Biochemical Pharmacology, Vol. 25, pp. 2197–2201. Pergamon Press, 1976. Printed in Great Britain.

THE EFFECT OF INHIBITORS OF ALCOHOL METABOLISM UPON THE CHANGES IN THE HEPATIC MICROSOMAL METABOLISM OF FOREIGN COMPOUNDS PRODUCED BY THE ACUTE ADMINISTRATION OF SOME ALCOHOLS TO THE RAT

GARTH POWIS and LINDSAY GRANT
Department of Pharmacology, University of Glasgow, Glasgow G12 8QQ, Scotland

(*Received* 16 *September* 1975; *accepted* 18 *March* 1976)

Abstract—Pyrazole administered to rats 24 hr previously produced an increase in hepatic microsomal aniline hydroxylation and a decrease in aminopyrine demethylation. This was a direct effect and not a consequence of the inhibition of the metabolism of endogenously produced ethanol. Acetaldehyde oxime had no effect upon aniline hydroxylation but produced a small decrease in aminopyrine demethylation. Both inhibitors of alcohol dehydrogenase potentiated the increase in aniline hydroxylation produced by submaximal doses of methanol and ethanol, suggesting that this was a direct effect produced by the alcohols themselves. The decrease in aminopyrine demethylation produced by methanol and ethanol was partly reversed by inhibitors of alcohol dehydrogenase, suggesting that this effect resulted from the metabolism of the alcohols. Formaldehyde and acetaldehyde administered to rats had no effect upon aniline hydroxylation but both produced a decrease in aminopyrine demethylation and in the type I spectral change. The inhibitory effect of submaximal doses of the aldehydes upon aminopyrine demethylation was potentiated by disulphiram, an inhibitor of aldehyde dehydrogenase. Inhibitors of alcohol dehydrogenase had no effect upon the increase in aniline hydroxylation produced by propan-2-ol, nor was their effect upon aminopyrine demethylation modified in any consistent way by propan-2-ol.

The administration to rats of a single oral dose of ethanol, and to a greater extent methanol, has been found in this laboratory to produce a selective induction of hepatic microsomal aniline hydroxylation, and a decrease in aminopyrine demethylation. There is, however, no change in any of the components of the microsomal mixed function oxidase. Propan-2-ol produces an increase in hepatic microsomal aniline hydroxylation, NADPH-cytochrome *c* reductase, NADPH-cytochrome P-450 reductase, with no effect or a small increase in the levels of aminopyrine demethylation [1]. The major pathway for the metabolism of ethanol and propan-2-ol *in vivo* in the rat, is thought to be through alcohol dehydrogenase [2, 3], whilst the metabolism of methanol may be through the concerted action of catalase and alcohol dehydrogenase [2]. Inhibitors of alcohol dehydrogenase are known to produce a marked decrease in the metabolism of all three alcohols *in vivo* [2, 3, 4].

The present study is an investigation of the direct effects of inhibitors of alcohol dehydrogenase upon the hepatic microsomal mixed function oxidase, and their action upon the effects of methanol, ethanol and propan-2-ol on this system. It was hoped that this might lead to an explanation of the differential effects of the alcohols upon the metabolism of various substrates, possibly in terms of different effects produced by the parent alcohols and their metabolites.

MATERIALS AND METHODS

Administration of drugs and chemicals. Alcohols and aldehydes were administered by stomach tube to unanaesthetised male Wistar albino rats weighing 200–250 g, as a 25% (v/v) solution in water. Pyrazole (200 mg/kg) and acetaldehyde oxime (200 mg/kg) dissolved in 0.9% NaCl, and 4-iodopyrazole (200 mg/kg) and dilsulphiram (300 mg/kg) dissolved in corn oil were administered by intraperitoneal injection 5 min before the administration of the alcohols or 2 hr before the administration of the aldehydes. Control animals were fed water or injected with saline or corn oil as appropriate. In some studies rats were fed for 5 days with neomycin sulphate added to the drinking water, giving a mean dose of 140 mg/day, and nystatin mixed with the standard diet, giving a mean dose of 15 mg/day, as described by Krebs and Perkins [13].

