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EFFECTS OF CHRONIC ETHANOL ADMINISTRATION IN THE RAT: RELATIVE DEPENDENCY ON DIETARY LIPIDS—I.

INDUCTION OF HEPATIC DRUG-METABOLIZING ENZYMES *IN VITRO**

JEAN-GIL JOLY† and CLAUDE HÉTU

Clinical Research Center and Section of Liver Diseases, Hôpital Saint-Luc and Department of Medicine, Hôpital Saint-Luc and Université de Montréal, Montréal, Canada

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Abstract—Female Sprague–Dawley rats were fed nutritionally adequate liquid diets with or without ethanol, at two ethanol concentrations, 5 and 6% (w/v). In other animals, various degrees of caloric deficiency were obtained by replacing ethanol by water in one animal of a pair. Ethanol given as a 5% (w/v) solution with high amounts of dietary fat increased cytochrome P-450, the activities of NADPH-cytochrome P-450 reductase, benzphetamine demethylation, aniline hydroxylation and microsomal ethanol-oxidizing system (MEOS). When ethanol was given with a low fat diet as a 5% (w/v) solution, the increase in cytochrome P-450 and P-450 reductase was much less than with a high fat diet; the other enzyme activities, however, were enhanced to a level comparable to that achieved with the high fat diet. When ethanol was administered as a 6% (w/v) solution in presence of a low fat diet, caloric deficiency was observed and no significant induction of any parameter except aniline hydroxylation could be found. When it was given with a high fat diet, in spite of caloric deficiency and lower ethanol ingestion, cytochrome P-450 and P-450 reductase activities were enhanced while that of MEOS was not. Ingestion of ethanol as a 6% (w/v) solution with a high fat diet resulted in a negligible weight gain. Higher basal levels of cytochrome P-450, P-450 reductase and benzphetamine demethylation activities were found in animals rendered caloric-deficient. Ethanol is associated with a greater induction of drug-metabolizing enzyme activities in the high fat model compared to the low fat model. Induction of drug-metabolizing enzymes by ethanol is partly dependent on dietary lipids as well as on the amounts of ethanol ingested and on the caloric status of the animal.

Using a model in which ethanol is incorporated into a totally liquid diet [1], chronic ethanol administration has been shown to be associated with proliferation of the smooth endoplasmic reticulum of the hepatocytes [2–5] and with increased hepatic drug-metabolizing enzymes [4–6], predominantly in the smooth microsomes [7]; ethanol has also been found to increase the activity of the microsomal ethanol-oxidizing system (MEOS) [8, 9]. These reports were confirmed by some studies [10, 11] and challenged by others [12–15] in which ethanol was given as a 10% or higher solution in the drinking water of animals fed laboratory Chow. Such conflicting results may be explained by the important differences between experimental models. The latter model of ethanol administration differs from the liquid diet model by the lipid content of the diet, the daily intake of ethanol [16], and because of the degree of undernutrition associated with the intake of a greater than 5% (w/v) ethanol solution [16–18]. We have therefore studied the importance of dietary lipid [19], daily ethanol intake and undernutrition [20–22] on the effects of ethanol on drug-metabolizing enzyme activities *in vitro*. Our findings showed that dietary manipulations can lead to apparently conflicting results on the effects of ethanol on drug-metabolizing enzymes.

MATERIALS AND METHODS

Animals and diets. Female Sprague–Dawley littermate rats were fed laboratory Chow and tap water *ad lib.* until the start of the experiment, when they reached a weight of 120–150 g. They were then pair-fed nutritionally adequate liquid diets [1]. Six groups of animals were studied. The composition of the diets is given in Table 1. All diets contained at least 2 per cent of calories as linoleate, this amount of unsaturated fatty acids having been reported to be optimal for induction of drug metabolism [23]. Animals of groups 1–4 were fed for periods of 5–7 weeks. Animals of groups 5 and 6 were fed for periods of 21 and 28 days respectively.

