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SIGNIFICANCE *IN VIVO* OF THE INCREASE IN MICROSOMAL ETHANOL-OXIDIZING SYSTEM AFTER CHRONIC ADMINISTRATION OF ETHANOL, PHENOBARBITAL AND CHLORCYCLIZINE

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Abstract—The administration of phenobarbital (100 mg/kg) or chlorcyclizine (50 mg/kg) to rats by stomach tube daily for 1 week resulted in significant increases in liver weight, microsomal protein concentration and the activity of the NADPH-dependent hepatic microsomal ethanol-oxidizing system (MEOS). Pretreatment with either of these agents, however, had no significant effect on ethanol metabolism *in vitro* by liver slices. Pretreatment with phenobarbital had no effect on the rate of ethanol metabolism *in vivo* as measured in the whole body or as estimated from the rate of decrease of blood ethanol concentration, at either 24 or 48 hr after the last dose of phenobarbital. Chronic treatment with ethanol in a high fat adequate protein liquid diet or in a high protein low fat liquid diet resulted in a significant increase in the rate of disappearance of ethanol from blood and the calculated rate of ethanol metabolism. However, no increase in MEOS occurred on a high protein, low fat diet. It is concluded that changes in MEOS bear no relationship to metabolic tolerance to ethanol or to cross-tolerance between ethanol and drugs.

IT IS frequently stated that alcoholics are resistant to barbiturates and other CNS depressants (for references see Kalant *et al.*[1,2]). Barbiturate addicts showed only moderate signs of intoxication after very large doses of alcohol when it was substituted for barbiturates.[3] The nature of this cross-tolerance in chronically treated subjects is not entirely clear. The mechanisms could conceivably involve alterations in distribution or metabolism of the drugs, or in sensitivity of the central nervous system.

A microsomal ethanol-oxidizing system (MEOS) capable of oxidizing ethanol to acetaldehyde *in vitro* in presence of NADPH and O_2 has been described.[4–8] Administration of ethanol and a variety of drugs including phenobarbital, butylated hydroxytoluene and 3-methylcholanthrene has been reported to produce a significant increase in the activity of MEOS, and in the rate of pentobarbital and meprobamate metabolism both *in vitro* and *in vivo*.[9–15] Further, pretreatment of rats with phenobarbital for several days was reported to increase the rate of disappearance of ethanol from the blood.[16] The increase in hepatic MEOS activity after administration of alcohol or drugs has been invoked to explain, at least in part, the following: (1) metabolic tolerance to ethanol in alcoholics, (2) the resistance of alcoholics to hypnotics and anaesthetics and (3) the lesser degree of intoxication with ethanol in barbiturate addicts. The finding by Rubin *et al.*[14] that MEOS activity is significantly inhibited by other drugs, and that ethanol also inhibits the hydroxylation of pentobarbital and

of aniline by microsomes *in vitro*, has been interpreted as evidence that ethanol and these drugs are metabolized by the same microsomal enzyme system.

The results of several other investigators do not seem consistent with these interpretations. Inhibitors and inducers of hepatic microsomal drug-metabolizing systems (MDMS) did not affect ethanol metabolism *in vivo*,[17–21] and barbiturate metabolism *in vivo* and *in vitro* was not affected by chronic alcohol administration.[1] While this evidence cast doubt on the functional significance of MEOS activity *in vivo*, it did not permit definite conclusions because MEOS activity and metabolism of ethanol *in vivo* were not compared in the same studies. After the present work was started, Mezey[21] reported discrepancy between changes in MEOS activity and in blood ethanol disappearance curves *in vivo*.

The present experiments were designed to investigate the following: (1) Under what conditions does chronic administration of ethanol or of inducers of MDMS increase MEOS activity? (2) Does the increase in MEOS produced by ethanol and by inducers of MDMS also result in an enhancement in ethanol metabolism?

