Biochemical Pharmacology, Vol. 25, pp. 329–335. Pergamon Press, 1976. Printed in Great Britain.

EFFECT OF CHRONIC ETHANOL TREATMENT ON METABOLISM OF DRUGS *IN VITRO* AND *IN VIVO*

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(*Received* 21 *January* 1975; *accepted* 11 *April* 1975)

Abstract—A daily intake of ethanol ranging from 10 to 12 g/kg for 1 month in either high-fat adequate protein (HFAP) or low-fat high protein (LFHP) liquid diets resulted in significant increases in liver weight, microsomal protein and microsomal metabolism of aminopyrine, zoxazolamine, aniline and meprobamate, when related to 100 g body weight. Morphine metabolism was increased only after the HFAP diet; pentobarbital metabolism was studied only after HFAP diet, and was increased. The increase *in vitro* was highest with aminopyrine and lowest with pentobarbital. The analysis of variance showed highly significant differences among the various drugs. There were significant interaction effects of drugs × ethanol and drugs × fat content, but no significant interaction between ethanol × fat or drugs × ethanol × fat except in the case of morphine. The marked between-drug variability in induction of metabolism *in vitro* by ethanol is also reflected *in vivo* among the various drugs examined. The increase *in vivo* was greatest with meprobamate, intermediate with aniline and zoxazolamine, low with aminopyrine and absent with pentobarbital. Moreover, there was variability in inductive effect of ethanol *in vivo* as compared to *in vitro*. It is, therefore, concluded that chronic ethanol administration does increase the metabolism of drugs *in vitro* and *in vivo*, but the diversity of effects on different drugs cannot be explained by a single mechanism based on an increase in the amount of cytochrome P-450 or other component of the mixed function oxidase system.

In a previous study [1] we reported that cross-tolerance to pentobarbital in alcohol-treated rats resulted primarily from reduced sensitivity of the central nervous system, and that changes in barbiturate metabolism or distribution do not play an important role. Similar findings have also been recently reported by Hatfield *et al.* [2]. On the other hand, it has also been reported that chronic administration of ethanol causes proliferation of the smooth endoplasmic reticulum [3–5] resulting in an increased ability of the microsomes to metabolize a variety of drugs such as pentobarbital, aniline, benzpyrene, nitrobenzoic acid and aminopyrine [4, 6–12].

However, it has not been established whether the inductive action of ethanol on metabolism *in vitro* is comparable to that on metabolism *in vivo*. In fact, only two studies [12, 13] are available which compare the effect of chronic alcohol on rates of microsomal enzyme activity *in vitro* and of drug disappearance *in vivo*. In one of these studies [12], the clearance of meprobamate was studied in both rats and humans and pentobarbital disappearance was examined only in humans. In the other study [13], warfarin, zoxazolamine and hexobarbital metabolism *in vitro* (by microsomes) and *in vivo* (clearance from blood) was studied in rats 17 hr after a single dose (4·71 g/kg) of ethanol and after regular administration for 7–11 days. No changes were observed with either treatment in metabolism of warfarin *in vitro* or *in vivo*. However, the authors found marked increases in metabolism of the other two drugs *in vitro* and *in vivo* after even a single dose of ethanol. Surprisingly enough, a single dose, which causes no hypertrophy of the smooth endoplasmic reticulum (SER), produced the maximum increase in drug metabolism, and chronic ethanol intake did not result in further increase. This report appears to conflict with the work of Kato and Chiesara [14], who found no effect of a single dose of ethanol (4 g/kg) on pentobarbital metabolism *in vivo* or *in vitro* 48 hr later. Since a single dose of corticosterone has been reported to increase the rate of metabolism of hexobarbital and ethylmorphine [15,16], the apparent discrepancy may be related to the duration of a stress response after a single large dose of ethanol. The report of Mallov and Baesl [13] also appears to disagree with the finding of increased warfarin metabolism in human alcoholics [17].

The finding of increased activity of microsomal enzymes after chronic administration of ethanol has also been questioned recently. Nakanishi *et al.* [18] reported identical metabolism *in vitro* of hexobarbital, aminopyrine and aniline in ethanol-treated and control rats, when ethanol was replaced by tap water 24 hr prior to sacrifice. They did, however, report an increase in aniline hydroxylase activity in rats continuing to drink ethanol *ad lib.* up to the time of sacrifice. Ramsey and Fallon [19] also found increased cytochrome P-450 and aniline hydroxylase, but reported a decrease in aminopyrine and benzphetamine demethylation in both male and female rats chronically treated with ethanol. Ioannides and Parke [20] reported no change in the half-life of plasma pentobarbital after chronic ingestion of 10% ethanol by rats.

