Biochemical Pharmacology, Vol. 25, pp. 337–342. Pergamon Press, 1976. Printed in Great Britain.

ETHANOL—A DIRECT INDUCER OF DRUG METABOLISM

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

Abstract—The inductive effects of chronic phenobarbital and ethanol ingestion on hepatic microsomal drug metabolism were examined in rats consuming different proportions of dietary fat. Eight groups of rats were fed for 1 week on matched amounts of nutritionally adequate liquid diets providing 2, 5, 10 or 41% of calories as fat (safflower oil), two groups at each level. One group at each level received phenobarbital (80 mg/kg daily by intubation), the other getting water. In a parallel experiment, one group at each fat level received 36% of calories as ethanol, the other as sucrose, and these regimens were continued for 1 month. Dietary fat content *per se* had no significant effect on body weight, liver weight, microsomal protein, cytochrome P-450 content, or hepatic triglycerides (TG) during the 1-week experiment, but significantly increased all these measures in the 1-month experiment. Both phenobarbital and ethanol treatment increased all these measures except body weight, but phenobarbital had a greater effect on liver weight, microsomal protein and cytochrome P-450, while ethanol had a greater effect on hepatic TG and a significant interaction with dietary fat content in relation to TG levels. In contrast, microsomal metabolism of aminopyrine, aniline and meprobamate *in vitro* was increased approximately equally by phenobarbital and ethanol, while dietary fat content had no effect alone, and no significant interaction with phenobarbital or ethanol. Ethanol, like phenobarbital, therefore, appears to induce microsomal drug metabolism independently of its effects on TG metabolism.

Chronic administration of ethanol to rats and humans has been shown to cause proliferation of the smooth endoplasmic reticulum (SER). It also causes increases in microsomal protein content, as well as in many of the constituents of the drug oxidizing system, and results in an increased drug-metabolizing ability of the microsomes (for references, see [1]).

Since ethanol is known to cause lipid accumulation in the liver [2, 3], it is conceivable that ethanol may act on the microsomes indirectly, by disrupting the triglyceride (TG) metabolism. The TG accumulation might be the direct stimulus to the endoplasmic reticulum, resulting in production of more protein for the formation and release of lipoprotein into the bloodstream. The increase in drug metabolism might be a 'by-product' of this SER stimulation. Consonant with the above hypothesis is the observation that the inductive effects on SER and on the drug-oxidizing system are more pronounced when the ethanol is accompanied by a protein- or choline-deficient diet than by an adequate diet [4, 5]. However, other investigators have reported a directly opposite effect, i.e., the induction of SER and some of its enzymatic activities by ethanol was reduced in animals receiving a choline-deficient diet [6, 7]. It is well known that lipid accumulation is potentiated by alcohol diets which are low in protein and/or lipotropic factors [8].

Dietary fat content and composition also influence the metabolism of various drugs by the rat, even in the absence of ethanol [9–12]. Three weeks feeding of a fat-free diet results in depression of microsomal drug-oxidizing activity as well as a depressed level of cytochrome P-450. Provision of as little as 2–3% of calories in the form of polyunsaturated fatty acid in the diet is said to permit the recovery of normal drug-metabolizing values, as well as the full inductive expression of phenobarbital on cytochrome P-450 and the hydroxylating systems [13, 14].

Manipulation of the amount of dietary fat can also influence dramatically the response of the liver to the steatogenic effect of ethanol [15]. Rats were fed for 24 days on diets providing 18% of the total calories as protein and 36% as ethanol or carbohydrate. The remainder of the calories came from varying amounts of fat (2, 5, 10, 15, 25, 35 and 43%) and complementary amounts of carbohydrate. Lieber and DeCarli found that the extent of TG accumulation correlated with the level of dietary fat, when this was 25% or more. However, no significant decrease in hepatic lipid content was achieved by reduction of dietary fat below 25% of calories.

