pm$ 8.7
Phenobarbitone+propan-2-ol	24.3 $\pm$ 1.4	223.3 $\pm$ 22.1
20-Methylcholanthrene+methanol	28.7 $\pm$ 0.9	38.9 $\pm$ 5.0
20-Methylcholanthrene+ethanol	17.9 $\pm$ 2.7	29.0 $\pm$ 5.4
20-Methylcholanthrene+propan-2-ol	14.8 $\pm$ 1.0	23.7 $\pm$ 5.3

Modification of the effects of alcohols by other drugs

The simultaneous intraperitoneal administration of cycloheximide, an inhibitor of drug-induced protein synthesis (Miller & Jondorf, 1973), completely blocked the increase in microsomal aniline hydroxylation produced by the oral administration of methanol, ethanol and propan-2-ol, giving a value not significantly different from the control value (Table 3). Aminopyrine demethylation was inhibited by the administration of cycloheximide itself, and was further inhibited by the administration of methanol and ethanol. The increase in aminopyrine demethylation produced by propan-2-ol was completely blocked by cycloheximide.

The effects of methanol, ethanol and propan-2-ol administered simultaneously with phenobarbitone or 20-methylcholanthrene in doses sufficient to produce a maximum induction of the microsomal mixed-function oxidase (Orrenius, 1965; Conney, 1967) are shown in Table 4. Aniline hydroxylation was stimulated by both phenobarbitone and 20-methylcholanthrene although neither compound, when administered simultaneously with an alcohol, was able to increase the rate significantly above that found with alcohol alone. In fact the activity after the administration of propan-2-ol was significantly inhibited by the further administration of phenobarbitone or 20-methylcholanthrene. Phenobarbitone administration increased the rate of aminopyrine

demethylation and overcame the inhibition produced by pretreatment with methanol and ethanol, but did not further increase the rate when administered together with propan-2-ol. The administration of 20-methylcholanthrene markedly inhibited aminopyrine demethylation, which was not significantly further inhibited by the simultaneous administration of the alcohols.

Discussion

The effects of a single dose of ethanol have received less attention than the effects of chronic ethanol administration. Single doses of ethanol (around 100mmol/kg), 16–24h before death, have been reported to have no effect on hepatic microsomal cytochrome *P*-450 (Ariyoshi *et al.*, 1970; Tobon & Mezey, 1971) or on aminopyrine demethylation (Ariyoshi *et al.*, 1970), but to increase aniline hydroxylation (Ariyoshi *et al.*, 1970; Tobon & Mezey, 1971) and the metabolism of zoxazolamine and hexobarbitone (Mallov & Baesl, 1972).

In the present study a single dose of ethanol, and to a greater extent methanol, was found to produce a selective increase in aniline hydroxylation with no change in liver weight, microsomal protein, cytochrome *P*-450, NADPH–cytochrome *c* reductase or NADPH–cytochrome *P*-450 reductase, whereas the metabolism of aminopyrine and hexobarbitone was inhibited. Alcohols are known to inhibit the microsomal mixed-function oxidase directly (Rubin *et al.*, 1970; Cohen & Mannering, 1973) although it is unlikely that sufficient ethanol remained after 24h to exert a direct action on the microsomal fraction. Strubelt *et al.* (1974) have reported that in the rat an oral dose of 140mmol of ethanol/kg is completely cleared from the blood in 24h. Methanol on the other hand is metabolized at only one-tenth to one-fifteenth of the rate of ethanol (Lester & Benson, 1970; Feytmans & Leighton, 1973) so that appreciable quantities of methanol might be present in the animal even after 36h. It is difficult, however, to explain the time-course of inhibition of aminopyrine demethylation in terms of a direct inhibition by alcohol, and also why aniline hydroxylation, which is more sensitive to the inhibitory effects *in vitro* of alcohols at low concentrations, than aminopyrine demethylation (Rubin *et al.*, 1970; Cohen & Mannering, 1973) is not also inhibited.

The administration of propan-2-ol led to an increase in both aniline hydroxylation and aminopyrine demethylation associated with small increases in the yield of microsomal protein, in NADPH–cytochrome *c* reductase and in NADPH–cytochrome *P*-450 reductase, although the concentrations of cytochrome *P*-450 were unchanged. Sipes *et al.* (1973) have reported that propan-2-ol administration increases dimethylnitrosamine demethylation, has no effect on microsomal cytochrome *P*-450 and

their results also show a small increase in NADPH–cytochrome *c* reductase. Propan-2-ol is metabolized in the rat to acetone, a dose of 30mmol of propan-2-ol/kg being almost cleared from the blood in 18h (Traiger & Plaa, 1972). Acetone may account for some of the effects of propan-2-ol; it has been found to induce microsomal aniline hydroxylation and NADPH–cytochrome *c* reductase but has no effect on cytochrome *P*-450 and aminopyrine demethylation (Sipes *et al.*, 1973; Clark & Powis, 1974).