Dietary composition is also known to have a marked effect on hepatic drug metabolism [21–24] and its inducibility [25, 26]. Moreover, the level of fat in the diet has been reported to influence markedly the degree of SER proliferation and increased microsomal drug-metabolizing activity caused by chronic ingestion of ethanol [27, 28].

Because of the limited number of studies available on the influence of chronic ethanol administration on drug metabolism *in vivo*, and the apparently conflicting findings on microsomal induction, we have compared the effects of chronic ethanol ingestion on the metabolism both *in vitro* and *in vivo* of a variety of drugs involving different enzymatic activities; the effects on metabolism *in vitro* were further compared in rats fed diets of different composition.

MATERIALS AND METHODS

Animals. Male Wistar Rats, weighing 100–150 g, obtained from High Oak Ranch, Goodwood, Ontario, were used in all experiments.

Chronic ethanol treatment. Ethanol was administered in a liquid diet, modified from that devised by Lieber *et al.* [29], as described previously [30]. Two types of diets were used. In the first (high-fat, adequate protein), ethanol provided 35% of the total calories, fat 41%, protein hydrolysate 19% and sucrose 5%. In the second (low-fat, high-protein diet) ethanol provided 35% of the total calories, fat 10%, protein hydrolysate 25% and sucrose 30%. In the corresponding control diets, ethanol was replaced with equicaloric concentrations of sucrose. The amount of liquid diet consumed by each rat in the ethanol group was measured daily, and an equal amount of the sucrose liquid diet was given to its pair-fed control. These diets were administered for 4 weeks and the daily intake of ethanol ranged from 10 to 12 g/kg throughout the experimental period. The diets were replaced by tap water in both ethanol and control groups 16hr prior to the studies *in vitro* and *in vivo* described below.

Preparation of microsomes. The rats were killed by decapitation and livers were removed, weighed and homogenized with 2 vol. of 1·15% KCl in a glass Potter–Elvehjem homogenizer with a motor-driven Teflon pestle. The homogenates were centrifuged at 9,000 *g* for 20 min in the SM head of a Servall RC-2B. The supernatants were decanted and recentrifuged for an additional 20 min. The supernatants were again decanted and then centrifuged at 105,000 *g* in the A 147 head of a model B-60 International preparation ultracentrifuge for 1 hr at 4°. The resulting supernatant was then poured off and the pellet suspended in 1·15% KCl solution. Microsomal protein content was determined by the biuret reaction [31].

Drug metabolism in vitro. For all the drugs mentioned below, preliminary experiments were carried out to determine the limits within which the rate of metabolism was linearly related to time and to the amount of microsomal preparation. These parameters were then kept well within the determined limits in all subsequent experiments.

The rate of demethylation of aminopyrine was measured in a system containing 300 μmoles phosphate buffer, pH 7·4; NADP, 6 μmoles; glucose 6-phosphate, 60 μmoles; nicotinamide, 50 μmoles; semicarbazide HCl, 50 μmoles; $MgCl_2$, 50 μmoles; glucose 6-phosphate dehydrogenase, 5 units; and aminopyrine, 10 μmoles, in a final volume of 4·2 ml. The reaction mixture was preincubated for 5 min at 37° in air in a Dubnoff shaker and the reaction was started by adding 0·8 ml microsome suspension (equivalent to 0·4 g liver). Samples were incubated for 20 min and the reaction was stopped by adding 2 ml of $ZnSO_4$ (3%) solution. After 3 min of shaking, 2 ml saturated $Ba(OH)_2$ solution was added with mixing and the flasks were shaken for another 5 min. The content of the flask was then transferred to a centrifuge tube and centrifuged at 12,000 *g* for 10 min. Formaldehyde formed was measured in the supernatant by the procedure of Nash [32]. Appropriate microsomal and reagent blanks were run with each experiment. In all experiments, each sample was run in duplicate.

Morphine demethylation was measured with the same incubation system as used for aminopyrine; 25 μmoles morphine sulfate was used in each incubation flask.