Differential centrifugation. At the end of the experiment, the animals were killed by decapitation, the liver was quickly perfused with ice-cold 0·15 M KCl, excised and homogenized in 4 vol. of 0·25 M sucrose by means of a glass homogenizer with a Teflon pestle. The homogenate was centrifuged at 600 *g* for 5 min, the remaining supernatant at 15,700 *g* for 15 min, and

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† Send reprint requests to: Hôpital Saint-Luc, 1058 St-Denis St., Montréal, Qué. H2X 3J4, Canada.

Table 1. Composition of liquid diets (in % of total calories) for the various groups of animals studied*

Diet components	Group 1 (5% E-HFD)		Group 2 (5% E-LFD)		Group 3 (6% E-HFD)		Group 4 (6% E-LFD)		Group 5 (hypocaloric *ad lib.*)		Group 6 (hypocaloric restricted)†	
	C‡	E‡	C‡	E‡	C‡	E‡	C‡	E‡	C‡	E§	C‡	E§
Protein	18	18	18	18	18	18	18	18	18	28	18	28
Carbohydrate‖	47	11	80	44	47	5	80	38	47	17	47	17
Lipid												
Corn oil: olive oil mixture	33	33			33	33			33	52	33	52
Linoleate	2	2	2	2	2	2	2	2	2	3	2	3
Ethanol		36		36		42		42				
Duration of pair-feeding	5–7 weeks		5–7 weeks		5–7 weeks		5–7 weeks		21 days		28 days	

* 5% E-HFD = 5% (w/v) ethanol, high fat diet; 5% E-LFD = 5% (w/v) ethanol, low fat diet; 6% E-HFD = 6% (w/v) ethanol, high fat diet; 6% E-LFD = 6% (w/v) ethanol, low fat diet. C = control animal; E = experimental animal.
† Restriction of the experimental animal to less than 45 calories/day.
‡ 1 ml of diet = 1 calorie.
§ 1 ml of diet = 0·64 calorie.
‖ Dextri-maltose (Mead-Johnson, Evansville, Ind.).

the post-mitochondrial supernatant at 105,000 *g* for 60 min; microsomes were washed once and resuspended in 0·15 M KCl.

Enzyme assays and biochemical analyses. Activities of microsomal demethylation were measured according to Holtzman *et al.* for aminopyrine [24] and according to Hewick and Fouts for benzphetamine [25]. After 10 min of incubation, the reaction was stopped with 1 ml of 10% (w/v) trichloroacetic acid. Formaldehyde production was determined by the method of Nash [26]. The demethylase-dependent formaldehyde production was calculated by subtraction of the amount of formaldehyde produced in the absence of substrate. Aniline hydroxylation was measured by the method of Chhabra *et al.* [27]. NADPH-cytochrome P-450 reductase was assayed by the method of Holtzman and Carr [28]; all measurements were done in triplicate. NADPH-cytochrome *c* reductase was measured as described by Masters *et al.* [29]. MEOS was measured according to Lieber and DeCarli [8]. Cytochrome P-450 was measured in homogenates by the method of Greim [30] and in microsomal suspensions according to Omura and Sato [31], using an Aminco DW-2 UV/VIS spectrophotometer (American Instruments, Silver Spring, Md.) in the split-beam mode. Protein was determined according to Lowry *et al.* [32] and phospholipid by the method of Bartlett [33].

Correction for microsomal losses. The ratio of cytochrome P-450 (nmoles/g of liver) of microsomes over cytochrome P-450 (nmoles/g of liver) of homogenate yielded a value of microsomal recovery. This value was used to correct for microsomal losses [34, 35]. All values of microsomal protein per g of liver reported in this study are corrected values.

Statistical analyses. The mean of individual differences was tested by the Student *t*-test for pairs [36].