Preparation of the microsomal fraction and assays. The hepatic microsomal subcellular fraction was prepared by the method of Ernster *et al.* [5] in 0.25 M sucrose containing 0.05 M Tris HCl buffer, pH 7.4 and adjusted to give a protein concentration of 10 mg/ml. Protein was determined by the method of Lowry *et al.* [6] with crystalline bovine serum albumin in 0.25 M sucrose containing 0.05 M Tris–HCl buffer, pH 7.4, as a standard. The metabolism of foreign compounds by the microsomal fraction was measured over 30 min at 37° with a reaction mixture containing 4 mg of microsomal protein, Tris–HCl buffer, pH 7.4 (112 μmole), $MgCl_2$ (10 μmole), nicotinamide (50 μmole), glucose 6-phosphate (25 μmole), glucose 6-phosphate:$NADP^+$ oxidoreductase (EC 1.1.1.49) (2 units), $NADP^+$ (1 μmole) and either aminopyrine (4-dimethylaminoantipyrine) (10 μmole) or aniline (5 μmole), all in a final incubation volume of 2 ml. Formaldehyde formed from aminopyrine was

Vol. 25, No. 10

trapped with semicarbazide hydrochloride (10 μmole) and measured by the method of Nash [7]. The formation of *p*-aminophenol from aniline was measured by the method of Schenkman *et al.* [8]. The spectral changes produced by the interaction of 5 mM aminopyrine (type I compound) or 5 mM aniline (type II compound) with the microsomal fraction were measured by the method of Schenkman *et al.* [8].

Statistical treatment of results. Groups of data were subjected to a Student's *t*-test [9] to determine if a significant difference existed between the means of the groups of data, at the 5% level.

RESULTS

Effect of maximal doses of alcohols and inhibitors of alcohol dehydrogenase upon the hepatic microsomal metabolism of aniline and aminopyrine. The doses of alcohols which were found to be just sufficient to produce a maximal induction of aniline hydroxylation were methanol 125 m-mole/kg, ethanol 150 m-mole/kg and propan-2-ol 30 m-mole/kg. Doses of ethanol up to 200 m-mole/kg failed to produce a larger increase in aniline hydroxylation, despite the fact that it represented about only half the increase produced by smaller doses of methanol or propan-2-ol. Ethanol was, however, the most active in producing a decrease in aminopyrine demethylation, followed by methanol, whilst propan-2-ol had no significant effect (Table 1). Pyrazole, an inhibitor of alcohol dehydrogenase [10] produced an increase in aniline hydroxylation, thus confirming the observation of Lieber *et al.* [11], and a decrease in aminopyrine demethylation (Table 1). 4-Iodopyrazole (200 mg/kg), also an inhibitor of alcohol dehydrogenase [12], produced changes in metabolising activity similar to those of pyrazole (results not shown). Another reported inhibitor of alcohol dehydrogenase acetaldehyde oxime [4], had no effect on aniline hydroxylation and produced only a small non-significant decrease in aminopyrine demethylation.

The combination of an inhibitor of alcohol dehydrogenase and ethanol at a dose of 150 m-mole/kg, invariably proved fatal. The rats were, however, able to tolerate an inhibitor of alcohol dehydrogenase and methanol at a dose of 150 m-mole/kg and propan-2-ol at a dose of 40 m-mole/kg. Inhibition of alcohol dehydrogenase did not significantly potentiate the increase in aniline hydroxylation produced by either methanol or propan-2-ol. The decrease in aminopyrine demethylation produced by pyrazole was completely blocked when it was administered together with methanol, and reduced when administered together with propan-2-ol. Similar, although non-significant changes were observed when acetaldehyde oxime was administered together with either methanol or propan-2-ol. The ability to produce supramaximal changes in hepatic microsomal metabolism by the simultaneous administration of different agents has been regarded as evidence of different mechanisms of action [13]. The present work suggests that alcohols and inhibitors of alcohol dehydrogenase produce an increase in aniline hydroxylation by a similar mechanism, or it could be that some other factor indirectly involved in the increase becomes rate limiting. A combination of methanol and an inhibitor of alcohol dehydrogenase acted to prevent the decrease in aminopyrine demethylation found with either agent alone. It might be that methanol acts to produce an increase in aminopyrine demethylation, but that this is normally completely masked by some inhibitory effect dependent upon the metabolism of methanol.