Zoxazolamine hydroxylation by microsomes was studied under the conditions described by Mallov and Baesl [13] and zoxazolamine was estimated by the method of Burns *et al.* [33]. The selected parameters were: microsomes equivalent to 0·12 g liver, 0·59 μmole zoxazolamine and 20 min of incubation in a total volume of 3 ml.

Aniline hydroxylase activity was determined by measuring the formation of *p*-aminophenol as described by Schenkman *et al.* [34]. Incubation parameters were: microsomes equivalent to 0·15 g liver, 25 μmoles aniline and 20 min of incubation in a final volume of 3 ml.

For studies on meprobamate hydroxylation, each flack contained 150 μmoles phosphate buffer, pH 7·4; liver microsomes equivalent to 0·5 g liver; nicotinamide, 20 μmoles; $MgCl_2$, 6 H_2O, 50 μmoles; glucose 6-phosphate, 50 μmoles; NADP, 8 μmoles; and glucose 6-phosphate dehydrogenase, 5 units, in a final volume of 4 ml. After a 5-min preincubation period at 37° in a Dubnoff shaker bath, the reaction was started by adding 1 ml [^{14}C]meprobamate (final concentration 0·3 mM). Incubation was stopped at 15 min by immersing the flasks in ice water. Each liver preparation was tested in duplicate, accompanied by appropriate standards and blanks. Residual unchanged meprobamate was extracted by the method of Hoffman and Ludwig [35], and measured by scintillation counting with a Nuclear Chicago, model 725, liquid scintillation counter.

The incubation mixture and procedure for studies on pentobarbital metabolism have been described recently [36].

Pentobarbital and meprobamate disappearance in vivo. The method used for measuring the rate of [^{14}C]pentobarbital disappearance from the blood after intravenous injection has been described elsewhere [36]. The same procedure was used for studying [^{14}C]meprobamate disappearance. A dose of 30 mg/kg of [^{14}C]meprobamate (sp. act., 0·24 μCi/mg; Mallinkrodt Nuclear, St Louis, Mo.) was dissolved in saline, in a volume of 0·25 ml/100 g body weight, for intravenous injection. Blood samples (100 μl) were taken from the cut tip of the tail at various intervals up to 180 min after injection, for drug assay. Unmetabolized meprobamate was extracted by the method of Hoffman and Ludwig [35]; the method for pentobarbital has been described [36].

Whole body metabolism of drugs. The disappearance of drugs from the whole body was studied in ethanol-

Table 1. Effect of chronic ethanol treatment (with high- and low-fat diets) on body weight, liver weight and microsomal protein*

	High-fat diet (n = 6)			Low-fat diet (n = 6)		
	Control	Ethanol	P	Control	Ethanol	P
Body wt	278 ± 11	275 ± 11	NS	311 ± 12	314 ± 10	NS
Liver wt/body wt (g/100 g)	3·52 ± 0·06	4·70 ± 0·08	< 0·001	3·74 ± 0·10	4.17 ± 0·12	< 0·01
Microsomal protein (mg/g liver)	35·9 ± 1·3	45·5 ± 7·4	NS	30·1 ± 1·4	41·5 ± 3	< 0·05
Microsomal protein (in total wt of fresh liver/100 g body wt)	126·1 ± 3·7	211·7 ± 31	< 0·05	112·3 ± 3·8	173·4 ± 14·7	< 0·02

* Values shown are mean ± S. E. M. P refers to the significance of difference between chronic ethanol group and the pair-fed controls as estimated by the *t*-test for paired data. NS = not significant (P > 0·05).

treated and pair-fed rats after intraperitoneal injection of the drug in question. The rats were killed by a blow on the head, immediately dropped into a pre-cooled industrial Waring blender and homogenized. The homogenate was filtered through several layers of gauze and the amount of the drug remaining in an aliquot of the homogenate was determined. The dose of the drug and time of killing are described in Table 4. Aminopyrine, zoxazolamine and aniline were determined by the methods of Brodie and Axelrod [37], Burns *et al.* [33] and Brodie and Axelrod [38] respectively.

