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EFFECTS OF CHRONIC ETHANOL ADMINISTRATION IN THE RAT: RELATIVE DEPENDENCY ON DIETARY LIPIDS—III. *IN VIVO* TOLERANCE TO HEXOBARBITAL *

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Abstract—Chronic administration of ethanol with either a high-fat (35% cal., including 2% cal. as linoleate) or a low-fat (2% cal. as linoleate) diet reduces similarly the hexobarbital sleeping times in the rat. Ethanol decreased the plasma and total body half-lives of hexobarbital in both dietary models, but they were decreased significantly more with the high-fat diet. A good correlation between hexobarbital plasma half-life and sleeping time was found only with the high-fat dietary model; the sleeping time was not a good index of hexobarbital metabolism in the low-fat model. Ethanol-fed rats awaken with significantly higher hexobarbital concentrations in brain and other tissues; this phenomenon is significantly more important in the high-fat model. Ethanol, administered chronically in nutritionally adequate liquid diets, increases tolerance to hexobarbital by increasing drug disposition and by decreasing central nervous system sensitivity. Both factors in tolerance are altered by modifications of the dietary lipid intake.

Chronic ethanol administration produces a proliferation of the smooth endoplasmic reticulum [1–3], as well as an increase in microsomal protein and phospholipid mass [4]. *In vitro* drug-metabolizing enzyme systems are dependent on at least three components: cytochrome P-450, NADPH cytochrome P-450 reductase and phospholipids [5–7]. Chronic ethanol administration increases hepatic cytochrome P-450 [8, 9], NADPH cytochrome P-450 reductase [9] and microsomal phospholipids [4]. A number of microsomal drug-metabolizing enzyme activities are also induced by chronic ethanol administration [3, 8], particularly in the smooth endoplasmic reticulum microsomes [9].

Plasma clearances of certain drugs are increased after chronic ethanol administration, both in experimental animals and man [10]; this effect has been attributed, in part, to increased activities of components responsible for hepatic drug biotransformation. On the other hand, tolerance to barbiturates after ethanol administration has also been shown to be due to a decrease in central nervous system sensitivity [11], a sex-dependent phenomenon [12].

Lipids play a role in drug-metabolizing enzyme activities [13–17] and in microsomal membrane proliferation, following phenobarbital administration [18, 19]. Variations in the type of dietary lipid ingested are also associated with variations in microsomal membrane composition [20–22], while the chronic administration of ethanol is known to alter hepatic lipid metabolism [23, 24]. In recent studies, we observed that dietary fat might play a significant role in the induction of drug-metabolizing enzymes *in vitro* by ethanol [25–27]. This prompted us to verify the effects of chronic ethanol administration and dietary lipids on hexobarbital metabolism *in vivo*.

MATERIALS AND METHODS

Animals and diets. Female Sprague–Dawley rats were purchased from the Canadian Breeding Laboratories (Saint-Constant, Quebec) in groups of weanling littermates and fed laboratory chow and tap water *ad lib.* until the start of the experiment, when they had reached a weight of 120–150 g. They were then housed in individual wired-bottom cages and isocalorically fed nutritionally adequate liquid diets [28] in pairs or in groups of four rats. These diets contained 18% of total calories as protein, 35% or 2% as fat, always including 2% of total calories as linoleate, 11 or 44% as carbohydrate (Dextri-Maltose) and 36% of the remaining calories either as additional carbohydrates (controls) or as ethanol (5%, w/v, solution). Diets containing 35% of total calories as fat will be referred to as high-fat diets (HFD) and those containing 2% of total calories as fat, as low-fat diets (LFD). The duration of feeding periods and the caloric status of the animals are given in Results for each series of experiments. The average daily intake of ethanol varied between 13 and 17 g/kg/day and the last dose of ethanol was given 24 hr before the administration of hexobarbital so that, when the studies were done, no remaining ethanol was present in the blood samples.