MATERIALS AND METHODS

Chronic drug treatments. Adult male Wistar rats, obtained from Canadian Breeding Laboratories, were used in all experiments. Those used in experiments involving chronic phenobarbital or chlorcyclizine treatment were allowed Purina chow and tap water *ad lib.*

Separate groups of animals were given phenobarbital sodium (100 mg/kg) or chlorcyclizine (50 mg/kg) dissolved in distilled water by stomach tube daily for 1 week. The control rats received an equal volume of water. The last dose of drug was given either 24 or 48 hr before tests, as detailed below for individual experiments. Water was available *ad lib.*, but food was withheld for 24 hr before all tests, except in one experiment which is specified below.

Chronic ethanol treatment. Ethanol was administered in a liquid diet, modified from that devised by Lieber *et al.*,[22] as described previously.[23] Two types of diets were used. In the first (high fat, adequate protein diet) ethanol provided 35 per cent of the total calories, fat 41, protein hydrolysate 19 and sucrose 5 per cent. The lipotropic value of the diet expressed as mg/100 Kcal was 73. In the second (low fat, high protein diet) ethanol provided 35 per cent of the total calories, fat 10, protein hydrolysate 25 and sucrose 30 per cent. The lipotropic value of the diet was 110. In the corresponding control diets, ethanol was replaced isocalorically with 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. Liquid diets were administered for 3–4 weeks. The daily intake of ethanol was 10–12 g/kg throughout the experimental period. The diets were replaced by tap water in both ethanol and control groups 24 hr prior to measurement of MEOS activity or ethanol metabolism *in vivo*.

MEOS activity. The liver was removed, and preparation of liver microsomes (105,000 *g* pellet) and assay of MEOS activity were carried out as described previously.[5,7] Microsomal protein was measured by the biuret method.[24]

Ethanol metabolism in vitro *by liver slices.* The livers were removed and liver slices prepared. Slices totaling 250–300 mg/flask were incubated in 5·0 ml of Krebs–Ringer phosphate solution containing 4·0 mg of ethanol. The uptake of ethanol *in vitro*

was determined by measuring the amount of ethanol left in the medium after incubation of liver slices for appropriate periods. The details of the procedure have been reported elsewhere recently.[25] One drug-treated animal and one control were used in each separate replication of the experiment.

Ethanol metabolism in vivo. The disappearance of ethanol from the whole body was studied in phenobarbital-treated and control rats after intraperitoneal injection of a test dose of ethanol (3 g/kg, as a 20%, v/v, solution in saline), given 24 or 48 hr after the last dose of phenobarbital. Food was withheld for 24 hr before administration of ethanol. Three hours after the ethanol injection the rats were killed by a blow on the head, immediately dropped into a precooled industrial Waring blender and homogenized with 2000 ml of cold distilled water. The supernatant fraction was obtained and analyzed for ethanol as described previously.[25]

Ethanol disappearance from the blood was also studied in chronically alcohol-treated and control rats, as well as in phenobarbital-treated and control rats, after intraperitoneal injection of a test dose (2·5 g/kg) of ethanol (20%, v/v, in saline), given 24 and 48 hr after the last dose of phenobarbital. Since the same animals were used both times, food was withheld for only 12 hr before each injection of ethanol. Hourly samples of 0·05 ml of blood were taken from the tail of each animal for 5–6 hr after ethanol administration. Blood ethanol concentration was measured by the internal standard technique of gas–liquid chromatography.[26] The disappearance rate of blood ethanol (β) and the rate of ethanol metabolism in milligrams per kilogram per hour were calculated as described previously.[17]

Measurement of phenobarbital in blood. In some of the experiments involving phenobarbital pretreatment, phenobarbital level in the blood was measured at the end of the alcohol disappearance study. For this purpose, 3·0 ml of blood, obtained by decapitation, was extracted and measured spectrophotometrically by the method of Williams and Zak.[27]

RESULTS

Effect of phenobarbital, chlorcyclizine and ethanol treatment on liver weight, microsomal protein and MEOS activity

In comparison with control rats of comparable weight and age, the phenobarbital-treated group showed significant increases in liver weight, microsomal protein and MEOS activity (Table 1). Two separate experiments were carried out with animals of different initial weight. MEOS activity per milligram of microsomal protein increased significantly by approximately 25 per cent in both experiments (Table 1). Much greater increases were obtained when the MEOS activity was calculated per gram of liver (79 per cent in exp. I and 122 per cent in exp. II) or when related to 100 g body wt. (148 per cent in exp. I and 224 per cent in exp. II). The different magnitude of increase in the two experiments was apparently a reflection of age or weight of the animals.