RESULTS

Effect of chronic ethanol treatment on body weight, liver weight and microsomal protein. Table 1 shows the body weights, liver weights and microsomal protein content of the control and alcohol-treated groups on high-fat and low-fat diets. With either diet, there was no significant difference in body weight between the ethanol-treated and pair-fed controls. However, the total gain, as reflected by the final body weight, was greater in the alcohol groups fed the low-fat diet than in those on the high-fat diet (< 0·05). The differ-

Table 2. Effect of chronic ethanol treatment (with high-fat diets) on drug metabolism *in vitro* by microsomes*

Drug	No. of pairs	Control	Ethanol	% Change	P
Aminopyrine†	6				
Activity/mg protein		0·102 ± 0·009	0·117 ± 0·013	+15	NS
Activity/g liver		3·68 ± 0·39	4·96 ± 0·57	+35	< 0·005
Activity/100 g body wt		12·90 ± 1·25	23·3 ± 2·6	+81	< 0·001
Morphine†	6				
Activity/mg protein		0·064 ± 0·003	0·059 ± 0·005	−19	NS
Activity/g liver		2·28 ± 0·123	2·51 ± 0·20	+10	NS
Activity/100 g body wt		8·01 ± 0·30	11·8 ± 0·81	+47	< 0·01
Zoxazolamine‡	6				
Activity/mg protein		0·052 ± 0·002	0·049 ± 0·004	−6	NS
Activity/g liver		1·87 ± 0·10	2·08 ± 0·17	+11	NS
Activity/100 g body wt		6·56 ± 0·30	9·75 ± 0·71	+49	< 0·01
Aniline§	6				
Activity/mg protein		0·028 ± 0·001	0·027 ± 0·003	−4	NS
Activity/g liver		1·00 ± 0·04	1·13 ± 0·08	+13	NS
Activity/100 g body wt		3·56 ± 0·15	5·29 ± 0·37	+49	< 0·005
Meprobamate‖	10				
Activity/mg protein		0·0144 ± 0·0012	0·0134 ± 0·0007	−6·9	NS
Activity/g liver		0·410 ± 0·023	0·458 ± 0·041	+11·7	NS
Activity/100 g body wt		1·33 ± 0·08	2·20 ± 0·22	+65	< 0·005
Pentobarbital¶	7				
Activity/g liver		0·751 ± 0·026	0·769 ± 0·034	+2·4	NS
Activity/100 g body wt		3·07 ± 0·12	3·84 ± 0·14	+25	< 0·005

* Values shown are mean ± S. E. M. P refers to the significance of difference between chronic ethanol group and the pair-fed controls as estimated by the *t*-test for paired data. NS = not significant (P > 0·05).

† Expressed as μmoles HCHO produced/20 min of incubation.

‡ Expressed as μmoles zoxazolamine metabolized/20 min of incubation.

§ Expressed as μmoles of *para*-aminophenol formed/20 min of incubation.

‖ Expressed as μmoles meprobamate metabolized/15 min of incubation.

¶ Expressed as μmoles pentobarbital metabolized in 20 min using the 9,000 *g* supernatant.

Table 3. Effect of chronic ethanol treatment (with low-fat diets) on drug metabolism *in vitro* by microsomes*

Drug	No. of pairs	Control	Ethanol	% Change	P
Aminopyrine	6				
Activity/mg protein		0·097 ± 0·010	0·084 ± 0·016	−13	NS
Activity/g liver		2·83 ± 0·23	4·05 ± 0·45	+43	< 0·05
Activity/100 g body wt		10·61 ± 0·97	16·9 ± 1·95	+59	< 0·02
Morphine	6				
Activity/mg protein		0·080 ± 0·003	0·06 ± 0·003	−25	NS
Activity/g liver		2·42 ± 0·09	2·5 ± 0·18	+3	NS
Activity/100 g body wt		9·04 ± 0·35	10·27 ± 0·70	+14	NS
Zoxazolamine	6				
Activity/mg protein		0·048 ± 0·003	0·046 ± 0·004	−4	NS
Activity/g liver		1·46 ± 0·11	1·84 ± 0·06	+26	< 0·025
Activity/100 g body wt		5·41 ± 0·29	7·64 ± 0·26	+41	< 0·005
Aniline	6				
Activity/mg protein		0·026 ± 0·002	0·023 ± 0·001	−12	NS
Activity/g liver		0·784 ± 0·07	0·93 ± 0·06	+19	NS
Activity/100 g body wt		2·91 ± 0·51	3·89 ± 0·26	+34	< 0·02
Meprobamate	11				
Activity/mg protein		0·0143 ± 0·0005	0·0137 ± 0·0005	−4·2	NS
Activity/g liver		0·408 ± 0·014	0·526 ± 0·021	+28·9	< 0·001
Activity/100 g body wt		1·15 ± 0·04	2·07 ± 0·10	+37·1	< 0·001
Pentobarbital					
Activity/g liver			ND		
Activity/100 g body wt			ND		

* Enzyme activities and statistical comparisons as defined in Table 2.
ND = not determined.

ence for the corresponding controls fell just short of statistical significance.