Action of pyrazole in antibiotic pretreated animals. Because of the marked similarity between the effects of ethanol and pyrazole on the hepatic microsomal metabolising activity, it was considered a possibility that pyrazole might be producing its effects by preventing the metabolism of ethanol which is known to be continuously produced in the rat by the fermentation of the gastrointestinal contents [14]. Rats were, therefore, fed a combination of neomycin sulphate and nystatin which effectively sterilized the gastrointestinal tract, and which suppresses endogenous ethanol production [14]. Whilst this treatment itself decreased the hepatic microsomal metabolising activity, pyrazole still produced a significant increase in aniline hydroxylation and a decrease in aminopyrine demeth-

Table 1. Effect of inhibitors of alcohol dehydrogenase upon the changes in the hepatic microsomal metabolism of aniline and aminopyrine produced by supramaximal doses of alcohols

		Methanol	Ethanol	Propan-2-ol
	Control	Aniline hydroxylation (nmole/30 min per mg)		
No drug	11.1 ± 0.8	20.4 ± 0.4†	15.7 ± 0.9†	19.6 ± 1.8†
Pyrazole	15.4 ± 0.7*	18.6 ± 1.1†		20.7 ± 2.2†
Acetaldehyde oxime	11.4 ± 0.5	18.5 ± 1.0†	—	20.6 ± 1.9†
		Aminopyrine demethylation (nmole/30 min per mg)		
No drug	111.8 ± 11.7	75.2 ± 11.3†	54.7 ± 9.3†	118.9 ± 5.1
Pyrazole	58.1 ± 5.0*	116.9 ± 5.8*†	—	86.5 ± 11.6*†
Acetaldehyde oxime	78.3 ± 13.6	94.3 ± 4.9	—	91.2 ± 4.7*

Pyrazole (200 mg/kg) or acetaldehyde oxime (200 mg/kg) were administered to unanaesthetised male rats by i.p. injection 5 min before the oral administration of a dose of alcohol more than sufficient to produce a maximal increase in aniline hydroxylation, viz methanol, 150 m-mole/kg; ethanol, 150 m-mole/kg; and propan-2-ol, 40 m-mole/kg). The combination of ethanol at this dose and an inhibitor of alcohol dehydrogenase was, however, fatal. The hepatic microsomal fraction was prepared 24 hr later and metabolising activity determined as described in the text. Values represent the mean ± S.E.M. of six animals. *$P < 0.05$ compared with the value in the absence of an inhibitor of alcohol dehydrogenase. †$P < 0.05$ compared with the control value in the absence of an alcohol.

Table 2. Effect of antibiotic pretreatment upon the metabolism of aniline and aminopyrine by the hepatic microsomal fraction of rats administered pyrazole

	Aniline hydroxylation (nmole/30 min per mg)	Aminopyrine demethylation (nmole/30 min per mg)
Antibiotic fed	4.9 ± 0.6	34.9 ± 5.3
Antibiotic fed + pyrazole	10.0 ± 0.6*	22.9 ± 1.5*

Rats were fed with neomycin sulphate (140 mg/day) and nystatin (15 mg/day) in their food for five days, as described in the text. Pyrazole (200 mg/kg) was administered by i.p. injection 24 hr before the preparation of the hepatic microsomal fraction. Metabolism was determined as described in the text. Values represent the mean ± S.E.M. of eight animals *$P < 0.01$ compared with the values in the absence of pyrazole.

ylation (Table 2), indicating that it was not acting through endogenously produced ethanol.

Effect of inhibitors of alcohol dehydrogenase on the changes in hepatic microsomal metabolism produced by submaximal doses of alcohols. The submaximal doses of the alcohols used in this part of the study produced little or no change in microsomal aniline hydroxylation (Table 3). Both pyrazole and acetaldehyde oxime potentiated the effects of ethanol on aniline hydroxylation, transforming a small inhibition into a marked stimulation, greater than had been observed with even the highest doses of ethanol. Similar, although less marked, effects were observed on the changes produced by methanol with both pyrazole and acetaldehyde oxime. The effects of these inhibitors and propan-2-ol did not, however, appear to be synergistic.

The effect of inhibitors of alcohol dehydrogenase on the changes in aminopyrine demethylase activity were more difficult to interpret. Only acetaldehyde oxime and methanol interacted to produce a positive synergistic effect, transforming a small inhibition into a small but significant potentiation. Acetaldehyde oxime, however, appeared to have no effect upon aminopyrine demethylation in the presence of ethanol or propan-2-ol. Pyrazole had no effect upon the changes produced by either methanol or ethanol, but produced a greater inhibition when administered together with propan-2-ol.