Significant increases in liver weight and MEOS activity were also observed in two separate experiments with rats or different weights or ages, treated with chlorcyclizine (Table 2). Microsomal protein increased significantly in the drug-treated group in one experiment in which the treated animals showed a significant fall in body weight, but not in the other experiment in which no weight loss occurred. The reason for the difference is not clear, but this phenomenon was noted with chlorcyclizine previously.[17]

TABLE 1. EFFECT OF CHRONIC PHENOBARBITAL TREATMENT ON LIVER WEIGHT, MICROSOMAL PROTEIN AND ACTIVITY OF NADPH-DEPENDENT MICROSOMAL ETHANOL-OXIDIZING SYSTEM (MEOS)*

	Body wt. (g)	No. of rats	Liver wt./ body wt. (g/100 g)	Microsomal protein (mg/g liver)	MEOS activity (nmoles acetaldehyde produced/min)		
					Per mg of microsomal protein	Per g of fresh liver	In total wt. of fresh liver/100 g body wt.
Experiment I							
Controls	192 ± 4	6	4·64 ± 0·09	21·0 ± 1·7	3·18 ± 0·24	66 ± 5	304 ± 22
Treated	185 ± 5	6	6·39 ± 0·10	29·4 ± 1·4	4·02 ± 0·22	118 ± 10	754 ± 55
P	NS		< 0·001	< 0·005	< 0·05	< 0·001	< 0·001
Experiment II							
Controls	299 ± 6	7	2·95 ± 0·08	32·3 ± 1·7	3·80 ± 0·06	121 ± 5	359 ± 18
Treated	290 ± 8	7	4·25 ± 0·06	57·8 ± 2·5	4·70 ± 0·02	269 ± 11	1163 ± 67
P	NS		< 0·001	< 0·001	< 0·001	< 0·001	< 0·001

* Values shown are mean ± standard error. P values are for the difference between treated and control group; NS = not significant (P > 0·05).

TABLE 2. EFFECT OF CHRONIC CHLORCYCLIZINE TREATMENT ON LIVER WEIGHT, MICROSOMAL PROTEIN AND ACTIVITY OF NADPH-DEPENDENT MICROSOMAL ETHANOL-OXIDIZING SYSTEM (MEOS)*

	Body wt.	No. of rats	Liver wt./ body wt. (g/100 g)	Microsomal protein (mg/g liver)	MEOS activity (nmoles acetaldehyde produced/min) Per mg of microsomal protein	Per g of fresh liver	In total wt. of fresh liver/100 g body wt.
Experiment I							
Controls	201 ± 3	6	4·26 ± 0·10	18·8 ± 1·1	2·70 ± 0·18	51·0 ± 5	213 ± 24
Treated	199 ± 2	6	5·34 ± 0·12	23·2 ± 2·2	3·40 ± 0·16	80·0 ± 8	422 ± 42
P	NS		< 0·001	NS	< 0·02	< 0·02	< 0·005
Experiment II							
Controls	174 ± 3	6	3·04 ± 0·11	33·6 ± 1·3	3·32 ± 0·12	110 ± 2	336 ± 14
Treated	139 ± 4	6	5·03 ± 0·10	56·2 ± 4·2	4·40 ± 0·26	242 ± 16	1222 ± 91
P	< 0·001		< 0·001	< 0·005	< 0·005	< 0·001	< 0·001

* Values shown are mean ± standard error. P values are for the difference between treated and control group; NS = not significant (P > 0·05).