Hexobarbital sleeping time. The measurements of sleeping time and plasma half-life of the drug were carried out after administration of sodium hexobarbital (100 mg/kg, i.p.) as a 20 mg/ml solution in 0.9% saline. The duration of the sleeping time was estimated in rats by determination of the interval, in minutes, between the loss and the recovery of the righting reflex. The righting reflex was considered present if the animal was able to raise itself on its four legs after being placed on its back three times consecutively within 1 min.

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Hexobarbital plasma half-life measurements. The concentration of unchanged drug was measured at 10-min intervals, starting 20 min after the administration of hexobarbital. For this set of experiments, blood (150 μl) was collected from the tail vein into heparinized capillary tubes and centrifuged. Volumes of 50 μl of plasma were placed in 0.5-ml tubes and kept frozen until analysis. Hexobarbital was extracted according to the micro method of Flanagan and Withers [29]. The extraction was performed after the addition of 5 μl of 4.0 M NaH_2PO_4 to each plasma sample, followed by 50 μl chloroform containing 30 μg/ml of glutethimide as an internal standard. The contents of the tubes were mixed thoroughly for 30 sec on a vortex mixer and centrifuged. One-μl aliquots of the chloroform extracts were used for quantitation by gas chromatographic analysis. Calibration was done using a set of standard solutions containing from 10 to 75 μg/ml of hexobarbital (acid) and 30 μg/ml of glutethimide. The ratio of the peak height of the drug to the peak height of the internal standard was linear over the stated concentration range.

Gas chromatography. A Fisher Victoreen series 4400 gas chromatograph equipped with a flame ionization detector and a Mettler GA 10 recorder was used. The column was 4 ft × 2 mm i.d. silane-treated glass, with silanized glass-wool plugs, packed with 3% PPE-20 (Poly-M-Phenoxylene) on 100/120 mesh Supelcoport. Temperatures were 250° at the injection port, 220° in the column oven and 325° in the detector. The following flow rates were used: helium (carrier gas), 37 ml/min; hydrogen, 22 ml/min and oxygen, 400 ml/min. Retention times for hexobarbital and glutethimide were 1.5 and 1.8 min, respectively, under these conditions.

Hexobarbital tissue distribution upon awakening. The animals were given 100 mg/kg of sodium hexobarbital-[2-^{14}C] (0.135 μCi/mg) intraperitoneally. They were decapitated 1 min after awakening. Blood was collected in heparinized tubes, centrifuged, and the plasma samples were transferred to plastic vials and frozen in dry ice–acetone. Tissue samples were transferred quickly to plastic vials and also frozen in dry ice–acetone. All samples were stored at −40° until analysis.

Total body half-life of hexobarbital. The animals were given 100 mg/kg, i.p., of sodium hexobarbital-[2-^{14}C] (0.164 μCi/mg). They were killed in groups at 1, 20, 35, 50 and 65 min after injection. Each rat was stunned and homogenized with 700 ml of 0.1 M phosphate buffer, pH 6.8, in a 1-gallon Waring blender for 3 min. The homogenate was filtered through cheesecloth and samples were removed and frozen until assay of hexobarbital.

Hexobarbital-[2-^{14}C] measurements. Hexobarbital-[2-^{14}C] was separated from its metabolites by solvent extraction [30]. Plasma samples were diluted with, and tissues were homogenized in, 4 vol. of 0.1 M phosphate buffer, pH 6.8, containing a known amount of sodium hexobarbital-[^{3}H] as an internal recovery standard. The samples were then extracted with an equal volume of 1-chlorobutane for a 1-hr period. The organic layer was then washed twice with fresh buffer for 15-min periods, and 3-ml samples were then transferred to glass scintillation vials for counting in Bray's solution. Counting was done in an Isocap/300 scintillation counter (Nuclear Chicago) equipped with a PDS 3 data reduction system. Recovery of hexobarbital in this system averaged 80 per cent.

Lipid analyses. The total lipid content of whole body homogenates was determined gravimetrically after extraction and washing of the extracts according to Folch *et al.* [31]. The Dade Diagnostics, Inc., Tri-25 triglyceride method was used for the measurement of the triglyceride content of the body homogenates.