The aim of the present study was to determine whether ethanol *per se* acts as an inducer of microsomal drug metabolism or acts only indirectly through production of TG accumulation. For this purpose, microsomal drug metabolism, as well as the degree of increase in it produced by chronic ethanol or phenobarbital, was studied in groups of rats fed diets with different levels of fat, producing different levels of TG accumulation in the liver.

MATERIALS AND METHODS

Male Wistar rats (High Oak Ranch, Ontario) weighing 200 ± 5 g were maintained on nutritionally adequate liquid diets [16]. The amount of fat in the diets was set at 2, 5, 10 and 41% of total calories provided as safflower oil (GBC). The lowest level (2%)

Table 1. Effect of phenobarbital treatment (80 mg/kg/day for 1 week) on body weight, liver weight, microsomal protein, TG and P-450*

	2% Fat		5% Fat		10% Fat		41% Fat	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated
Body wt	236 ± 6	234 ± 22	239 ± 9	230 ± 13	250 ± 13	240 ± 9	231 ± 11	257 ± 10
Liver wt/body wt (g/100 g)	3·92 ± 0·26	4·13 ± 0·15	3·42 ± 0·09	4·9 ± 0·25	3·54 ± 0·10	4·4 ± 0·11	3·30 ± 0·07	4·46 ± 0·11
Microsomal protein (mg/g liver)	24·9 ± 1·3	46·1 ± 4·0	24·1 ± 1·2	38·6 ± 1·8	25·2 ± 0·5	39·3 ± 1·8	26·0 ± 1·8	41·3 ± 1·9
Microsomal protein (in total liver/100 g body wt)	97·6 ± 8·0	191·7 ± 17·9	83·0 ± 5·6	189·7 ± 13·1	89·5 ± 2·7	122·7 ± 9·0	86·3 ± 7·7	185·2 ± 12·1
TG (mg/g liver)	10·5 ± 2·4	8·1 ± 2·4	6·8 ± 2·4	13·9 ± 1·6	9·9 ± 3·0	14·9 ± 2·4	8·0 ± 2·6	14·8 ± 1·7
TG (in total liver/ 100 g body wt)	45·1 ± 13·4	34·0 ± 10·3	23·2 ± 4·4	54·3 ± 27·1	37·0 ± 11·1	64·6 ± 10·2	35·3 ± 5·8	79·2 ± 12·9
P-450 (nmoles/mg protein)	1·19 ± 0·09	2·89 ± 0·25	1·44 ± 0·12	2·56 ± 0·20	1·29 ± 0·09	2·47 ± 0·45	1·40 ± 0·09	2·82 ± 0·20
P-450 (nmoles/g liver)	29·6 ± 2·7	134·9 ± 20·3	35·2 ± 3·0	93·3 ± 6·4	31·6 ± 2·9	110·1 ± 12·5	36·4 ± 4·0	115·7 ± 7·7
P-450 (nmoles in total liver/100 g body wt)	112·9 ± 6·22	564·9 ± 90·28	118·3 ± 10·2	459·0 ± 40·6	111·2 ± 8·57	484·4 ± 57·1	120·4 ± 13·86	516·9 ± 39·17

* Values shown in Tables 1–4 are mean ± S. E. M.; n = 6 in each case.

is known to be sufficient to prevent essential fatty acid deficiency [17]. Protein provided 19% of total calories, and the rest was carbohydrate (Dextri-maltose, Mead Johnson).

Two groups of six animals each were supplied with each of the diets described. One group on each diet was treated with phenobarbital (80 mg/kg/day, p.o.) while the other (control) group received water to balance the stress due to intubation. Animals in all the other seven groups were pair-fed with those in the phenobarbital-treated group on the 2% fat diet. The duration of treatment was 7 days, after which the animals were fasted for 16 hr and then killed for biochemical analyses.