In comparison with the control rats, the ethanol-treated groups on both diets showed significant increases in liver weight and microsomal protein when related to body weight. The increase of liver weight and microsomal protein after ethanol was, however, more pronounced with a high-fat diet than with a low-fat diet.

The effects of chronic ethanol treatment on metabolism *in vitro* of various drugs by microsomes are shown in Tables 2 and 3. With both types of diet, metabolism of aminopyrine, morphine, zoxazolamine, aniline and meprobamate by microsomes from the ethanol-treated animals was not significantly different from their pair-fed controls when the drug-metabolizing activity was calculated per mg of microsomal protein. When expressed per g of liver, significant increases were obtained with aminopyrine, meprobamate and zoxazolamine after the alcohol low-fat diet, and only with aminopyrine after the alcohol high-fat diet. No such differences in activity were seen with respect to other drugs examined. When related to 100 g body weight, significant increases in drug metabolism were obtained with all the drugs studied (except morphine on a low-fat diet). The increase was highest with aminopyrine (81 per cent) and lowest with pentobarbital (25 per cent).

Metabolism of the first four drugs listed in Tables 2 and 3 was studied in parallel with each individual liver preparation. It was, therefore, possible to carry out an analysis of variance for the separate effects of ethanol and of dietary fat level across all four drugs. The analysis confirmed the highly significant differences among the various drugs, when the respective activities were expressed in terms of total liver per 100 g body weight (F, 602·75; df 3, 30; $P < 0·001$). The activities were consistently increased by ethanol (F, 100·37; df 1, 10; $P < 0·001$) and by a high-fat diet (F, 6·160; df 1, 10; $P < 0·05$). Each of these factors affected the metabolism of the various drugs to different degrees, as shown by significant interaction effects of drugs × ethanol (F, 8·258; df 3, 30; $P < 0·001$) and drugs × fat content (F, 4·087; df 3, 30; $P < 0·05$). But there was no significant interaction between ethanol and fat content, or drugs × ethanol × fat content, except in the case of morphine. Essentially the same results were obtained when the analysis of variance was applied to the activities per g of liver. A separate comparison for meprobamate metabolism, which was studied in different animals, confirmed that the increase produced by ethanol on the high-fat diet did not differ significantly from that produced on the low-fat diet.

[^{14}C]*pentobarbital clearance* in vivo. Figure 1 shows the disappearance from blood of [^{14}C]pentobarbital, injected intravenously, in ethanol-treated and pair-fed controls on a high-fat diet. The apparent volumes of distribution of pentobarbital in the two groups are the same, since the extrapolated disappearance curves have identical vertical intercepts. There is also no difference between the two groups with respect to the disappearance of pentobarbital as the calculated slopes (ethanol, $-0·0069 \pm 0·0004$; controls, $-0·0066 \pm 0·0002$) are not significantly different.

[^{14}C]*meprobamate disappearance* in vivo. The results on meprobamate disappearance are shown in Fig. 2. The mean half-life of meprobamate blood disappearance in chronically ethanol-treated rats was $44·67 \pm 2·77$ min, as compared to $61·56 \pm 3·66$ in the controls. The difference is statistically significant ($P < 0·001$). Again, comparison of the meprobamate levels at zero time in the ethanol-treated and pair-fed controls showed no significant difference; this indi-

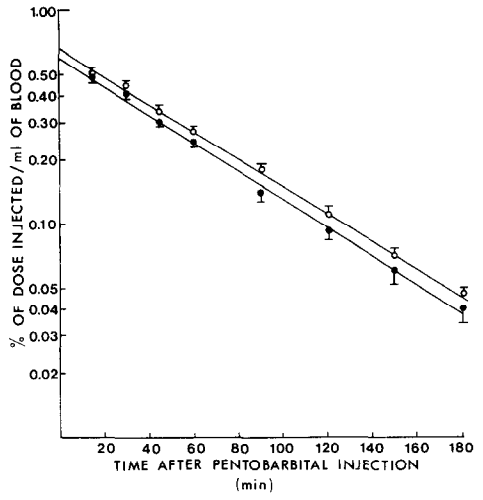

Fig. 1. Disappearance of [^{14}C]pentobarbital from blood in ethanol-treated (●——●) and pair-fed controls (○——○); n = 9 pairs. Vertical bars represent S. E. M. Regression lines were calculated by the method of least mean squares.

cated that the differences in half-life were not due to changes in distribution.