Chemicals. Benzphetamine was kindly supplied by the Upjohn Co. (Kalamazoo, Mich.). Aminopyrine was bought from K & K Laboratories (Plainview,

Table 2. Effects of chronic alcohol feeding (36% cal. or 5% w/v) with various amounts of dietary fat on weight gain, caloric intake, liver weight, microsomal protein and phospholipid (mean ± S.E.)

	35% Cal. as fat (5% E-HFD)			2% Cal. as fat (5% E-LFD)		
	n*	Control	Ethanol	n*	Control	Ethanol
Weight gain (g/day)	30	2·24 ± 0·19	1·84 ± 0·17†	22	2·08 ± 0·16	2·09 ± 0·123
Caloric intake (cal./day)	30	54·45 ± 1·24	54·30 ± 1·25	22	63·87 ± 1·20	63·77 ± 0·94
Approximate ethanol intake (g/kg/day)	30		13–15	22		14–16
Liver wt (g/100 g body wt)	30	3·75 ± 0·08	4·08 ± 0·14†	22	4·03 ± 0·09	4·03 ± 0·09
Microsomal proteins (mg/g of liver)	30	45·15 ± 2·00	44·54 ± 2·21	22	42·29 ± 1·13	38·71 ± 1·76‡
Microsomal phospholipids (mg/mg protein)	29	0·421 ± 0·022	0·447 ± 0·095	22	0·420 ± 0·020	0·460 ± 0·020
(mg/100 g body wt)	29	70·58 ± 5·69	83·93 ± 7·23‡	22	73·69 ± 5·31	73·50 ± 6·02

* Number of pairs.
† $P < 0.001$.
‡ $P < 0.025$.

Table 3. Effects of chronic alcohol feeding (36% cal. or 5% w/v) with various amounts of dietary fat on microsomal recovery, cytochrome P-450 and the activities of NADPH-cytochrome *c* or cytochrome P-450 reductase, aminopyrine and benzphetamine demethylase, aniline hydroxylase and of the MEOS (mean ± S.E.)

		35% Cal. as fat (5% E-HFD)			2% Cal. as fat (5% E-LFD)		
		n*	Control	Ethanol	n*	Control	Ethanol
Microsomal recovery†		30	0·320 ± 0·014	0·367 ± 0·016‡	22	0·312 ± 0·011	0·356 ± 0·017§
Cytochrome P-450	(nmoles/g liver)	30	40·32 ± 1·90	61·84 ± 5·23‡	22	35·35 ± 1·95	39·89 ± 2·51§
Cytochrome P-450	(nmoles/mg protein)	30	0·959 ± 9·052	1·457 ± 0·118‡	22	0·819 ± 0·032	1·030 ± 0·044‡
NADPH-cytochrome *c* reductase	nmoles/mg protein		77·01 ± 4·96	81·69 ± 7·13		74·17 ± 3·38	71 75 ± 4·79
(nmoles cytochrome *c* reduced		20			13		
min^{-1})	(nmoles/100 g body wt)		(12035 ± 892)	13731 ± 1163		(11621 ± 736)	(9890 ± 1076)‖
NADPH-cytochrome P-450 reductase	Δ O.D./mg protein		24·54 ± 1·85	41·72 ± 4·39¶		26 37 ± 1·09	30·62 ± 2·17
		9			9		
(Δ O.D. 465–450 $sec^{-1} \times 10^{-3}$)	(Δ O.D./100 g body wt)		(4679 ± 427)	(9748 ± 1600)¶		(4992 ± 173)	(5443 ± 516)
Benzphetamine demethylase	nmoles/mg protein		5·24 ± 0·53	6·46 ± 0·54‡		4·68 ± 0·47	6·32 ± 0·73‡
(nmoles formaldehyde formed		23			21		
min^{-1})	(nmoles/100 g body wt)		(907 ± 115)	(1178 ± 135)‡		(831 ± 196)	(1023 ± 142)¶
Aniline hydroxylase	nmoles/mg protein		0·855 ± 0·032	2·300 ± 0·080‡		0·810 ± 0·106	1·830 ± 0·080‡
(nmoles *p*-aminophenol formed		26			22		
min^{-1})	(nmoles/100 g body wt)		(137 ± 5)	(391 ± 20)‡		(135 ± 15)	(280 ± 18)‡
MEOS	nmoles/mg protein		10·08 ± 0·88	13·25 ± 1·11‡		7·29 ± 0·45	9·97 ± 0·90‡
(nmoles acetaldehyde formed		27			22		
min^{-1})	(nmoles/100 g body wt)		(1621 ± 126)	(2397 ± 186)‡		(1233 ± 83)	(1493 ± 142)¶