The chronic ethanol experiment also consisted of eight groups of six animals each. Four of these groups were maintained on ethanol (35% calories) diets containing 2, 5, 10 and 41% fat, with the normal complement of protein (19%) and varying amounts of carbohydrate. In the four corresponding control groups, ethanol was replaced isocalorically with carbohydrate. All animals were pair-fed for 1 month with the group receiving ethanol in the 2% fat diet. After a 16-hr fast, the animals were killed by decapitation, the livers were excised and rinsed in ice-cold 1·15% KCl, blotted dry and weighed for subsequent analyses.

A portion of each liver (about 2 g) was excised, weighed and set aside for TG determination. The rest of the liver was homogenized in 4 vol. of ice-cold 1·15% KCl by means of a glass homogenizer with a Teflon pestle. The microsomal fraction from each liver was isolated as described previously [1] and diluted to a final concentration of 6 mg protein/ml for drug metabolism studies. Microsomal protein was determined by the biuret method [18].

Microsomal cytochrome P-450 content was measured by the procedure of Omura and Sato [19]. Activities of microsomal *N*-demethylation of aminopyrine, and aniline and meprobamate hydroxylation were determined as described previously [1].

RESULTS

The results presented in Tables 1–4 were subjected to analysis of variance with a 2 × 4 factorial design. In the phenobarbital experiment, neither the phenobarbital treatment nor the variation in dietary fat level had any significant effect on body weights (Table 1). On the other hand, irrespective of the level of dietary fat, all phenobarbital-treated animals have significantly higher values for the ratio of liver weight/body weight ($F = 61{\cdot}13$; df 1, 38; $P < 0{\cdot}001$), and there was no significant interaction between phenobarbital and diet. Phenobarbital also caused highly significant increases in the amount of microsomal protein and cytochrome P-450, regardless of how expressed. In contrast, the dietary fat level had no significant effect on these various measures, and no interaction with the phenobarbital effect. An unexpected finding was a small but significant increase in liver TG concentration due to phenobarbital treat-

Table 2. Effect of chronic phenobarbital treatment (80 mg/kg/day for 1 week) with various amounts of dietary fat on drug metabolism *in vitro*

	2% Fat		5% Fat		10% Fat		41% Fat	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated
Aminopyrine*								
Activity/mg protein	0·084 ± 0·012	0·155 ± 0·010	0·098 ± 0·011	0·163 ± 0·017	0·099 ± 0·005	0·152 ± 0·011	0·108 ± 0·015	0·168 ± 0·013
Activity/g liver	2·04 ± 0·22	7·62 ± 0·86	2·35 ± 0·25	6·23 ± 0·60	2·50 ± 0·15	6·05 ± 0·72	2·79 ± 0·39	6·95 ± 0·66
Activity/100 g body wt in total liver	7·24 ± 1·07	30·96 ± 2·18	8·03 ± 0·93	31·09 ± 4·24	8·85 ± 0·52	26·54 ± 3·04	9·50 ± 1·58	31·13 ± 3·17
Aniline†								
Activity/mg protein	0·028 ± 0·002	0·039 ± 0·003	0·035 ± 0·004	0·048 ± 0·004	0·028 ± 0·003	0·038 ± 0·003	0·029 ± 0·002	0·043 ± 0·004
Activity/g liver	0·70 ± 0·06	1·88 ± 0·12	0·83 ± 0·09	1·88 ± 0·21	0·71 ± 0·07	1·52 ± 0·16	0·75 ± 0·04	1·79 ± 0·21
Activity/100 g body wt in total liver	2·76 ± 0·32	7·78 ± 0·46	2·82 ± 0·26	9·29 ± 1·25	2·50 ± 0·25	6·70 ± 0·74	2·47 ± 0·18	8·07 ± 2·44
Meprobamate‡								
Activity/mg protein	0·0083 ± 0·0014	0·0190 ± 0·0021	0·0090 ± 0·0012	0·0188 ± 0·0021	0·0090 ± 0·0007	0·0156 ± 0·0014	0·0080 ± 0·0007	0·0155 ± 0·0002
Activity/g liver	0·203 ± 0·027	0·843 ± 0·167	0·227 ± 0·028	0·720 ± 0·100	0·227 ± 0·021	0·617 ± 0·060	0·211 ± 0·015	0·638 ± 0·064
Activity/100 g body wt in total liver	0·78 ± 0·10	3·70 ± 0·77	0·74 ± 0·10	3·60 ± 0·54	0·79 ± 0·57	2·63 ± 0·25	0·69 ± 0·09	2·84 ± 0·12