Effect of aldehydes on hepatic microsomal metabolism. Formaldehyde and acetaldehyde were administered to rats 24 hr and 18 hr before the preparation of the hepatic microsomal fraction, to simulate more closely the continuous production of aldehyde from a large dose of alcohol. Neither formaldehyde nor acetaldehyde produced any significant change in the levels of microsomal aniline hydroxylase but both led to a significant decrease in aminopyrine demethylation (Table 4). A single dose of formaldehyde or acetaldehyde given 24 hr before death produced similar but less marked changes (results not shown). Both aldehydes produced a decrease in the type I spectral change, which might explain the decrease in the type I spectral change previously noted after administration of methanol or ethanol [1]. Formaldehyde produced a small although non-significant, and acetaldehyde a significant, decrease in the type II spectral change.

Effect of an inhibitor of aldehyde dehydrogenase on the changes in hepatic microsomal metabolism produced by submaximal doses of aldehydes. Disulphiram, an inhibitor of aldehyde dehydrogenase [15], itself produced an inhibition of aniline hydroxylation and aminopyrine demethylation (Table 5). Honjo and Netter [10] have previously reported that disulphiram administered to rats will inhibit hepatic microsomal aminopyrine demethylation, although only after a lag period of about 15 hr. Whilst formaldehyde and ace-

Table 3. Effect of inhibitors of alcohol dehydrogenase on the changes in the metabolism of aniline and aminopyrine produced by submaximal doses of methanol, ethanol, and propan-2-ol

		Methanol	Ethanol	Propan-2-ol
	Control	Aniline hydroxylation (n-mole/30 min per mg)		
No drug	11.1 ± 0.4	11.7 ± 1.0	9.4 ± 0.4*	13.1 ± 0.7†
Pyrazole	16.7 ± 0.8*	18.4 ± 0.9*	20.3 ± 1.4*†	16.1 ± 1.1*
Acetaldehyde oxime	12.3 ± 1.0	16.8 ± 0.8*†	21.9 ± 1.3*†	14.2 ± 1.0
		Aminopyrine demethylation (n-mole/30 min per mg)		
No drug	110.8 ± 5.1	90.8 ± 6.5†	82.9 ± 5.3†	104.6 ± 4.3
Pyrazole	78.5 ± 7.5*	86.3 ± 7.8	85.0 ± 6.5	60.9 ± 3.7*
Acetaldehyde oxime	89.6 ± 8.7	125.9 ± 3.1*†	86.1 ± 4.8	102.5 ± 4.8

Pyrazole (200 mg/kg) or acetaldehyde oxime (200 mg/kg) were administered to unanaesthetised male rats by i.p. injection 5 min before the oral administration of a submaximal dose of methanol or ethanol (85 m-mole/kg) or propan-2-ol (20 m-mole/kg). The hepatic microsomal fraction was prepared 24 hr later and metabolising activity determined as described in the text. Values represent the mean ± S.E.M. of six animals. *$P < 0.05$ compared with the value in the absence of an inhibitor of alcohol dehydrogenase. †$P < 0.05$ compared with the control value in the absence of an alcohol.

Table 4. Effect of formaldehyde and acetaldehyde on the hepatic microsomal metabolism of aniline and aminopyrine

	Aniline hydroxylation (n-mole/30 min per mg)	Aminopyrine demethylation (n-mole/30 min per mg)	Spectral Changes	
			Type I (E385–417/g)	Type II (E429–395/g)
Control	11.2 ± 0.6	105.3 ± 5.8	7.3 ± 0.9	18.5 ± 2.0
Formaldehyde	14.0 ± 1.2	61.6 ± 5.1*	2.1 ± 0.5*	15.0 ± 1.1
Acetaldehyde	10.6 ± 0.7	75.5 ± 5.3*	3.3 ± 0.6*	11.4 ± 0.9*

Formaldehyde (13 m-mole/kg) and acetaldehyde (22 m-mole/kg) were fed by stomach tube to unanaesthetised male rats 24 hr and 18 hr before death and preparation of the hepatic microsomal fraction. Metabolising activity was determined as described in the text. The spectral changes represent the peak-to-trough changes caused by the addition of 5 mM aminopyrine (type I) and 5 mM aniline (type II) to a cuvette containing the microsomal fraction (2 mg protein/ml) and for convenience these results are expressed per g of microsomal protein. Values represent the mean ± S.E.M. of six animals. *$P < 0.01$ compared with the control value.

taldehyde at these lower doses produced only a small decrease in aminopyrine demethylation, in the presence of disulphiram there was a marked increase in the fractional inhibition of aminopyrine demethylation produced by both aldehydes.