Statistical analyses. Reported results are given as mean ± S.E. The determination of statistical significance and interaction for the effects of the two variables studied was done using a computer program for non-orthogonal analysis of variance for factorial designs as described by Armitage [32]. Correlation coefficients and linear regression lines were calculated with a Compucorp 340 Statistician (Computer Design Corp., Los Angeles, CA).

Reagents. Dextri-Maltose was purchased from Mead-Johnson (Evansville, IN). Sodium hexobarbital was obtained from Winthrop Laboratories (New York, NY), and glutethimide from Ciba Co. Ltd (Dorval, Quebec). Hexobarbital[2-^{14}C] and hexobarbital[^{3}H] were obtained from the New England Nuclear Corp. (Lachine, Quebec). Columns for gas chromatographic analyses were purchased from Supelco, Inc. (Bellefonte, PA).

RESULTS

The nutritional status of the animals is described in Table 1. Rats used in the first set of experiments (group 1) were submitted to a pair-feeding schedule, resulting in a 12 per cent higher caloric intake in animals receiving the LFD as compared to those on the HFD. Similarly, ethanol consumption was 12 per cent higher in ethanol-treated rats receiving the LFD than in rats given ethanol with the HFD. Therefore, in subsequent experiments, groups 2 and 3 were fed isocalorically in sub-groups of four rats (LFD, E-LFD, HFD, E-HFD), their daily caloric intake being adjusted to that of the least consuming rat, usually the one receiving ethanol with the HFD. In each set of experiments, rats fed with the HFD and the LFD were studied at the same time so that experimental conditions such as age of animals, stocks of diets, room temperature and stress were similar and could not account for differences in results between the high-fat and low-fat models.

As seen in Table 1, similar growth rates were observed in control rats from both dietary models. Ethanol with a HFD significantly impaired weight gain ($P < 0.001$) and this, by contrast, was not observed with ethanol and the LFD. The effect of ethanol is significantly potentiated by the high dietary fat content ($P < 0.005$).

Hexobarbital sleeping time. The effects of chronic ethanol administration on the hexobarbital sleeping time, given in Table 2, were first studied in the pair-fed rats from group 1. The administration of ethanol slightly increased the sleep induction time and significantly reduced the sleeping time ($P < 0.001$) in this group to approximately half of control values in both dietary models, although rats given ethanol with the HFD showed, both as a group and as individual pairs with their controls, a tendency toward a greater, though not statistically significant, reduction in sleeping time than those given ethanol with the LFD. Since these

Table 1. Nutritional status of the animals after chronic ethanol feeding with various amounts of dietary fat

Group No.	No. of pairs	Type of diet	Duration (weeks)	Initial weight (g)	Caloric intake (cal./day)	Weight gain g/day	Ethanol / Control
1	10	LFD*	9	149.5 ± 4.6†	64.5 ± 1.0†	1.51 ± 0.11†	0.894
		E-LFD	9	155.0 ± 4.7	63.9 ± 1.6	1.35 ± 0.07	
	8	HFD*	9	134.2 ± 11.4	57.8 ± 2.9	1.56 ± 0.09	0.743
		E-HFD	9	141.1 ± 10.7	57.1 ± 3.0	1.16 ± 0.07	
2	12	LFD	5	133.1 ± 3.7	51.3 ± 1.3	1.73 ± 0.07	0.929
		E-LFD	5	140.7 ± 5.0	51.5 ± 1.1	1.61 ± 0.09	
	12	HFD	5	132.5 ± 1.8	50.9 ± 0.8	1.85 ± 0.06	0.747
		E-HFD	5	138.2 ± 2.3	48.6 ± 0.8	1.38 ± 0.11	
3	21	LFD	4.5	152.2 ± 3.5	56.6 ± 0.4	1.82 ± 0.08	0.890
		E-LFD	4.5	157.9 ± 3.6	54.3 ± 0.6	1.62 ± 0.10	
	21	HFD	4.5	152.3 ± 3.6	56.7 ± 0.4	1.74 ± 0.10	0.678
		E-HFD	4.5	158.3 ± 3.6	54.0 ± 0.7	1.18 ± 0.10	