* Expressed as μmoles HCHO produced/20 min of incubation.
† Expressed as μmoles *para*-aminophenol formed/20 min of incubation.
‡ Expressed as μmoles meprobamate metabolized/15 min of incubation.

ment ($F = 6·23$; df 1, 22; $P < 0·025$), while dietary fat had no such effect of its own, and no significant interaction with phenobarbital. The same was true of total hepatic TG relative to body weight.

Microsomal drug metabolism (Table 2) was unaffected by the per cent of fat in the diets, regardless of how the activity was expressed. In contrast, there was a highly significant induction by phenobarbital, which was unaffected by dietary fat content. This induction was evident for all three drugs studied, and for all three bases of calculation, viz. specific activity per mg of microsomal protein or per g of liver, and total activity per 100 g body weight. Since the latter includes the effects of increase in specific activity of the microsomes, increased SER per cell, and increased liver size, it naturally shows the largest percentage increase.

After 1 month on the various ethanol and control diets (Table 3), it was found that ethanol had no significant effect on body weight, but the dietary fat level did ($F = 4·79$; df 3, 40; $P < 0·01$). There was no significant interaction between ethanol and diet in this respect, both the alcohol and control animals showing maximum gain on the 5% fat diet, and appreciably less on either the very high- or low-fat diets. In contrast, liver size in relation to body weight was not significantly affected by dietary fat alone, but was significantly increased by ethanol ($F = 21·0$; df 1, 40; $P < 0·001$). This effect was especially marked on the high-fat (41%) diet, so that the interaction between ethanol and diet was also significant ($F = 9·79$; df 3, 40; $P < 0·001$).

Microsomal protein was not altered by any of the treatments, in terms of either mg/g of liver or in total liver per 100 g of body weight. Cytochrome P-450, whether expressed in relation to microsomal protein, to unit weight of liver, or to total liver per 100 g body weight, was significantly increased by ethanol ($P < 0·001$ in each case) but not by dietary fat level. In contrast, liver TG concentration was influenced significantly both by ethanol ($P < 0·01$) and by the dietary fat content ($P < 0·001$), and there was a significant interaction effect ($F = 5·27$; df 3, 40; $P < 0·01$) reflecting the marked increases produced by the combination of ethanol with the two highest dietary fat levels. The same was true of total hepatic TG per 100 g body weight.

Drug metabolism *in vitro*, regardless of how the activity was expressed, showed a remarkably consistent pattern. With all three drugs, there was a highly significant increase in metabolism attributable to chronic ethanol ingestion, but no significant effect of dietary fat and no interaction between the latter and ethanol (Table 4). Moreover, there was no signifi-

Table 3. Effect of chronic alcohol feeding (35% of calories) with various amounts of dietary fat on body weight, liver weight, microsomal protein, TG and P-450