TABLE 3. EFFECT OF CHRONIC ETHANOL TREATMENT ON LIVER WEIGHT, MICROSOMAL PROTEIN AND ACTIVITY OF NADPH-DEPENDENT MICROSOMAL ETHANOL-OXIDIZING SYSTEM (MEOS)*

	Body wt. (g)	No. of rats	Liver wt./ body wt. (g/100 g)	Microsomal protein (mg/g liver)	MEOS activity (nmoles acetaldehyde produced/min) Per mg of microsomal protein	Per g of fresh liver	In total wt. of fresh liver/100 g body wt.
High fat, adequate protein diet							
Experiment I							
Control	195 ± 28	5	3·18 ± 0·09	38·8 ± 4·6	4·29 ± 0·16	167 ± 24	529 ± 75
Treated	168 ± 26	5	3·95 ± 0·15	43·2 ± 3·1	4·84 ± 0·10	210 ± 18	837 ± 100
P	< 0·02		< 0·02	NS	< 0·025	< 0·01	< 0·005
Experiment II							
Control	334 ± 9	7	3·36 ± 0·06	34·9 ± 1·1	4·35 ± 0·27	152 ± 9	509 ± 30
Treated	280 ± 15	7	4·38 ± 0·11	39·2 ± 2·2	5·08 ± 0·21	199 ± 12	868 ± 53
P	< 0·001		< 0·001	NS	< 0·01	< 0·005	< 0·001
Low fat, High protein diet							
Experiment III							
Control	277 ± 5	8	3·70 ± 0·13	30·4 ± 1·1	5·26 ± 0·10	160 ± 7	581 ± 19
Treated	306 ± 7	8	4·10 ± 0·22	32·4 ± 1·6	5·16 ± 0·24	161 ± 7	661 ± 56
P	NS		NS	NS	NS	NS	NS
Experiment IV							
Control	285 ± 9	8	4·00 ± 0·08	21·8 ± 0·9	5·15 ± 0·10	115 ± 4	465 ± 19
Treated	320 ± 13	8	4·00 ± 0·12	25·2 ± 1·1	5·56 ± 0·15	140 ± 8	556 ± 30
P	NS		NS	NS	NS	NS	NS

* Results are expressed as mean ± standard error. Since the alcohol-treated and control animals were paired, the significance of differences between them was estimated by the Student's *t*-test for paired data. NS = not significant (P > 0·05). The P refers to Student's *t*-test on paired data for body weight gain rather than final body weights, since the initial weights were not completely identical.

The MEOS activity per milligram of microsomal protein increased significantly by 26–33 per cent. When MEOS activity was expressed per gram of liver or in total liver per 100 g of body wt., the increases were similar to those seen after phenobarbital treatment.

The effect of chronic ethanol treatment on liver weight, microsomal protein and MEOS activity was also examined in four separate experiments (Table 3). The increase of liver weight and MEOS activity after ethanol given in a high fat, adequate protein diet, though significant, was not as pronounced as after phenobarbital or chlorcyclizine, and there was no significant change in microsomal protein content of the liver. MEOS activity expressed per milligram of microsomal protein increased by only 13–17 per cent. When MEOS activity was expressed per gram of liver or in total liver per 100 g of body wt. the increases were 26–31 and 58–71 per cent respectively. In contrast, there was no significant change in liver weight, microsomal protein or any measure of MEOS activity in alcohol-treated rats on high protein, low fat diet, compared with their pair-fed sucrose controls. In experiments I and II (Table 3) the ethanol-treated animals did not grow as well as the controls, but showed increased MEOS activity; in experiments III and IV, they grew at least as well as the controls, and MEOS activity was unchanged. The significance of this observation is not yet apparent.

Effect of phenobarbital, chlorcyclizine and ethanol treatment on ethanol metabolism by liver slices and in vivo

Figure 1 summarizes the results of ethanol uptake experiments with rat liver slices from chlorcyclizine- and phenobarbital-treated rats and their controls. The point