Whole body metabolism. The results on whole body metabolism are summarized in Table 4. Comparison of chronic ethanol-treated vs controls showed a significant increase in metabolism with the three drugs examined. In this experiment, in contrast to that corresponding to Table 1, the body weights of the controls were significantly greater than those of the alcohol-treated animals.

DISCUSSION

The absence of any significant effect of chronic ethanol treatment on the rate of disappearance of pentobarbital from the blood (Fig.1) is in agreement with our earlier finding [1]. The present work, involving larger numbers of animals, more sampling times, and a more accurate measurement technique (radioactivity counting vs gas–liquid chromatography) for the range of drug concentrations used, lends greater weight to the negative finding.

The present results also confirm a variability, noted in previous work by ourselves [39] and others, in the ability of ethanol-containing diets to sustain growth equal to that of the pair-fed controls. In the earlier study [39], less growth than in controls was found in animals receiving ethanol in high-fat diet, while equal growth was noted when the ethanol was in a high-protein, low-fat diet. In the present study, however, the same two diets both supported growth

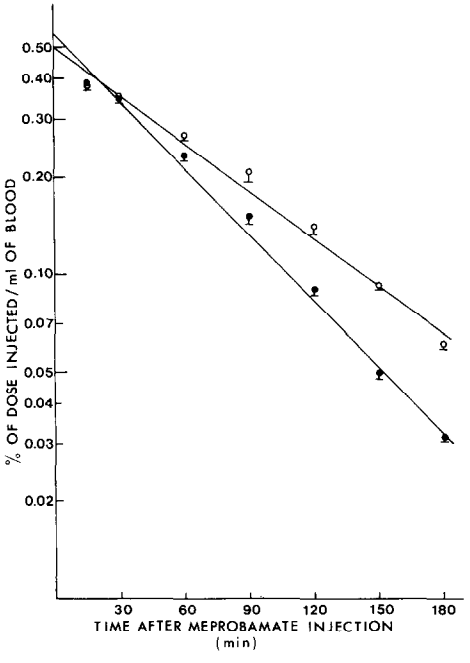

Fig. 2. Disappearance of [^{14}C]meprobamate from blood in ethanol-treated (●——●) and pair-fed controls (○——○); n = 8 pairs. Vertical bars represent S. E. M. Regression lines were calculated by the method of least mean squares.

Table 4. Whole body metabolism of aniline, zoxazolamine and aminopyrine in ethanol-treated animals and pair-fed controls
(High-fat diet)

Drug	No. of rats	Body wt* (g)	Dose (mg/kg)	Time of sacrifice (min)	Metabolism (mg/100 g body wt)	% Increase
Aniline						
Controls	9	227 + 7	30	30	1·68 ± 0·05	
Ethanol-treated	9	200 + 11	30	30	2·33 + 0·11	38
P					< 0·001	
Zoxazolamine						
Controls	7	242 ± 6	75	60	3·46 ± 0·19	
Ethanol-treated	7	213 ± 6	75	60	4·44 ± 0·35	32
P					< 0·01	
Aminopyrine						
Controls	5	205 ± 5	100	60	6·70 ± 0·50	
Ethanol-treated	5	186 ± 12	100	60	7·79 ± 0·60	16
P					< 0·025	

* Since there was a single pool of ethanol-treated animals and a single pool of controls from which the individual subjects for the three drug studies were drawn, body weight comparison was made between the two pools. The controls were significantly heavier than alcohol-treated animals (P < 0·001).

rates equal to those in the corresponding controls in one experiment (Table 1), while less growth was again found in the alcohol high-fat group in another experiment (Table 4). No explanation is yet available for this variability, but it is obvious that some factor other than known dietary composition and alcohol must be involved.

