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EFFECTS OF A CHOLINE-DEFICIENT DIET ON THE INDUCTION OF DRUG- AND ETHANOL-METABOLIZING ENZYMES AND ON THE ALTERATION OF RATES OF ETHANOL DEGRADATION BY ETHANOL AND PHENOBARBITAL*

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Abstract—Phosphatidylcholine has been shown to be an essential component for electron transport to cytochrome P-450 and for hydroxylation of a number of substrates by microsomes *in vitro*. A choline-deficient diet was fed to rats for 3 weeks in order to study the effect *in vivo* of alterations of liver phospholipids on the activity of microsomal enzymes, on parameters of ethanol metabolism, and on the adaptive responses of both to ethanol and phenobarbital administration. Choline deficiency resulted in an increase in total liver lipids and triglycerides, but in a decrease in total phospholipids, due mostly to a decrease in phosphatidylcholine. Choline deficiency did not result in changes in microsomal enzymes or parameters of ethanol metabolism. However, it did prevent optimal induction of aniline hydroxylase activity and cytochrome P-450, by both ethanol and phenobarbital, and of microsomal protein concentration and cytochrome b_5 by phenobarbital; it also prevented ethanol-induced increases both in the activity of the microsomal ethanol-oxidizing system and in the rates of ethanol disappearance from the blood. Alcohol dehydrogenase activity remained unchanged. This study demonstrates that dietary choline is required for optimal induction of microsomal enzymes by both ethanol and phenobarbital, and for increases in ethanol metabolism induced by ethanol administration. It is suggested that a decrease in available hepatic phosphatidylcholine, due to choline deficiency, is a cause of inhibition of the optimal induction of microsomal enzymes.

Phospholipids are structural components of biological membranes and have been shown to be required for the activity of a number of membrane-bound enzymes [1]. The membranes of the endoplasmic reticulum of hepatocytes are particularly rich in phospholipids [2], and the induction of hepatic microsomal enzymes by drugs such as ethanol and phenobarbital is associated with an increase in microsomal phospholipids [3–5]. Ethanol, while principally metabolized by alcohol dehydrogenase which is present in the soluble fraction of liver homogenates [6], is also oxidized *in vitro* by a microsomal enzyme system [7]. The microsomal enzyme system and the rates of ethanol disappearance from the blood, but not alcohol dehydrogenase activity, have been shown to be increased by the administration of both ethanol [7, 8] and phenobarbital [9]. Recently, phosphatidylcholine was identified as the essential phospholipid for the hydroxylation of a number of substrates *in vitro* [10]. Feeding of a choline-deficient diet is known to result in an increase in total hepatic lipids and triglycerides, but in a decrease of phospholipids, in particular of phosphatidylcholine [11, 12].

In the present study, a choline-deficient diet was fed to rats to determine the effect *in vivo* of alterations in phospholipids on microsomal enzyme systems, on ethanol-oxidizing enzymes, on rates of ethanol metabolism, and on the adaptive responses of all parameters to ethanol and phenobarbital administration.

METHODS

Animals and diets

Male albino Wistar rats, weighing between 110 and 130 g initially, were kept in individual wire cages in an air-conditioned room at a constant temperature. The following two choline deficiency experiments were carried out, each with four groups of eight rats and lasting 3 weeks.

Ethanol experiment. The rats were fed either a choline-deficient or a choline-supplemented diet with either ethanol or sucrose making up 36 per cent of the calories. The choline-deficient diets were prepared by adding a solution of ethanol (35·7%) or sucrose (50·2%) to the French choline-deficient diet† [13] supplemented with casein (1·8 ml of the solution per g of French diet supplemented with 0·20 g of vitamin-free casein†) and homogenizing the mixture in a Waring blender. The final caloric compositions of the choline-deficient diets per 100 g of diet were: protein, 15·3 per cent (12·8 g); carbohydrate, 18·3 per cent (15·3 g); fat, 30·4 per cent (11·3 g); and either ethanol

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† Purchased from General Biochemicals, Chagrin Falls, Ohio.