Attempts to produce a superinduction of aniline hydroxylation by the simultaneous administration of alcohols and phenobarbitone or 20-methylcholanthrene were unsuccessful. The ability to produce a superinduction of hepatic microsomal metabolism by the simultaneous administration of different inducers has been regarded as evidence of different mechanisms of induction (Conney, 1967). The results of the present study suggest that either there is a common mechanism of induction, or that some other factor involved in the synthesis of new enzyme becomes rate-limiting.

The most characteristic effect on the microsomal mixed-function oxidase produced by the administration of methanol or ethanol was to decrease the type I binding to cytochrome *P*-450. Propan-2-ol also decreased the type I binding. All of these alcohols themselves bound to cytochrome *P*-450 producing a reverse type I spectral change (Imai & Sato, 1967), but no significant difference could be detected in the reverse type I spectral change produced by acetone added to the microsomal fraction from control and alcohol-pretreated animals. Alcohols might be producing some of their effects on the activity of the microsomal mixed-function oxidase by affecting the turnover of microsomal phospholipids. Ariyoshi & Takabatake (1971) have reported that the acute administration of ethanol to rats leads to a decrease in total hepatic microsomal phospholipids and particularly in phosphatidylcholine. Phosphatidylcholine is an essential component for the binding of type I substrates to cytochrome *P*-450 and the removal of phosphatidylcholine by the incubation of the microsomal fraction with phospholipase C (Chaplin & Mannering, 1970) or by isoctane extraction (Leibman & Estabrook, 1971) leads to a decrease in the type I spectral change and in the metabolism of type I substrates. Pretreatment with alcohols leads to a failure of type I substrates to stimulate the reduction of cytochrome *P*-450 by NADPH which is thought to be rate-limiting in the overall reaction involving the microsomal metabolism of foreign compounds (Gillette & Gram, 1969). This may explain why the metabolism of type I substrates is inhibited after the administration of methanol and ethanol. The increase in NADPH–cytochrome *P*-450 reductase after the administration of propan-2-ol may more than compensate for this

inhibition leading to the observed increase in the metabolism of type I substrates after the administration of propan-2-ol.

An increase in NADPH-cytochrome *P*-450 reductase might also explain the increase in aniline hydroxylation produced by the administration of propan-2-ol. The increase in aniline hydroxylation produced by the administration of methanol and ethanol is more difficult to explain. Methanol pretreatment leads to a decrease in the binding of type II substrates and consequently there is a failure of aniline to inhibit NADPH-cytochrome *P*-450 reductase. This may then result in an increase in the rate of aniline hydroxylation. However, pretreatment with either ethanol or propan-2-ol resulted in only a small insignificant decrease in the type II spectral change.

All of the alcohols act on aniline hydroxylation by increasing *V* rather than affecting *K_m* which would tend to rule out the possibility of a specific induction, or activation, of one of the two forms of aniline hydroxylase, which it is suggested exists in the microsomal fraction (Anders, 1968).

The administration of a single dose of methanol, ethanol and propan-2-ol to rats has been shown to potentiate the hepatotoxicity of a dose of carbon tetrachloride given 20 h later (Traiger & Plaa, 1971). It has been suggested that the toxicity of carbon tetrachloride results from a chemical attack by metabolites of carbon tetrachloride on the protein components of the endoplasmic reticulum (Sipes *et al.*, 1973) and that NADPH-cytochrome *c* reductase is the point of interaction of carbon tetrachloride with the microsomal electron-transport chain (Slater & Sawyer, 1971). It is thus possible that the increase in carbon tetrachloride toxicity produced by propan-2-ol is related to the increase in NADPH-cytochrome *c* reductase. However, this cannot explain the actions of methanol and ethanol in potentiating toxicity and suggests that some other mechanism, apart from an increase in NADPH-cytochrome *c* reductase, must be involved.

A grant from the Rankin Research Fund of Glasgow University is gratefully acknowledged.