	2% Fat		5% Fat		10% Fat		41% Fat	
	Control	Ethanol	Control	Ethanol	Control	Ethanol	Control	Ethanol
Body wt	333 ± 13	345 ± 17	363 ± 5	385 ± 9	350 ± 14	372 ± 12	352 ± 11	317 ± 14
Liver wt/body wt (g/100 g)	3·40 ± 0·12	3·36 ± 0·08	3·25 ± 0·07	3·35 ± 0·06	3·26 ± 0·09	3·46 ± 0·07	3·07 ± 0·05	3·86 ± 0·09
Microsomal protein (mg/g liver)	31·7 ± 2·2	29·0 ± 1·0	28·9 ± 1·8	28·1 ± 1·3	29·9 ± 2·1	28·6 ± 1·7	27·1 ± 1·4	27·9 ± 1·4
Microsomal protein (in total liver/ 100 g body wt)	108·5 ± 9·56	97·7 ± 4·81	94·2 ± 7·23	94·3 ± 5·58	97·4 ± 7·35	99·1 ± 7·13	83·5 ± 4·82	107·9 ± 6·66
TG (mg/g liver)	19·4 ± 2·2	20·0 ± 2·7	20·5 ± 2·1	19·7 ± 2·1	22·3 ± 1·5	29·3 ± 5·8	42·5 ± 3·8	67·3 ± 6·0
TG (in total liver/ 100 g body wt)	65·8 ± 7·9	66·7 ± 8·7	65·4 ± 7·0	66·3 ± 7·0	72·1 ± 3·2	100·3 ± 18·3	130·9 ± 12·3	259·3 ± 22·0
P-450 (nmoles/mg protein)	1·30 ± 0·05	2·53 ± 0·07	1·67 ± 0·08	2·27 ± 0·13	1·69 ± 0·14	2·35 ± 0·24	1·90 ± 0·11	2·52 ± 0·05
P-450 (nmoles/g liver)	41·3 ± 3·4	73·3 ± 1·7	48·1 ± 3·4	63·0 ± 2·9	49·4 ± 2·5	65·4 ± 4·2	51·2 ± 2·6	70·3 ± 3·9
P-450 (nmoles/100 g body wt)	141·5 ± 14·13	246·5 ± 9·51	156·7 ± 12·17	211·8 ± 12·36	160·5 ± 7·76	226·0 ± 13·1	158·0 ± 8·57	272·2 ± 18·78

cant correlation between the various drug-metabolizing activities and the hepatic TG concentration, microsomal protein concentration, or the TG:protein ratio, regardless of how these were expressed. Thus, in the same animals, there was no parallelism between the changes in drug metabolism and those of either liver size or liver TG.

DISCUSSION

The present findings, in agreement with those of previous investigators, reveal that chronic treatment with either phenobarbital or ethanol resulted in increased liver size relative to body weight, increased hepatic concentration and total content of cytochrome P-450, and increased rates of microsomal drug metabolism *in vitro* (for references, see [1]). All of these measures were increased substantially more by the 7-day treatment with phenobarbital than by the 30-day treatment with ethanol. Consistent with this, phenobarbital markedly increased the concentration and total amount of microsomal protein, regardless of diet, while ethanol did not increase microsomal protein concentration at all, and increased the total content significantly ($P < 0{\cdot}02$) only on the highest fat diet (Table 3).

Despite these differences, phenobarbital and ethanol produced fairly comparable increases in microsomal drug-metabolizing activity *in vitro*, expressed per mg of microsomal protein. With all three drug substrates, and on all four diets, the increases in specific activity were of the order of 50 per cent. This differs from the result of the accompanying study [1], in which specific activities were not increased by ethanol on either high- or low-fat diets. However, the basic diets in the two studies differed, in that the major source of carbohydrate in the present work was mixed dextrins rather than sucrose. The possible importance of this factor has not yet been established. The much greater increase in drug metabolizing activity, relative to liver weight or body weight, after phenobarbital is, therefore, proportional to the greater increase in formation of new SER. Caution is necessary on one point, however. No attempt was made to correct for microsomal recovery in the present work. Ishii *et al.* [20] found no effect of chronic ethanol treatment on recoverability of microsomal cytochrome P-450 from liver homogenates, but this does not rule out the possibility that recovery might have been significantly higher from phenobarbital-treated animals.