(17·2 g) or sucrose (30·1 g), 36 per cent. The final concentrations of vitamins per 100 g of the diets were as follows: ascorbic acid, 45·0 mg; *p*-aminobenzoic acid, 5·0 mg; biotin, 0·02 mg; calcium pantothenate, 3·0 mg; folic acid, 0·09 mg; inositol, 5·0 mg; menadione, 2·3 mg; nicotinic acid, 4·5 mg; pyridoxine–HCl, 1·0 mg; riboflavin, 1·0 mg; thiamine-HCl, 1·0 mg; vitamin A, 900 i.u.; vitamin D, 300 i.u.; and vitamin E acetate, 5·3 i.u. Minerals were present in adequate amounts. The choline-supplemented diets were prepared similarly, except for the addition of 2·0 g of choline dihydrogen citrate per 100 g of the final diet. The choline-deficient diet containing ethanol was fed *ad lib.* to one group of animals, while the other three groups of animals were pair-fed the other diets. The animals were sacrificed after 14 hr of fasting. The mean intake of the diets was 12·7 ± 4·7 (S.D.) g per day. Animals on the ethanol diets were calculated to consume 15·6 ± 5·8 g ethanol per kg of body weight per day.

Phenobarbital experiment. Rats were fed the choline-deficient and the choline-supplemented diets of French for a period of 3 weeks. The caloric composition per 100 g of the choline-deficient diet was as follows: protein, 8·9 per cent (12·0 g); carbohydrate, 34·2 per cent (45·9 g); and fat, 56·9 per cent (34·0 g). The choline-supplemented diet contained choline dihydrogen citrate in the amount of 2·0 g per 100 g of diet. The choline-deficient diet was fed *ad lib.* to one group of 16 rats, while the other group of 16 rats was pair-fed the choline-supplemented diet. For 5 days during the last week of feeding, one-half of the animals in the choline-deficient and choline-supplemented groups were given intraperitoneal injections of sodium phenobarbital (80 mg/kg), and the other half intraperitoneal injections of normal saline. The animals were sacrificed 60 hr after the last injection. The mean intake of the diets was 14·5 ± 4·2 g per day.

Tissue preparation

The animals were sacrificed by decapitation after 14 hr of fasting. The livers were immediately removed, rinsed in ice-cold physiologic saline and in 1·15% KCl, and weighed. A large section of about 500 mg was separated, weighed, wrapped in foil, placed in a beaker under nitrogen, and frozen for later determinations of lipids. The remainder of the liver was cut into small pieces and homogenized with a Potter–Elvehjem homogenizer in a buffer equivalent to four times the liver weight. The composition of the buffer was as follows: Tris–HCl (0·05 M), KCl (0·08 M), $MgCl_2$ (0·01 M) and sucrose (0·25 M), pH 7·8. The homogenate was centrifuged at 700 *g* for 10 min, and the resulting supernatant recentrifuged at 9000 *g* for 10 min, in both cases at 4° in a Sorvall refrigerated centrifuge. The resulting precipitate was discarded and the supernatant centrifuged at 106,000 *g* for 60 min at 4° in a B-50 International refrigerated centrifuge (Rotor 211A). The supernatant obtained at this point was separated for determination of alcohol dehydrogenase activity. The microsomal pellet was washed once with the above buffer, recentrifuged and then suspended in 8·0 ml of 0·1 M NaH_2PO_4–K_2HPO_4 buffer, pH 7·4.

Enzyme assays

Alcohol dehydrogenase activity was determined by the method of Bonnichsen and Brink [14], the microsomal NADPH-dependent ethanol-oxidizing system (MEOS) activity as described by Lieber and DeCarli [15], aniline hydroxylase according to Imai and Sato [16], and glucose 6-phosphatase by the method of Harper [17], except that the released inorganic phosphorus was measured according to Chen *et al.* [18]. Cytochromes P-450 and b_5 were determined by the methods of Omura and Sato [19], NADPH-cytochrome c reductase by the method of Masters *et al.* [20], and protein concentration according to Lowry *et al.* [21] with bovine serum albumin used as a standard. The activity of alcohol dehydrogenase is expressed per mg of supernatant protein, while the activities of the microsomal enzymes are expressed per mg of microsomal protein.