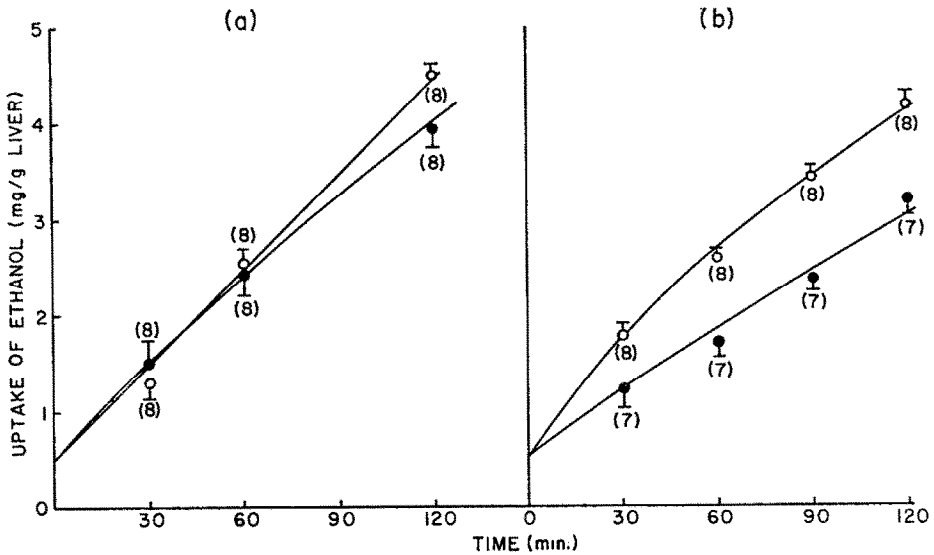

FIG. 1. Uptake of ethanol *in vitro* by liver slices from rats pretreated in various ways: (a) phenobarbita group (●——●) received 100 mg/kg by stomach tube daily for 7 days, while controls (○——○) received water; (b) chlorcyclizine group (●——●) received 50 mg/kg by stomach tube daily for 7 days, while controls (○——○) received water. Phenobarbital and chlorcyclizine, as well as food, were withdrawn 24 hr before sacrifice. Number of animals for each point shown in parentheses. Vertical lines indicate positive or negative half of the standard errors. Regression lines calculated by the method of least mean squares.

from which the curves are shown to originate at zero time represents the theoretical uptake which would correspond to simple diffusion of ethanol from the medium into the tissue water of the slices. This has been discussed elsewhere.[25] If linear regressions are calculated for the flat portions of the uptake curves, using only the experimentally determined points, the slopes (representing rates of metabolism of ethanol) are 1·61 ± 0·16 mg/g/hr for the phenobarbital-treated group (Fig. 1a) and 2·11 ± 0·14 for the controls ($P < 0·05$). The corresponding slopes for chlorcyclizine-treated animals and their controls (Fig. 1b) are 1·33 ± 0·14 and 1·61 ± 0·09 respectively; these are not significantly different. If the curved lines are valid, they would indicate in both cases a more rapid decline in ethanol metabolism by the slices from the drug-treated animals than by those from controls. Since pretreatment with phenobarbital and chlorcyclizine produced a marked increase in liver weight, ethanol uptake by the slices was also calculated on the basis of milligrams of ethanol taken up by total liver per 100 g

TABLE 4. EFFECT OF PHENOBARBITAL TREATMENT ON DISAPPEARANCE OF ETHANOL FROM THE WHOLE CARCASS*

Treatment	No. of rats	Body wt. (g)	Ethanol disappearance rate (mg/kg/hr)
Phenobarbital withdrawn 24 hr			
Controls	8	184 ± 8	249 ± 26
Treated	8	188 ± 7	254 ± 23
P		NS	NS
Phenobarbital withdrawn 48 hr			
Controls	8	192 ± 10	285 ± 19
Treated	9	184 ± 6	301 ± 20
P			NS

* Results are expressed as mean ± standard error. P values are for the difference between control and phenobarbital-treated group; NS = not significant ($P > 0·05$).

TABLE 5. EFFECT OF PHENOBARBITAL TREATMENT ON DISAPPEARANCE OF BLOOD ETHANOL AND CALCULATED RATE OF ETHANOL METABOLISM *in vivo**

Treatment	No. of rats	Body wt. (g)	Disappearance of ethanol from blood (mg/100 ml/hr)	Calculated rate of ethanol metabolism (mg/kg/hr)
Phenobarbital withdrawn 24 hr				
Controls	6	223 ± 4	30·9 ± 4·5	209 ± 23
Treated	7	224 ± 2	33·0 ± 2·5	222 ± 9
P		NS	NS	NS
Phenobarbital withdrawn 48 hr				
Controls	7	209 ± 8	31·6 ± 2·4	237 ± 16
Treated	9	207 ± 2	36·5 ± 2·1	239 ± 9
P		NS	NS	NS

* Results are expressed as mean ± standard error. P values are for difference between control and phenobarbital-treated group; NS = not significant ($P > 0·05$).