* Number of pairs.
† Microsomal recovery factor: nmoles cytochrome P-450/g of liver in microsomes/nmoles cytochrome P-450/g of liver in homogenate.
‡ $P < 0.001$.
§ $P < 0.05$.
‖ $P < 0.020$.
¶ $P < 0.01$.

N.Y.) and aniline from Eastman (Rochester, N.Y.). NADPH, sodium glucose 6-phosphate, sodium isocitrate, glucose 6-phosphate dehydrogenase, isocitric dehydrogenase (crude type IV), nicotinamide and cytochrome *c* were purchased from Sigma Chemical Co. (St. Louis, Mo.). All other chemicals were of reagent grade and were obtained from Fisher Scientific (Montreal, Canada).

RESULTS

Results obtained in rats fed the 5% (w/v) ethanol liquid diet. High-fat diets resulted in a lower daily caloric (17 per cent) and ethanol (7 per cent) intake (Table 2). In groups fed ethanol with high-fat diets (E-HFD), differences between results expressed per mg of microsomal protein become greater when expressed per 100 g of body weight because of an increase in liver weight. The ethanol-induced increases in cytochrome P-450 content and NADPH-cytochrome P-450 reductase activity were greater in presence of a high fat diet. The other enzyme activities were enhanced by ethanol to the same degree whether administered with high fat or with low fat diets. When administered with low fat diets, ethanol significantly decreased the microsomal protein content and differences in activities per mg of microsomal protein were reduced when expressed per 100 g of body weight (Table 3).

Table 4. Effects of chronic alcohol feeding (42% cal. or 6% w/v) with various amounts of dietary fat on weight gain, caloric intake, liver weight, microsomal protein and phospholipid (mean ± S.E.)

	35% Cal. as fat (6% E-HFD)			2% Cal. as fat (6% E-LFD)		
	n*	Control	Ethanol	n*	Control	Ethanol
Weight gain (g/day)	9	1·85 ± 0·08	1·36 ± 0·07†	10	2·39 ± 0·36	2·14 ± 0·39‡
Caloric intake (cal./day)	9	41·96 ± 1·25	41·84 ± 1·81	10	51·46 ± 1·17	51·39 ± 1·21
Approximate ethanol intake (g/kg/day)	9		10–12	10		13–15
Liver weight (g/100 g body wt)	9	2·83 ± 0·11	3·30 ± 0·10§	10	3·08 ± 0·06	2·88 ± 0·08
Microsomal proteins (mg/g of liver)	9	43·27 ± 3·41	42·84 ± 2·51	10	46·26 ± 3·43	42·80 ± 3·46‡
Microsomal phospholipids (mg/mg protein)	9	0·415 ± 0·019	0·469 ± 0·015§	10	0·521 ± 0·034	0·530 ± 0·037
(mg/100 g body wt)	9	52·38 ± 2·94	69·36 ± 4·31†	10	69·94 ± 5·59	60·91 ± 7·51‡

* Number of pairs.
† $P < 0.001$.
‡ $P < 0.05$.
§ $P < 0.01$.

Table 5. Effects of chronic alcohol feeding (42% cal. or 6% w/v) with various amounts of dietary fat on microsomal recovery, cytochrome P-450 and the activities of NADPH-cytochrome *c* reductase, aminopyrine demethylase, aniline hydroxylase and of the MEOS (mean ± S.E.)