Liver lipids

Hepatic lipids were extracted by the method of Folch *et al.* [22]. Total hepatic lipids were measured by the method of Amenta [23], triglycerides were determined by the method of Van Handel and Zilversmit [24], and phospholipids by the method of Chen *et al.* [18]. The phospholipids were separated by thin-layer chromatography on Silica gel plates in a solvent system consisting of chloroform–methanol–water (65:25:4) [25]. The plates were air-dried at room temperature for 20 min and the spots detected with iodine vapors. The spots corresponding to phosphatidylcholine and phosphatidylethanolamine were identified by comparison with chromatography of appropriate standards, and further verified with modified Dragendorff reagent and ninhydrin respectively [25]. The spots were then marked by encircling them with a fine needle, scraped out, eluted, and their phosphorus content determined according to Chen *et al.* [18].

Rates of ethanol disappearance

The rates of ethanol disappearance from the blood were determined 2 days prior to sacrifice of the animals. Ethanol (3 g/kg) was given as a 20% solution in water by stomach tube. Eighty μl of blood was obtained from the retro-orbital plexus of each animal with a heparinized capillary tube hourly for 6 hr. After centrifugation at 2000 *g* for 10 min, the separated plasma samples were analyzed for ethanol concentration by gas–liquid chromatography [26]. Ethanol concentrations in the plasma, when plotted against time, followed a linear function. The rate of ethanol disappearance from the plasma was obtained from the slope of the regression line calculated by the method of least squares [27] and was expressed in mg ethanol cleared per 100 ml of plasma per hr.

Statistical analysis

The results are expressed as means ±1 S.D. The data were analyzed by the two-way analysis of variance and by Student's *t*-test [27].

RESULTS

Ethanol experiment. Weight gain was similar in all four groups of animals (Table 1). Choline deficiency

Table 1. Effects of a choline-deficient diet and ethanol on hepatic weight and lipids*

Determination	CS	CD	CS + E	CD + E
Weight gain (g/day)	1·5 ± 0·9	1·3 ± 0·5	1·3 ± 0·9	1·4 ± 0·8
Liver wt (g/100 g body wt)	2·8 ± 0·3	5·9 ± 1·6†	2·9 ± 0·3	4·8 ± 0·7
Total lipids (mg/g)	54·5 ± 11·8	259·2 ± 112·9†	52·1 ± 12·6	187·5 ± 50·4
Triglycerides (mg/g)	11·5 ± 6·1	100·4 ± 54·8†	13·9 ± 2·5	66·9 ± 44·3
Phospholipids (mg/g)	19·7 ± 1·2	14·1 ± 4·3‡	19·4 ± 2·3	18·4 ± 3·8
Phosphatidylcholine (mg/g)	11·3 ± 1·0	8·1 ± 2·7‡	11·1 ± 1·6	10·3 ± 2·9
Phosphatidylethanolamine (mg/g)	6·6 ± 1·0	5·9 ± 2·6	6·2 ± 0·7	6·1 ± 1·9

* CS, choline-supplemented; CD, choline-deficient; CS + E, choline-supplemented plus ethanol; CD + E, choline deficient plus ethanol. All values are expressed as means ±S.D.
† CS vs. CD, $P < 0.001$.
‡ CS vs. CD, $P < 0.05$.

resulted in marked increases in liver weight, total liver lipids and triglycerides ($P < 0.001$). On the other hand, liver phospholipids were lower in the choline-deficient as compared with the choline-supplemented animals ($P < 0.05$), due to a decrease in phosphatidylcholine ($P < 0.05$), while phosphatidylethanolamine remain unchanged. In the choline-supplemented rats, ethanol did not result in any increases in liver weight, total liver lipids, triglycerides or phospholipids. Choline deficiency plus ethanol resulted in increases in liver weight, total liver lipids and triglycerides, similar to the changes found with choline deficiency alone, except that liver phospholipids and phosphatidylcholine were not decreased.