TABLE 6. EFFECT OF CHRONIC ETHANOL TREATMENT ON ETHANOL METABOLISM IN RATS*

Treatment	No. of rats	Body wt. (g)	C_0† (mg/100 ml)	r‡	β§ (mg/100 ml/hr)	Calculated rate of ethanol metabolism (mg/kg/hr)
High fat, adequate protein diets						
Chronic ethanol group	10	202 ± 7	380 ± 17	0·699 ± 0·017	55·0 ± 2·2	382 ± 8
Pair-fed controls	10	244 ± 7	398 ± 12	0·669 ± 0·025	37·4 ± 2·7	248 ± 14
P		< 0·001	NS	NS	< 0·001	< 0·001
Low fat, high protein diets						
Chronic ethanol group	14	316 ± 8	377 ± 10	0·669 ± 0·017	46·5 ± 4·6	305 ± 25
Pair-fed controls	14	280 ± 6	330 ± 14	0·776 ± 0·032	32·6 ± 5·1	233 ± 28
P		NS	< 0·005	< 0·005	< 0·02	< 0·02

* Values shown are mean ± S. E. M. Since the alcohol-treated and control animals were pair-fed, the significance of differences between them was estimated by the Student's t-test for paired data. NS = not significant ($P > 0·05$). The P refers to Student's t-test on paired data; that for body weight refers to weight gain rather than final weights, since the initial weights were not completely identical.

† C_0 = Blood alcohol concentration at zero time, estimated by extrapolation of the line fitted to 3–5 hr values.

‡ r = Widmark "reduktionsfaktor", obtained by dividing C_0 into administered dose. This is an approximate substitute for the volume of distribution of alcohol in the body.

§ β = Disappearance rate of ethanol from blood.

of body wt. per hour. These calculated values were 8·70 ± 0·72 and 7·98 ± 0·78, respectively, for phenobarbital-treated animals and their controls, and 5·80 ± 0·60 and 4·91 ± 0·19 for chlorcyclizine-treated and control groups. There was no significant difference between treated and control groups with either drug.

The results pertaining to metabolism *in vivo* from phenobarbital-treated and control rats are shown in Tables 4 and 5. There was no difference between the two groups with respect to the disappearance of ethanol from the whole body, whether a 24- or a 48-hr interval was left between the last dose of phenobarbital and the test dose of ethanol (Table 4). There was also no apparent effect of phenobarbital pretreatment on the rate of ethanol metabolism as calculated from the slope of the blood ethanol disappearance curve (Table 5). Blood samples obtained at the end of the tests done 48 hr after the last dose of phenobarbital showed no detectable levels of barbiturate.

In contrast to phenobarbital and chlorcyclizine, chronic treatment with ethanol on high fat, adequate protein or low fat, high protein diets resulted in a significant increase in the rate of disappearance of ethanol from blood (β) and in the calculated rate of ethanol metabolism (Table 6). The approximate distribution volume for ethanol in the body (r) was significantly lower after chronic ethanol treatment than in pair-fed controls on high protein diet, but no difference was seen between the groups on high fat diet.

DISCUSSION

The present study shows that chronic pretreatment with phenobarbital and chlorcyclizine, which are known inducers of hepatic microsomal drug-metabolizing system (MDMS), results in a significant increase in MEOS activity. This confirms the findings of Lieber and DeCarli[9] and Mezey,[21] who reported a significant increase in MEOS activity after chronic treatment with inducers of the MDMS. However, our findings are not identical with theirs. They observed a significant increase in MEOS on the basis of activity per gram of liver or in total liver per 100 g of body wt, but not per milligram of microsomal protein. In the present study, highly significant increases in MEOS activity occurred, whichever way the activity was calculated. The reason for this discrepancy is not clear, but it may lie in the duration, dose or route of administration of the inducing agent. We administered phenobarbital (100 mg/kg, p.o.) for 7 days, whereas Lieber and De Carli[9] used 80 mg/kg, i.p., for 4 days, and Mezey[21] gave 100 mg/kg, i.p., for 3 and 5 days. The difference is probably not an important one, since the increase in MEOS per milligram of microsomal protein found by Lieber and DeCarli[9] approached statistical significance, with a P value between 0·05 and 0·10.