* LFD, low-fat diet (2% of cal. as fat); E-LFD, low-fat diet with ethanol (36% of cal.). HFD, high-fat diet (35% of cal. as fat); E-HFD, high-fat diet with ethanol (36% of cal.).
† Mean ± S.E.

differences could have been accounted for by differences in daily caloric and/or ethanol intake (Table 1) between the HFD and LFD rats in this experiment, we have also studied this phenomenon in a second group of animals (group 2) which was fed isocalorically in subgroups of four. Ethanol administration again significantly reduced hexobarbital sleeping time (Table 2), but to a similar extent (60 per cent decrease, $P < 0.001$) in both dietary models; it also slightly increased sleep induction time similarly with the HFD and LFD ($P < 0.05$). Statistical analyses reveal that in group 2 a high dietary fat content reduces the hexobarbital sleeping time ($P < 0.02$). This dietary fat effect was found to be additive to the ethanol effect and did not potentiate the latter.

Hexobarbital plasma half-life. The plasma half-life ($T_{\frac{1}{2}}$) of hexobarbital was also measured concurrently with sleeping time determinations in rats from group 1. Ethanol, when given with a HFD, significantly ($P < 0.005$) reduced the plasma $T_{\frac{1}{2}}$ of the barbiturate by 44 per cent (Fig. 1). This is of the same order of magnitude as the reduction in the sleeping time (52 per cent) in the same animals (Table 2). By contrast, ethanol administration with a LFD resulted in a decrease in the plasma $T_{\frac{1}{2}}$ of hexobarbital of only 26 per cent ($P < 0.05$) (Fig. 2), although a 41 per cent decrease in sleeping time was observed in these rats (Table 2). Plasma $T_{\frac{1}{2}}$ of controls fed the HFD does not differ significantly from plasma $T_{\frac{1}{2}}$ of controls fed the LFD (Figs. 1 and 2). These results, although suggestive of a potentiation of the ethanol effect by dietary fat, failed to show statistical significance in favor of such an interaction between these two factors.

Correlation between hexobarbital sleeping time and plasma half-life. Figure 3 illustrates the highly significant correlation (r: 0.99) found by regression analysis between the hexobarbital sleeping time and plasma $T_{\frac{1}{2}}$ in all rats of group 1 (Tables 1 and 2) fed the HFD, whether ethanol-treated or controls; however, no significant correlation between the same two parameters could be found in rats of this group fed the LFD.

Hexobarbital concentration in tissues upon awaken-

Table 2. Effects of chronic ethanol feeding with various amounts of dietary fat on the hexobarbital sleeping time

Group No.	Type of diet	Onset of sleep (min)	Ethanol / Control	Duration of sleep (min)	Ethanol / Control
1	LFD	3.1 ± 0.3*	1.19	26.4 ± 2.7*	0.59
	E-LFD	3.7 ± 0.3		15.7 ± 0.9	
	HFD	3.2 ± 0.3	1.06	26.2 ± 3.1	0.48
	E-HFD	3.4 ± 0.2		12.7 ± 1.4	
2	LFD	2.2 ± 0.1	1.14	50.0 ± 4.1	0.41
	E-LFD	2.5 ± 0.1		20.5 ± 2.2	
	HFD	2.2 ± 0.2	1.18	40.9 ± 2.5	0.38
	E-HFD	2.6 ± 0.2		15.7 ± 1.6	

* Mean ± S.E.