Choline deficiency resulted in a decrease in microsomal protein concentration, but produced no changes in aniline hydroxylase activity or in cytochromes P-450 and b_5 (Table 2). Ethanol resulted in increases in aniline hydroxylase activity and in cytochromes P-450 and b_5 in both the choline-supplemented and choline-deficient animals. However, the mean induced values obtained for aniline hydroxylase and cytochrome P-450, but not for cytochrome b_5, were significantly greater in the choline-supplemented as compared with the choline-deficient animals at $P < 0.05$ and $P < 0.01$ respectively. The activity of glucose 6-phosphatase was not affected by any of the treatments.

As regards parameters of ethanol metabolism, choline deficiency did not change the activities of MEOS and alcohol dehydrogenase or the rates of ethanol disappearance from the blood. However, ethanol resulted in significant increases in the activity of MEOS and in the rates of ethanol disappearance from the blood in the choline-supplemented but not in the choline-deficient animals. The activity of alcohol dehydrogenase was not changed by ethanol in either group of animals.

Phenobarbital experiment. There were no differences in weight gain among the four groups of pair-fed animals (Table 3). Liver weights were increased independently by choline deficiency and by phenobarbital. Therefore, the highest liver weights were found in the animals which had received both a choline-deficient diet and phenobarbital. As in the previous experiment, choline deficiency resulted in an increase in total liver lipids and triglycerides, and in a fall in phospholipids. However, the fall in phospholipids differed from that in the previous experiment in that it was due to decreases in both phosphatidylcholine

Table 2. Effects of a choline-deficient diet and ethanol on the activities of microsomal enzymes, on ethanol-metabolizing enzymes and on rates of ethanol disappearance from the blood*

Determination	CS	CD	CS + E	CD + E
Microsomal protein (mg/g)	25·5 ± 2·1	17·8 ± 2·5†	27·3 ± 4·6	22·1 ± 5·1
Aniline hydroxylase (nmoles/mg/hr)	11·0 ± 3·8	8·2 ± 3·5	18·7 ± 4·2‡§	14·0 ± 4·5‖
Cytochrome P-450 (nmoles/mg)	1·21 ± 0·17	1·00 ± 0·18	1·76 ± 0·19¶·**	1·43 ± 0·18††
Cytochrome b_5 (nmoles/mg)	0·34 ± 0·05	0·34 ± 0·10	0·51 ± 0·14‡	0·53 ± 0·08‡‡
Glucose 6-phosphatase (nmoles Pi/mg/min)	194·9 ± 32·6	203·0 ± 30·4	197·0 ± 46·5	178·0 ± 27·4
MEOS (nmoles/mg/min)	10·6 ± 3·0	8·7 ± 2·6	15·4 ± 3·1‡·**	10·5 ± 1·5
Alcohol dehydrogenase (μmoles/mg/hr)	0·89 ± 0·25	0·72 ± 0·21	0·90 ± 0·10	0·88 ± 0·13
Ethanol disappearance (mg/100 ml/hr)	36·3 ± 4·4	40·7 ± 5·0	49·2 ± 5·6¶	46·2 ± 6·3

* CS, choline-supplemented; CD, choline-deficient; CS + E, choline-supplemented plus ethanol; CD + E, choline-deficient plus ethanol. All values are expressed as means ±S.D.
† CS vs. CD, $P < 0.001$.
‡ CS vs. CS + E, $P < 0.01$.
§ CS + E vs. CD + E, $P < 0.05$.
‖ CD vs. CD + E, $P < 0.05$.
¶ CS vs. CS + E, $P < 0.001$.
** CS + E vs. CD + E, $P < 0.01$.
†† CD vs. CD + E, $P < 0.001$.
‡‡ CD vs. CD + E, $P < 0.01$.