However, the increase in MEOS activity does not appear to have any significance with respect to ethanol metabolism, because pretreatment with phenobarbital or chlorcyclizine in doses which increased MEOS activity had no effect on ethanol metabolism by liver slices (Fig. 1), and pretreatment with phenobarbital had no significant effect on the rate of ethanol metabolism *in vivo* (Table 4 and 5). Similar findings have also been reported by Tephley *et al.*,[18] Klaassen[19] and Mezey.[21] Despite limitations on interpretability of results obtained with liver slices,[25] all of these findings are in accord with the idea that MEOS activity does not occur to any significant extent in intact liver cells.

In a preliminary communication, Lieber and DeCarli[16] have reported results which appear to be at odds with these findings. In rats treated with phenobarbital (80 mg/kg/day) or saline intraperitoneally for 4 days, they studied blood ethanol disappearance after an oral dose of 3 g/kg given 24 and 48 hr after the last dose of phenobarbital. The rate of ethanol disappearance was enhanced when tested 48 hr after the last dose of phenobarbital, but not at 24 hr. In our study (Table 5) the slope of the blood ethanol curve also appeared to be increased after phenobarbital treatment, but the difference was not significant, and did not represent a true change in rate of ethanol metabolism, regardless of whether phenobarbital was withdrawn 24 or 48 hr before the ethanol test. This lack of difference cannot be attributed to inhibition of ethanol metabolism by residual phenobarbital[16] because no phenobarbital was detectable when tested after the 48-hr interval.

Repeated daily administration of phenobarbital or 3-methylcholanthrene has been shown recently to reduce significantly the sleeping time and the LD_{50} for ethanol in comparison with control values. However, ethanol metabolism *in vivo* was virtually identical in the two groups, so that the cross-tolerance was presumably due to changes in the nervous system.[20] Reinhard and Spector[20] did find a significant increase in metabolism of ethanol *in vitro* by the supernatant fraction of liver homogenates from rats pretreated with phenobarbital and 3-methylcholanthrene but not from those pretreated with 3,4-benzpyrene and phenylbutazone. The reason for the increased metabolism of ethanol *in vitro* by liver homogenates is not certain, but the extremely low activity which they observed makes any interpretation difficult. Their values are only 2–3 per cent of those obtained by other investigators using supernatant fractions from rat liver.

Earlier work from this laboratory had shown[28] that chronic treatment with ethanol resulted in an increase in the rate of ethanol metabolism *in vivo*. This finding has been confirmed by a number of investigators.[15,28–31] We cannot yet explain the difference in value of r between alcohol-treated and control groups on high protein diet, and the lack of difference on high fat diet; the finding deserves further study. However, the increase in β with chronic ethanol treatment is not a distribution artifact, since it appeared on both diets, and corresponded to an increase in calculated rate of alcohol metabolism. The mechanism of this increase needs further exploration. It may be due to adaptive increase in liver alcohol dehydrogenase as suggested by many studies[28,32,33] or to more rapid reoxidation of NADH to NAD as shown recently.[29] In the context of the present work, the relevant finding is that the increase occurred with both a high protein, low fat diet and a high fat, adequate protein diet (Table 6). In contrast, ethanol produced an increase in MEOS activity when given with the latter type of diet (Lieber and DeCarli[9] and present work), or with a low fat, normal protein diet,[34] but not when given in a high protein, low fat diet. Moreover, administration of phenobarbital and chlorcyclizine, both of which increased MEOS activity markedly, did not increase ethanol metabolism. Therefore, the changes in MEOS are evidently not related to change in ethanol metabolism, but probably reflect an increase in smooth endoplasmic reticulum.

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