		35% Cal as fat (6% E-HFD)			2% Cal as fat (6% E-LFD)		
		n*	Control	Ethanol	n*	Control	Ethanol
Microsomal recovery†		9	0·321 ± 0·023	0·410 ± 0·011‡	10	0·270 ± 0·014	0·321 ± 0·028
Cytochrome P-450	(nmoles/g liver)	9	30·77 ± 1·42	49·22 ± 3·04‡	10	31·55 ± 1·71	32·26 ± 1·36
Cytochrome P-450	(nmoles/mg protein)	9	0·740 ± 0·042	1·139 ± 0·071‡	10	0·706 ± 0·037	0·799 ± 0·056
NADPH-cytochrome *c* reductase	nmoles/mg protein		65·10 ± 4·07	80·52 ± 3·99§		50·97 ± 2·32	53·42 ± 2·26
(nmoles cytochrome *c* reduced min^{-1})		9			10		
	(nmoles/100 g body wt)		(7936 ± 772)	(11217 ± 599)‡		(7332 ± 742)	(6570 ± 577)
Aminopyrine déméthylase	nmoles/mg protein		4·47 ± 0·20	6·53 ± 0·99‡		4·12 ± 0·27	5·05 ± 0·23
(nmoles formaldehyde formed min^{-1})		9			10		
	(nmoles/100 g body wt)		(539 ± 40)	(916 ± 66)‡		(590 ± 63)	(615 ± 50)
Aniline hydroxylase	nmoles/mg protein		0·813 ± 0·071	3·818 ± 0·798§		0·667 ± 0·037	1·597 ± 0·140‡
(nmoles *p*-aminophenol formed min^{-1})		9			10		
	(nmoles/100 g body wt)		(97 ± 8)	(563 ± 144)‡		(96 ± 9)	(192 ± 17)‡
MEOS	nmoles/mg protein		7·85 ± 1·05	5·88 ± 0·46		5·45 ± 0·86	7·14 ± 0·54
(nmoles acetaldehyde formed min^{-1})		9			10		
	(nmoles/100 g body wt)		(944 ± 146)	(818 ± 65)		(823 ± 47)	(865 ± 82)

* Number of pairs.

† Microsomal recovery factor: nmoles cytochrome P-450/g liver in microsomes/nmoles cytochrome P-450/g liver in homogenate.

‡ $P < 0.001$.

§ $P < 0.005$.

Results obtained in rats fed the 6% (w/v) ethanol liquid diets. With a 6% (w/v) solution, rats drank less liquid diet and suffered undernutrition in the high fat group. As shown by the weight gain, this was less marked with low fat diets. Thus, the 6% E-HFD, in addition to showing lower weight gain, had a lower daily ethanol intake than that of the 6% ethanol low fat diet (E-LFD). Only in the 6% E-HFD group was liver weight significantly increased after ethanol. Microsomal proteins were again significantly decreased after ethanol and low fat diets (Table 4). Compared to those expressed per mg of microsomal protein, differences between results expressed per 100 g of body weight increased in rats fed high fat diets and decreased in animals fed the low fat diets. The degree of induction by ethanol was greater in the 6% E-HFD group in spite of a lower ethanol intake. MEOS showed low activities in these animals. After ethanol,

Table 6. Effects of caloric restriction on hepatic drug-metabolizing enzymes in two groups of rats (mean ± S.E.)