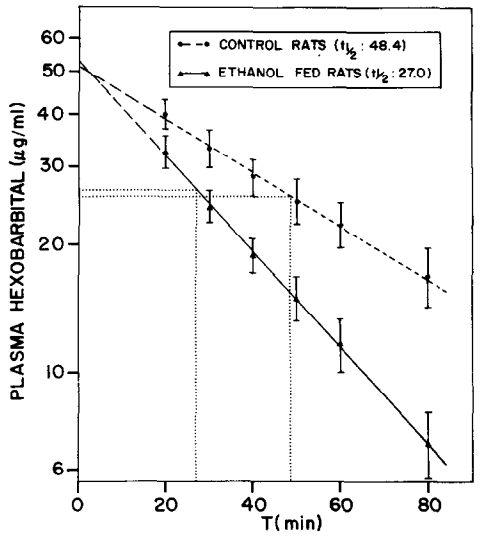

Fig. 1. Effect of chronic ethanol consumption with 35% cal. as fat on the plasma half-life of hexobarbital (100 mg/kg, i.p.) in rat littermates (eight pairs).

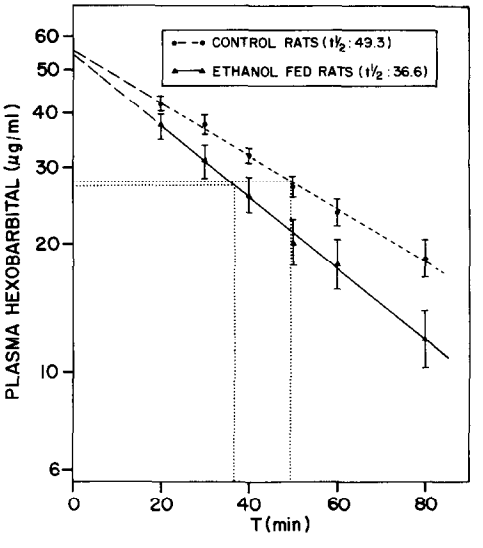

Fig. 2. Effect of chronic ethanol consumption with 2% cal. as fat (linoleate) on the plasma half-life of hexobarbital (100 mg/kg, i.p.) in rat littermates (ten pairs).

ing. The concentration of hexobarbital in tissues at the moment of awakening was determined in rats from group 2 (Table 1). The results are summarized in Fig. 4. In spite of similar daily caloric and ethanol intakes in the two dietary models, both chronic ethanol administration and dietary fat influence the tissue concentration of unmetabolized drug at awakening. For ethanol, the level of significance is the same ($P < 0.005$) for all tissues except for liver ($P < 0.05$). For dietary fat, the effect is significant ($P < 0.05$) for all tissues except for plasma, adipose tissue and muscle. An additive effect between ethanol and dietary fat led to higher unmetabolized hexobarbital tissue concentrations at awakening in animals fed the HFD.

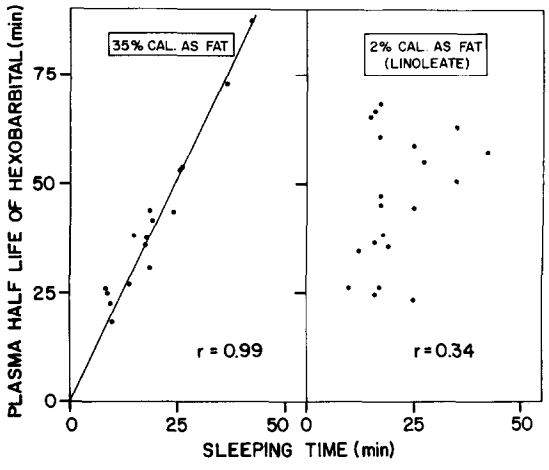

Fig. 3. Correlation between hexobarbital sleeping time and plasma half-life in rat littermates fed either the ethanol-containing or the control diets.

Overall examination of the results shows that drug levels at awakening in the four subgroups of rats follow a similar pattern for all tissues studied. Lowest tissue concentrations are found with the LFD, followed by the HFD, the E-LFD and the E-HFD which showed the highest concentrations.