DISCUSSION

Ethanol and methanol administered to rats as a single dose have the unusual effect of producing a selective induction of aniline hydroxylation, a decrease in aminopyrine demethylation, and no change in the components of the mixed function oxidase, apart from a decreased spectral change produced by the interaction of substrates with cytochrome P-450 [1]. Propan-2-ol on the other hand, produces an induction of aniline hydroxylation but has no effect upon aminopyrine demethylation, despite an increase in NADPH-cytochrome P-450 reductase, which it has been suggested is rate limiting in the microsomal metabolism of foreign compounds [17]. The present study reveals nothing of the molecular mechanisms underlying these changes, but it does show the relative contribution of the alcohols and their metabolites to the differential effects observed on the metabolism of aniline and aminopyrine.

Pyrazole, a widely used inhibitor of alcohol dehydrogenase [4, 10, 11], itself produced changes in the hepatic microsomal mixed function oxidase very similar to those produced by ethanol, so much so that it had to be considered that pyrazole might be producing its effects by allowing an accumulation of alcohol which is normally formed in, and absorbed from, the intestinal tract of the rat [14]. This possibility was, however, eliminated by the finding that pyrazole still exerted its effects in rats fed with antibiotics, which markedly reduce the amount of alcohol produced in the gastrointestinal tract [14]. Acetaldehyde oxime, an inhibitor of alcohol dehydrogenase equipotent with pyrazole [4] failed to give rise to an increase in aniline hydroxylation and produced only a small and nonsignificant decrease in aminopyrine demethylation. The finding that the effects of the inhibitors of alcohol dehydrogenase and methanol and propan-2-ol at supramaximal doses, were not additive, might suggest a similar mechanism of action, although it is also a possibility that some other factor only involved indirectly in the changes in metabolism might become rate limiting.

Inhibitors of alcohol dehydrogenase are known to produce a marked decrease in the metabolism of methanol, ethanol and propan-2-ol *in vivo*, the metabolism of ethanol showing the greatest inhibition [4]. Both pyrazole and acetaldehyde oxime potentiated the increase in aniline hydroxylation produced by a submaximal dose of ethanol, and to a lesser extent

Table 5. Effect of an inhibitor of aldehyde dehydrogenase upon the changes in the hepatic microsomal metabolism of aniline and aminopyrine produced by submaximal doses of aldehydes

	Control	Formaldehyde	Acetaldehyde
	Aniline hydroxylation (n-mole/30 min per mg)		
No drug	11.1 ± 0.3	11.1 ± 0.9	12.2 ± 0.7
Disulphiram	4.9 ± 0.4*	4.1 ± 0.4*	2.6 ± 0.2*†
	Aminopyrine demethylation (n-mole/30 min per mg)		
No drug	161.9 ± 5.7	136.8 ± 4.6†	157.0 ± 12.8
Disulphiram	70.9 ± 3.6*	37.2 ± 1.7*†	37.9 ± 5.1*†

Disulphiram (300 mg/kg) was administered to unanaesthetised male rats by i.p. injection 2 hr before the oral administration of formaldehyde (5 m-mole/kg) or acetaldehyde (11 m-mole/kg). A second oral dose of formaldehyde or acetaldehyde was given 6 hr later. The animals were killed and the hepatic microsomal fraction prepared 24 hr after the first dose of aldehyde. Metabolising activity was determined as described in the text. Values represent the ±S.E.M. of six animals. *$P < 0.05$ compared with the value in the absence of disulphiram. †$P < 0.05$ compared with the control value in the absence of an aldehyde.

methanol, and appeared to reverse, at least in part, the decrease in aminopyrine demethylation produced by both alcohols. These results suggest that the increase in aniline hydroxylation is a direct consequence of the action of methanol and ethanol themselves, whilst the decrease in aminopyrine demethylation may be a consequence of the metabolism of the alcohols by alcohol dehydrogenase. There is also some evidence which suggests that methanol, at least, might be producing an increase in aminopyrine demethylation but that this is normally masked by an inhibition which is dependent on the metabolism of methanol. The administration of formaldehyde and acetaldehyde, had no effect upon aniline hydroxylation but both aldehydes produced a decrease in aminopyrine demethylation. This is likely to be a direct effect of the aldehydes themselves since disulphiram, an inhibitor of aldehyde dehydrogenase, potentiated the inhibition produced by submaximal doses of both aldehydes.