References

- Anders, M. W. (1968) *Arch. Biochem. Biophys.* **126**, 269-275
- Ariyoshi, T. & Takabatake, E. (1971) *Chem. Pharm. Bull.* **20**, 170-174
- Ariyoshi, T., Takabatake, E. & Remmer, H. (1970) *Life Sci.* **9**, 361-369
- Chaplin, M. D. & Mannering, G. J. (1970) *Mol. Pharmacol.* **6**, 631-640
- Cinti, D. L., Grundin, R. & Orrenius, S. (1973) *Biochem. J.* **134**, 367-375
- Clark, H. & Powis, G. (1974) *Biochem. Pharmacol.* **23**, 1015-1019
- Cohen, G. M. & Mannering, G. J. (1973) *Mol. Pharmacol.* **9**, 383-397
- Conney, A. H. (1967) *Pharmacol. Rev.* **19**, 317-366
- Cooper, J. R., Axelrod, J. & Brodie, B. B. (1954) *J. Pharmacol. Exp. Ther.* **112**, 55-63
- Dallner, G. (1963) *Acta Path. Microbiol. Scand. Suppl.* **166**, 1-180
- Ernster, L., Siekevitz, P. & Palade, G. E. (1962) *J. Cell Biol.* **15**, 541-562
- Feytmans, E. & Leighton, F. (1973) *Biochem. Pharmacol.* **22**, 349-360
- Gigon, P. L., Gram, T. E. & Gillette, J. R. (1969) *Mol. Pharmacol.* **5**, 109-122
- Gillette, J. R. & Gram, T. E. (1969) in *Microsomes and Drug Oxidations* (Gillette, J. R., Conney, A. H., Cosmides, G. J., Estabrook, R. W., Fouts, J. R. & Mannering, G. J., eds.), pp. 133-149, Academic Press, New York
- Imai, Y. & Sato, R. (1967) *J. Biochem. (Tokyo)* **62**, 239-249
- Jansson, I. & Schenkman, J. B. (1973) *Mol. Pharmacol.* **9**, 840-845
- Joly, J. G., Ishii, H., Teschke, R., Hasumura, Y. & Lieber, C. S. (1973) *Biochem. Pharmacol.* **22**, 1532-1535
- Leibman, K. C. & Estabrook, R. W. (1971) *Mol. Pharmacol.* **7**, 26-32
- Lester, D. & Benson, G. D. (1970) *Science* **169**, 282-284
- Lieber, C. S. & DeCarli, L. M. (1973) *Drug. Metab. Dispos.* **1**, 428-440
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265-275
- Mallov, S. & Baesl, T. J. (1972) *Biochem. Pharmacol.* **21**, 1667-1678
- Mazel, P. (1971) in *Fundamentals of Drug Metabolism and Drug Disposition* (LaDu, B. N., Mandel, H. G. & Way, E. L., eds.), pp. 546-582, Williams and Wilkins, Baltimore
- Miller, H. H. & Jondorf, W. R. (1973) *J. Pharm. Pharmacol.* **25**, 337-338
- Misra, P. S., Lefèvre, A., Ishii, H., Rubin, E. & Lieber, C. S. (1971) *Amer. J. Med.* **51**, 346-351
- Nakanishi, S., Masamura, E., Tsukada, M. & Matsumura, R. (1971) *Jap. J. Pharmacol.* **21**, 303-309
- Nash, T. (1953) *Biochem. J.* **55**, 416-421
- Orrenius, S. (1965) *J. Cell Biol.* **26**, 725-733
- Remmer, H. (1959) *Naunyn-Schmiedeberg's Arch. Pathol.* **235**, 279-290
- Remmer, H., Schenkman, J. B., Estabrook, R. W., Sasame, H., Gillette, J., Narasimhulu, S., Cooper, D. Y. & Rosenthal, O. (1966) *Mol. Pharmacol.* **2**, 187-190
- Rubin, E. & Lieber, C. S. (1968) *Science* **162**, 690-691
- Rubin, E., Gang, H., Misra, P. S. & Lieber, C. S. (1970) *Amer. J. Med.* **49**, 801-806
- Sasame, H. A., Mitchell, J. R., Thorgeirsson, S. & Gillette, J. R. (1973) *Drug Metab. Dispos.* **1**, 150-155
- Schenkman, J. B., Remmer, H. & Estabrook, R. W. (1967) *Mol. Pharmacol.* **3**, 113-123
- Singlevitch, T. & Barboriak, J. J. (1971) *Toxicol. Appl. Pharmacol.* **20**, 284-290
- Sipes, I. G., Stripp, B., Krishna, G., Maling, H. M. & Gillette, J. R. (1973) *Proc. Soc. Exp. Biol. Med.* **142**, 237-240

- Slater, R. F. & Sawyer, B. C. (1971) *Biochem. J.* **123**, 815–821
- Snedecor, G. W. & Cochran, W. G. (1967) in *Statistical Methods*, Iowa State University Press, Ames
- Spector, W. S. (1956) in *Handbook of Toxicology*, vol. 1, Saunders Co., Philadelphia
- Strubelt, O., Siegers, C. P. & Breining, H. (1974) *Arch. Toxikol.* **32**, 83–95
- Thurman, R. G. & Scholz, R. (1973) *Drug Metab. Dispos.* **1**, 441–448
- Tobon, F. & Mezey, E. (1971) *J. Lab. Clin. Med.* **77**, 110–121
- Traiger, G. J. & Plaa, G. L. (1971) *Toxicol. Appl. Pharmacol.* **20**, 105–112
- Traiger, G. J. & Plaa, G. L. (1972) *J. Pharmacol. Exp. Ther.* **183**, 481–488