	Group A*		Group B†	
	Ad lib.	Hypocaloric	*Ad lib.*	Hypocaloric
Weight gain (g/day)	1·29 ± 0·29	0·44 ± 0·10‡	1·33 ± 0·02	0·19 ± 0·10‡
Caloric intake (cal./day)	82·81 ± 0·29	53·05 ± 0·19‡	77·89 ± 0·01	41·15 ± 0·01‡
Liver weight (g/100 g body wt)	3·84 ± 0·12	3·75 ± 0·11	3·49 ± 0·11	3·65 ± 0·10
Microsomal proteins (mg/g of liver)	45·88 ± 1·22	54·82 ± 3·33	41·28 ± 2·08	41·33 ± 1·88
Cytochrome P-450 (nmoles/g of liver)	39·04 ± 1·96	49·41 ± 1·91§	40·74 ± 1·43	48·06 ± 1·98§
Cytochrome P-450 (nmoles/mg protein)	0·853 ± 0·050	0·908 ± 0·029	1·000 ± 0·040	1·170 ± 0·060‖
NADPH-cytochrome P-450 reductase (Δ O.D. 465–450 $sec^{-1} \times 10^{-3}$/ mg protein)	26·27 ± 1·27	26·14 ± 1·49	33·48 ± 3·29	44·42 ± 3·78¶
Benzphetamine demethylase (nmoles formaldehyde formed min^{-1}/mg protein)	5·81 ± 0·47	6·22 ± 0·26	7·92 ± 3·36	10·06 ± 0·61‖
Aniline hydroxylase (nmoles *p*-aminophenol formed min^{-1}/mg protein)	0·858 ± 0·040	0·942 ± 0·058	0·850 ± 0·050	0·965 ± 0·040
MEOS (nmoles acetaldehyde formed min^{-1}/mg protein)	7·52 ± 1·25	7·41 ± 0·86	5·34 ± 0·71	5·34 ± 1·43

* Six pairs of rats; the experimental animals are given 36% fewer calories than the controls (Group 5).

† Six pairs of rats; the experimental animals are given 47% fewer calories than the controls (Group 6).

‡ $P < 0.001$.

§ $P < 0.025$.

‖ $P < 0.05$.

¶ $P < 0.005$.

MEOS activity decreased in the 6% E-HFD group and was slightly enhanced in the 6% E-LFD group (Table 5).

Results obtained in rats fed hypocaloric diets. In group 5, the caloric intake of the experimental animals was similar to that achieved in the 5% E-HFD and in the 6% E-LFD groups (Table 6). Growth was impaired 66 per cent. Cytochrome P-450/g of liver was 26 per cent higher in the hypocaloric animals, but no significant differences were found in the other parameters measured. In group 6, the caloric intake of the experimental animals was similar to that achieved in the 6% E-HFD group. Significant increases in cytochrome P-450, NADPH-cytochrome P-450 reductase activity and benzphetamine demethylation were found in the hypocaloric animals. Aniline hydroxylation was slightly higher (12 per cent) in hypocaloric rats, but this difference was not significant. Apparent lower weight gain in controls, when compared to those of Tables 2 and 4, are explained by the fact that these were measured in much younger and smaller animals.

DISCUSSION

With the present model, ethanol administration is associated with profound alterations of hepatic lipid metabolism [37, 38]. On the other hand, dietary lipids are known to participate in the induction of drug-metabolizing enzymes [23, 39–43]. Thus, ethanol effects might be dependent on dietary lipids. In other studies, conflicting results on the effects of ethanol on drug-metabolizing enzymes were reported, but different experimental models with different diets were used.

Chronic ethanol administration as a 5% solution in liquid diets is associated with a lower weight gain, when given in presence of high amounts of dietary lipids. These findings are similar to those reported by Lieber and DeCarli [16]. No difference in weight gain was observed, however, when ethanol was given with a low fat diet. The factors responsible for the impairment of weight gain after chronic ethanol administration have been discussed in detail elsewhere [18, 44]. The absence of impairment of weight gain by ethanol when given with low fat diets is unexplained.

The present data confirm the aversion of rats to ethanol, when given in greater than 5% (w/v) solution [16, 17]. A diet high in fat and containing 6% (w/v) ethanol resulted in undernutrition, as shown by the lower weight gain in contrast with the 6% (w/v) ethanol low fat liquid diet. Restricted food intake is known to alter hepatic drug-metabolizing enzyme activities [20] and inducibility [21, 22]; our data demonstrate that caloric restriction also results in higher activities of cytochrome P-450, NADPH-cytochrome P-450 reductase and benzphetamine demethylase. It is tempting to speculate on the fact that results in the 6% E-HFD may reflect a greater inducibility of drug-metabolizing enzymes by ethanol is spite of the relatively low ethanol intake in this group.