Dietary fat content *per se* had no significant effect on body weight and liver TG content in the control animals in the phenobarbital experiment, but significantly increased both measures in the ethanol experiment. This is perhaps not surprising, because the diets were continued for a month in the latter case, and only a week in the former. We have no explanation for the finding that the greatest weight gain occurred on the diet with 5% fat. An unexpected finding, however, is that both ethanol and phenobarbital treatment increased hepatic TG levels above those of the respective control groups, and by comparable proportions (about 100 per cent increase on the high-

Table 4. Effect of chronic alcohol feeding (35% of calories) with various amounts of dietary fat on drug metabolism *in vitro*

	2% Fat		5% Fat		10% Fat		41% Fat	
	Control	Ethanol	Control	Ethanol	Control	Ethanol	Control	Ethanol
Aminopyrine*								
Activity/mg protein	0·120 ± 0·023	0·222 ± 0·019	0·145 ± 0·007	0·214 ± 0·011	0·177 ± 0·024	0·219 ± 0·021	0·140 ± 0·007	0·217 ± 0·011
Activity/g liver	4·33 ± 0·58	6·49 ± 0·69	4·19 ± 0·031	6·07 ± 0·24	5·30 ± 0·84	6·30 ± 0·77	3·80 ± 0·31	6·11 ± 0·57
Activity/100 g body wt in total liver	14·60 ± 1·82	21·94 ± 2·63	13·59 ± 1·24	20·41 ± 2·02	17·36 ± 2·94	21·88 ± 2·82	11·73 ± 1·06	23·64 ± 2·33
Aniline*								
Activity/mg protein	0·023 ± 0·001	0·033 ± 0·002	0·026 ± 0·002	0·030 ± 0·002	0·025 ± 0·003	0·035 ± 0·004	0·030 ± 0·004	0·036 ± 0·002
Activity/g liver	0·73 ± 0·072	0·976 ± 0·08	0·744 ± 0·08	0·85 ± 0·09	0·73 ± 0·08	0·98 ± 0·10	0·84 ± 0·14	1·02 ± 0·10
Activity/100 g body wt in total liver	2·49 ± 0·27	3·29 ± 0·30	2·43 ± 0·31	2·87 ± 0·36	2·37 ± 0·28	3·41 ± 0·37	2·58 ± 0·45	3·94 ± 0·40
Meprobamate*								
Activity/mg protein	0·0096 ± 0·0007	0·0166 ± 0·0007	0·0104 ± 0·0007	0·0184 ± 0·0014	0·0109 ± 0·0007	0·0157 ± 0·0021	0·0115 ± 0·0048	0·0148 ± 0·0014
Activity/g liver	0·295 ± 0·023	0·476 ± 0·030	0·302 ± 0·030	0·394 ± 0·038	0·316 ± 0·015	0·436 ± 0·049	0·313 ± 0·011	0·419 ± 0·058
Activity/100 g body wt in total liver	1·02 ± 0·10	1·60 ± 0·10	0·98 ± 0·11	1·33 ± 0·13	1·02 ± 0·04	1·44 ± 0·19	0·96 ± 0·04	1·64 ± 0·25

* Activities as defined in Table 2.

est fat diet). This was contrary to our predictions, since another study [21] had indicated that phenobarbital had no effect on liver TG by itself, and even decreased the TG accumulation resulting from acute ethanol treatment, albeit by a rather indirect mechanism. At the present stage, any explanation of the increase in TG levels in the phenobarbital group would be mere speculation. But, in that vein, one may speculate that induction of SER includes induction of the fatty acid esterification system, leading to production of TG in excess of lipoprotein secretion. This has already been shown for ethanol [2].

With neither treatment was there proportionality between TG levels and rates of drug metabolism *in vitro* across the different diets. One must, therefore, conclude that ethanol is indeed an inducer of SER and of its drug-metabolizing activities, as reported by Rubin *et al.* [4], independently of an effect on hepatic TG. Though the present work does not permit any conclusion as to the identity or non-identity of the mechanisms of induction by ethanol and by phenobarbital, the end result appears to be closely similar. However, this carries no implications with respect to the claimed oxidation of ethanol by hepatic microsomal enzymes.

Acknowledgements—The authors are grateful to Dr. L. Endrenyi and Mr. F. Kwong for valuable help with the statistical calculations.

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