Pyrazole and acetaldehyde oxime had no significant effect upon the increase in aniline hydroxylation produced by propan-2-ol, nor was their inhibitory effect upon aminopyrine demethylation modified in any consistent way by the presence of propan-2-ol. Propan-2-ol is metabolised in the rat to acetone [3, 18] which may account for some of the effects of propan-2-ol, such as the potentiation of the hepatotoxic effects of carbon tetrachloride and chlorinated hydrocarbons [19, 20]. Acetone has been found to induce microsomal aniline hydroxylation but to have no effect upon aminopyrine demethylation [21, 22]. Assuming that, as has been reported, inhibitors of alcohol dehydrogenase effectively inhibit the metabolism of propan-2-ol [3], the present results may be interpreted to suggest that at low doses of propan-2-ol, acetone may account for a large part of the increase in aniline hydroxylation, whilst at higher dose propan-2-ol itself might exhibit some inducing activity.

Acknowledgement—We are grateful for a grant from the Rankin Research Fund of Glasgow University.

REFERENCES

1. G. Powis, *Biochem. J.* **148,** 269 (1975).
2. E. Feytmans and F. Leighton, *Biochem. Pharmac.* **22,** 349 (1973).
3. R. Nordmann, C. Ribiere, H. Rouach, F. Beauge, Y. Giudicellie and J. Nordmann, *Life Sci.* **13,** 919 (1973).
4. D. Lester and G. D. Benson, *Science, N.Y.* **169,** 282 (1970).
5. L. Ernster, P. Siekevitz and G. E. Palade, *J. Cell Biol.* **15,** 541 (1962).
6. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193,** 265 (1951).
7. T. Nash, *Biochem. J.* **55,** 416 (1953).
8. J. B. Schenkman, H. Remmer and R. W. Estabrook, *Molec. Pharmac.* **3,** 113 (1967).
9. G. W. Snedecor and W. G. Cochran, in *Statistical Methods.* Iowa State University Press, Ames (1967).
10. L. Goldberg and U. Rydberg, *Biochem. Pharmac.* **18,** 1749 (1969).
11. C. S. Lieber, E. Rubin, B. A. DeCarli, P. Misra and H. Gang, *Lab. Invest.* **22,** 615 (1970).
12. H. Theorell, T. Yonetani and G. Sjoberg, *Acta chem. scand.* **23,** 255 (1969).
13. A. H. Conney, *Pharmac. Rev.* **19,** 317 (1967).
14. H. A. Krebs and J. R. Perkins, *Biochem. J.* **118,** 635 (1970).
15. R. A. Deitrich and V. G. Erwin, *Molec. Pharmac.* **7,** 301 (1971).
16. T. Honjo and K. J. Netter, *Biochem. Pharmac.* **18,** 2681 (1969).
17. J. R. Gillette and T. E. Gram, in *Microsomes and Drug Oxidations* (Eds. J. R. Gillette, A. H. Conney, G. J. Cosmides, R. W. Estabrook, J. R. Fouts and G. J. Mannering) pp. 133–149. Academic Press, New York (1969).
18. G. J. Traiger and G. L. Plaa, *J. Pharmac. exp. Ther.* **183,** 481 (1972).
19. G. J. Traiger and G. L. Plaa, *Can. J. Physiol. Pharmac.* **51,** 291 (1973).
20. G. J. Traiger and G. L. Plaa, *Archs environ. Health* **28,** 276 (1974).
21. I. G. Sipes, B. Stripp, G. Krishna, H. M. Maling and J. R. Gillette, *Proc. Soc. exp. Biol. Med.* **142,** 237 (1973).
22. H. Clark and G. Powis, *Biochem. Pharmac.* **23,** 1015 (1974).