When given with high amounts of fat, more so than when given with low fat diets, ethanol increases cytochrome P-450, P-450 reductase activity and microsomal phospholipids per mg of microsomal protein. These components have been shown to be active in the reconstituted drug-metabolizing enzyme system, after solubilization of microsomes [45–48]. None of these microsomal constituents seems to be rate-limiting *in vitro* for the induction of benzphetamine demethylase, aniline hydroxylase and MEOS activities; these activities expressed per mg of microsomal protein are induced to a similar degree after ethanol (5%, w/v), whether given with a high fat or low fat diet. When results are expressed per 100 g of body weight, a difference in induction between the high fat and low fat models becomes apparent. These results are in keeping with recent studies *in vivo* showing that hexobarbital plasma clearance is accelerated after ethanol, more so if ethanol is given with a high fat rather than a low fat diet [49].

Greater differences in induction levels after ethanol are observed *in vitro* between the high fat and the low fat model after 6% (w/v) ethanol. No significant enzyme induction, except for aniline hydroxylase, is obtained after chronic ingestion of 13–15 g ethanol/kg/day with only 2 per cent of calories as linoleate. The greater induction in the 6% E-HFD model, in spite of relatively low ethanol ingestion (10–12 g/kg/day), may be attributed, at least in part, to a greater inducibility by ethanol when associated with undernutrition and/or caloric deficiency, which has also been shown to yield higher basal values.

Basal MEOS activity is probably lipid dependent. In high fat models, control values are 38–44 per cent higher than in low fat models. This cannot be explained by lower caloric intakes and the results obtained in groups 5 and 6 do not show any difference in MEOS activity in caloric deficiency. Thus MEOS appears to be relatively independent of the caloric intake. However, the ethanol-induced increase in MEOS activity, which was 30–37 per cent in all models except the 6% E-HFD model, appears to be unrelated to the dietary lipid intake. In the 6% E-HFD model, associated with caloric deficiency, MEOS decreased 25 per cent after ethanol. Other drug-metabolizing enzyme activities, on the contrary, were not depressed by the same dietary stress. Both ethanol ingestion in sufficient amounts and adequate caloric intake are thus needed for adequate MEOS induction by ethanol. Our data in the low fat models suggest that, in spite of using the reported optimal amounts of dietary linoleate [23], induction of drug-metabolizing enzymes by ethanol is still lipid dependent. Measurement of the linoleate content of our high fat diets by gas chromatographic analysis revealed that they contained 10·9 per cent of calories as linoleate. Our findings are at variance with those of Casters *et al.* [23], who reported, in male Sprague–Dawley rats, a sharp decrease of hexobarbital metabolism *in vitro* as well as *in vivo*, in rats fed diets containing more than 3 per cent of calories as linoleate. However, the optimal linoleate content for inducibility, using DeCarli and Lieber's [1] liquid diet, is as yet unknown.

The present data also stress the importance of knowing the amount of ethanol ingested, the type and composition of the diet used, and the caloric status of the animals, when effects of ethanol on hepatic drug metabolism are studied. Undernutrition and

caloric deficiency are present when ethanol is given in drinking water at a 10% (w/v) or higher concentration [10, 15–18]. Conflicting results concerning the effects of ethanol on drug-metabolizing enzymes *in vitro* [10–15] may be explained by such factors [10, 15].

Phenobarbital induction of drug-metabolizing enzymes has been shown to be lipid dependent; this may be due to changes in the composition of microsomal membrane phospholipids and constituent fatty acids [41, 43, 50, 51]. Since ethanol has been shown to alter microsomal membrane constituents [52], it might thus alter drug-metabolizing enzymes by mechanisms similar to those invoked for phenobarbital.

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