Significantly higher (6.1 to 13.8 per cent) drug levels are found in controls with HFD when compared to controls fed LFD in plasma, heart, kidney and muscle ($P < 0.005$) and less significantly in brain, liver, lung and adipose tissue ($P < 0.05$). Differences between ethanol-fed and control rats in the HFD model range from 6.6 to 22.4 per cent and are significant in plasma and muscle ($P < 0.01$) and highly significant in all other tissues ($P < 0.001$). Differences ranging from 6.6 to 28 per cent, found in the LFD model after ethanol,

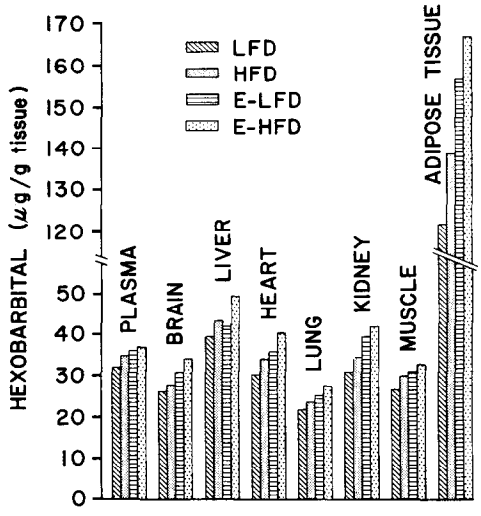

Fig. 4. Effect of chronic ethanol (E) consumption with 35% cal. as fat (HFD) or 2% cal. as fat (LFD) on the hexobarbital (100 mg/kg, i.p.) concentration in tissues at the moment of awakening.

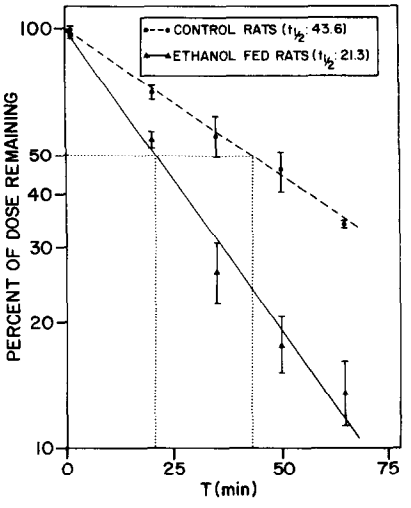

Fig. 5. Effect of chronic ethanol consumption with 35% cal. as fat on the total body clearance of hexobarbital (100 mg/kg, i.p.) in rat littermates (twenty-one pairs).

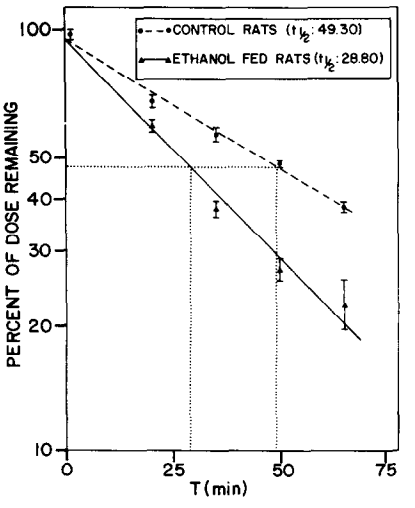

Fig. 6. Effect of chronic ethanol consumption with 2% cal. as fat (linoleate) on the total body clearance of hexobarbital (100 mg/kg, i.p.) in rat littermates (twenty-one pairs).

were highly significant in all tissues ($P < 0.001$) except liver. When ethanol-fed rats were compared with the LFD and HFD models, significantly higher tissue levels of hexobarbital were found in brain, liver, heart and lung ($P < 0.001$), and in kidney ($P < 0.05$) of HFD models. Hexobarbital was found to accumulate in adipose tissue in all animals; concentrations range from 3.8 to 4.5 times those found in plasma.