With respect to the effects of chronic ethanol ingestion and diet on drug metabolism, the analysis of variance showed clearly that the various drugs do not respond in the same manner. *In vitro*, there was no increase in specific activity of metabolism of any of the drugs (per mg of protein) with either diet. This finding differs from those of Idéo *et al.* [10] and Tobon and Mezey [40], as well as from our own findings in other experiments. This point is discussed in the accompanying paper [41]. The activity per g of liver was increased for aminopyrine on both diets, and for zoxazolamine and meprobamate only on the low-fat diet. The total activity in relation to body weight was increased for morphine only on high-fat diet, but for the other drugs on both diets. This between-drug variability in induction is also reflected among the three drugs in Table 4, and the difference in treatment effects shown in Figs. 1 and 2. If induction of drug metabolism by ethanol were explainable entirely on the basis of the demonstrated increases in activity of NADPH-cytochrome P-450 reductase [42], or in the concentration of cytochrome P-450 [7, 8, 12, 13], the effects on metabolism of the various drugs by the mixed function oxidase system would be expected to be proportionately the same. The observed differences suggest that some other factor is involved.

One obvious possibility is the influence of diet. However, though dietary fat content was found to have a significant influence of its own on the drug-metabolizing activities *in vitro*, it did not significantly modify the effects of ethanol. It is not even certain that the influence of diet was directly attributable to the fat level, since the low-fat diet was also a high-sucrose diet. Conceivably, the difference in activities found with the two diets may be due to an inhibitory effect of sucrose rather than a stimulatory effect of fat. Nevertheless, the significant drugs × diet interaction, like that of drugs × ethanol, raises the possibility that other constituents of the diet, as yet unrecognized, may play a role. In addition, the effect appears to be exerted through some mechanism distinct from a simple increase in the amount of the constituents of the drug hydroxylation system.

The latter statement is supported by the observation that metabolism of the different drugs by the same microsomal preparations shows different degrees of increase after chronic treatment with ethanol (Tables 2 and 3) or with phenobarbital [41]. Since the work *in vitro* was done at substrate concentrations within the ranges required for zero-order kinetics, the differences among drugs must be due to corresponding differences in drug binding to the cytochrome P-450, or in the catalytic activity of the latter with respect to the various drug substrates. Differential degrees of induction by ethanol would be compatible with conformational changes resulting in different degrees of alteration of binding affinity or catalytic activity with respect to the various drugs. This would be consistent with the observation that phenobarbital induction alters the K_m and K_s (spectral dissociation constant) differently for hexobarbital and aniline [26]. Similarly, steroid hormones affected the cytochrome P-450 binding of hexobarbital differently from that of ethylmorphine [43].

A further point of considerable importance for extrapolations *in vivo* is the highly variable relationship between metabolism of the same drug *in vitro* and *in vivo*. For example, the rate of metabolism *in vitro* of aminopyrine per 100 g body weight in the control animals (12·9 μmoles/20 min, Table 2) is approximately the same as the rate *in vivo* (6·70 mg/hr, Table 4; equivalent to 10·3 μmoles/20 min). The same is true for zoxazolamine. In contrast, the rate for aniline *in vivo* is about four times as high as the rate *in vitro*. This is probably most readily explained by the fact that the studies *in vitro* dealt only with the production of *p*-aminophenol, while *in vivo* there are other important routes of aniline metabolism, including formation of *N*-conjugation products [44]. Since these involve other enzyme systems with different K_m values and turnover rates, the relative proportions of products formed *in vivo* would be strongly dependent on the drug concentration in the body. This principle has been well established with other drugs, such as the salicylates [45]. The same consideration may, therefore, apply to the variability in inductive effect of ethanol *in vivo* as compared to *in vitro*, as seen in the percentage increases in Tables 2 and 4. Another possible factor is hepatic blood flow, which directly influences the access of drug to the liver. Depending on the initial rates of hepatic clearance, increased hepatic blood flow may have different effects on the rates of metabolism of different drugs [46]. Ethanol has been reported to have variable effects on hepatic blood flow, depending on the dose [47]. However, these findings were all obtained in acute studies and the circulatory effects of chronic ethanol administration are not adequately explored.

The present results, therefore, confirm the findings of various other groups, that chronic ethanol ingestion does increase the rate of metabolism of various drugs. But the diversity of effects on different drugs does not support the idea of a single common mechanism based on increase in the amount of cytochrome P-450 or other components of the mixed function oxidase system. This may explain the discrepancy in results obtained by different investigators, with respect to the effect of ethanol on metabolism of pentobarbital and other drugs.

Acknowledgements—The authors are grateful to Mr. G. Ho, Mr. F. Seymour and Mrs. J. Loth for expert technical assistance, and to Mr. F. Kwong for valuable help with the statistical calculations.

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