Hexobarbital total body half-life. The total body content of unmetabolized hexobarbital was determined in rats from group 3 (Table 1) after periods ranging from 1 to 65 min following injections of the drug. The results are summarized in Figs. 5 and 6. Chronic administration of ethanol with a HFD (Fig. 5) reduced the total body hexobarbital $T_{\frac{1}{2}}$ by 51 per cent when compared to control. A 41 per cent decrease was also observed after ethanol in the low fat model. Overall analysis of the data reveals that both ethanol ($P < 0.001$) and dietary fat ($P < 0.005$), as main effects, decrease total body $T_{\frac{1}{2}}$ of hexobarbital and that the interaction between these two factors is significant ($P < 0.01$). Point by point analysis showed that ethanol-fed rats had significantly lower body hexobarbital than their controls (HFD, $P < 0.005$; LFD, $P < 0.01$) at all times except in the first minute after drug administration. Hexobarbital levels in the homogenate were not significantly different in controls with the HFD and LFD models, but they were significantly lower in ethanol rats with the HFD compared to the LFD model between 35 and 70 min after hexobarbital administration ($P < 0.02$). These observations reveal that dietary fat, without influencing total body $T_{\frac{1}{2}}$ of the drug, *per se*, potentiates the action of ethanol.

Total body lipids. Because hexobarbital accumulated preferentially in adipose tissue, the total lipid content of body homogenates was measured in rats from group 3 (Table 1) in which total body $T_{\frac{1}{2}}$ measurements were obtained. These values are important for the proper assessment of the possible role of alterations of drug distribution in the overall effects of ethanol on hexobarbital plasma $T_{\frac{1}{2}}$. As can be seen in Table 3, rats given the HFD had higher, although not significantly, total body lipids than their LFD counterparts. In controls, this is accounted for by significantly higher total body triglycerides ($P < 0.05$) in the HFD model, but no such difference is found in the ethanol-fed animals. When compared with controls, animals fed ethanol with LFD or HFD show an 11 per cent lower content in total body lipids (NS). This is accounted for by a highly significant reduction in triglycerides after ethanol both in the LFD (46 per cent, $P < 0.005$) and the HFD (56 per cent, $P < 0.001$) model.

Table 3. Effects of chronic ethanol feeding with various amounts of dietary fat on whole body lipid and triglyceride content

Group No.	Type of Diet	Total lipids (mg/g body wt)	Triglycerides (mg/g body wt)	Total lipids-triglycerides (mg/g body wt)
3	LFD	87.81 ± 8.12*	15.27 ± 1.96*	72.54 ± 6.69*
	E-LFD	77.86 ± 5.57	8.23 ± 1.08	69.62 ± 4.96
	HFD	96.02 ± 6.89	20.12 ± 2.10	75.90 ± 6.30
	E-HFD	85.26 ± 6.72	8.94 ± 1.25	76.31 ± 6.20

* Mean ± S.E.

DISCUSSION

Chronic ethanol administration is associated with important alterations in the duration of the pharmacological effect of hexobarbital. Ethanol, given either with a HFD or a LFD, reduces the hexobarbital sleeping time to approximately half of the control value. In the first part of the present study (group 1), animals fed the LFD had a greater caloric and ethanol intake than those given the HFD as a result of the preference of rats for the LFD. If tolerance to hexobarbital were due only to alterations of the central nervous system sensitivity to drugs by ethanol, one would have expected shorter sleeping times in rats fed ethanol with a LFD, since these had ingested more ethanol than their HFD counterparts. While this was not the case, the hexobarbital plasma half-life was also found to be decreased by ethanol, an effect which tended to be more pronounced with a HFD than with a LFD. These observations suggested a possible influence of dietary lipids on drug disposition and action *in vivo*, in keeping with previous observations *in vitro* [25].

The close correlation found between hexobarbital sleeping time and plasma $T_{\frac{1}{2}}$ in rats from this group having received a HFD shows that the sleeping time is a good index of hexobarbital disposition in these animals. The lack of correlation between these parameters in the rats fed the LFD points, however, to the fact that the sleeping time is not always a good index of hexobarbital disposition and that factors other than drug disposition may play a role in the duration of the pharmacological effect of the drug.

In the second set of experiments (group 2), when the same amount of ethanol was given with either the HFD or the LFD, the hexobarbital sleeping time was reduced to the same extent after ethanol in rats fed either a LFD or a HFD when results were expressed as per cent of the control values. In this group, however, both ethanol and a high dietary fat content were shown to have, as main effects, a significant influence in decreasing sleeping times, showing that the dietary lipid content plays a role in the pharmacological action of the drug. Differences in daily caloric intake and in duration of the feeding period are probably responsible for differences in the time of sleep induction and in the sleeping time values between rats of groups 1 and 2.

Chronic ethanol administration has been shown to increase the *in vitro* activities of hepatic drug-metabolizing enzymes [9, 10], and it has been suggested that increased hepatic drug metabolism could explain, in part, the greater tolerance to drugs observed after ethanol administration [10]. The present data are consistent with these observations, as discussed previously. Increased biotransformation of hexobarbital appears to be the main factor responsible for the acceleration of its plasma clearance since, as seen in group 3, the total body hexobarbital $T_{\frac{1}{2}}$ is also reduced after ethanol in both dietary models. In agreement with previous *in vitro* findings, dietary lipids are found to play a significant role in hexobarbital disposition after ethanol. As seen in total body clearance studies, a significant difference in clearance of the drug is observed between the high- and low-fat models after ethanol, and dietary fat is found to potentiate the effect of ethanol. This effect of dietary fat was also suggested from the data on plasma T in which statistical significance was not reached. An effect of dietary lipids was also found on the sleeping time.

Ethanol-induced tolerance to barbiturates has been shown to be associated with modifications of the central nervous system sensitivity [11]. This might explain, in part, why the time for sleep induction tends to increase after ethanol treatment in both dietary models in the first experimental group and increases significantly after ethanol in the second group in which sleeping times were obtained. This cannot be explained satisfactorily by an increase in drug biotransformation. It is also in keeping with the tissue distribution pattern of hexobarbital observed at awakening. Ethanol-fed rats awaken with significantly higher hexobarbital concentrations than controls, in brain and other tissues, in both dietary models. This phenomenon is significantly more important in the high-fat than in the low-fat model. Hexobarbital tissue concentrations are higher in animals with shorter sleeping times and plasma $T_{\frac{1}{2}}$. This might be due to the lag period for the back diffusion of the drug from the peripheral tissues compartment to the plasma compartment in conditions of enhanced hexobarbital metabolism. Increased brain concentrations of hexobarbital at awakening in ethanol-fed rats do not allow definite conclusions on central nervous system sensitivity changes since they may not reflect the drug concentration in the brain structures responsible for the hypnotic effect of the drug.

On the basis of its lipid solubility, much higher concentrations of hexobarbital were expected and found in body fat than in any other tissue. Alterations in drug distribution secondary to chronic ethanol feeding do not explain, however, the acceleration of hexobarbital plasma clearance after ethanol since faster total body clearances of hexobarbital were also observed. A higher concentration of hexobarbital in adipose tissue after ethanol may also be explained by the reduction of total body triglyceride mass. Considering hexobarbital concentrations in adipose tissue and the body mass of lipid, the amount of hexobarbital calculated in body fat at awakening is similar in ethanol-fed and control animals.

In summary, sleeping time determination is not always a good index of drug disposition. Cross tolerance between ethanol and hexobarbital is associated with a faster plasma as well as total body clearance of hexobarbital in ethanol-fed animals. It is also associated with a slightly longer time for the induction of sleep and a greater brain drug concentration at awakening in ethanol-fed rats. Altered distribution of hexobarbital by ethanol cannot explain satisfactorily the acceleration of hexobarbital plasma clearance after chronic ethanol feeding. High dietary lipid intake enhances these ethanol-induced alterations of drug metabolism.

In conclusion, increased tolerance to hexobarbital after chronic ethanol feeding, in our model, is due to an important increase in drug disposition and also to a decrease in central nervous system sensitivity. Both factors of tolerance are altered by modifications of the dietary lipid intake.

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