Table 3. Effects of a choline-deficient diet and phenobarbital on hepatic weight and lipids*

Determination	CS	CD	CS + P	CD + P
Weight gain (g/day)	1·8 ± 0·4	1·9 ± 0·7	2·0 ± 0·7	1·4 ± 0·6
Liver wt (g/100 g body wt)	3·8 ± 0·5	5·8 ± 1·4†	5·4 ± 0·7‡	6·9 ± 0·7§
Total lipids (mg/g)	69·8 ± 33·9	253·2 ± 47·5‖	64·6 ± 8·2	237·6 ± 121·0¶
Triglycerides (mg/g)	6·1 ± 1·3	106·6 ± 29·1‖	8·9 ± 4·7	114·2 ± 40·3§
Phospholipids (mg/g)	19·5 ± 2·7	14·8 ± 2·9‖	20·7 ± 2·2	17·9 ± 3·5
Phosphatidylcholine (mg/g)	14·2 ± 1·0	7·9 ± 1·7‖	14·2 ± 1·4	9·6 ± 1·6§
Phosphatidylethanolamine (mg/g)	6·5 ± 0·6	3·9 ± 0·8‖	5·8 ± 1·8	4·4 ± 0·9§

* CD, choline-supplemented; CD, choline-deficient; CS + P, choline-supplemented plus phenobarbital; CD + P choline-deficient plus phenobarbital. All values are expressed as means ±S.D.
† CS vs. CD, $P < 0.01$.
‡ CS vs. CS + P, $P < 0.001$.
§ CS + P vs. CD + P, $P < 0.001$.
‖ CS vs. CD, $P < 0.001$.
¶ CS + P vs. CD + P, $P < 0.05$.

and phosphatidylethanolamine. Phenobarbital administration resulted in no change in total liver lipids, triglycerides or phospholipids in the choline-supplemented animals, and did not alter the increased concentrations of total liver lipids and triglycerides produced by the choline-deficient diet. Phenobarbital, however, partially reversed the lowering effect of choline deficiency on total phospholipids, so that their total concentration in the choline-deficient animals was not significantly different after phenobarbital administration from that found in the choline-supplemented animals; nevertheless, the concentrations of both phosphatidylcholine and phosphatidylethanolamine remained decreased.

Choline deficiency did not decrease microsomal protein concentration (Table 4), as was found in the previous experiment. This difference may be the result of the better dietary intake and weight gain of the animals in this second experiment. There were no differences in the activities of aniline hydroxylase, cytochromes P-450 and b_5, and cytochrome c reductase between the choline-deficient and choline-supplemented animals. Phenobarbital administration resulted in increases in microsomal protein concentration, aniline hydroxylase activity, cytochrome P-450 and cytochrome c reductase in both the choline-supplemented and choline-deficient animals. However, cytochrome b_5 was increased by phenobarbital only in the choline-supplemented animals and the induced values obtained for microsomal protein, aniline hydroxylase and cytochrome P-450 were significantly greater in the choline-supplemented as compared with the choline-deficient animals at $P < 0.05$. No significant difference was found in cytochrome c reductase between the choline-supplemented and choline-deficient animals receiving phenobarbital. The activity of glucose 6-phosphatase was not changed by choline deficiency. However, the administration of phenobarbital resulted in a paradoxic decrease in glucose 6-phosphatase activity in the choline-supplemented ($P < 0.05$) but not in the choline-deficient animals.

Table 4. Effects of a choline-deficient diet and phenobarbital on the activity of microsomal enzymes, on ethanol-metabolizing enzymes and on rates of ethanol disappearance from the blood*

Determination	CS	CD	CS + P	CD + P
Microsomal protein (mg/g)	22·2 ± 4·7	21·9 ± 3·4	31·2 ± 3·5†	27·5 ± 2·0‡
Aniline hydroxylase (nmoles/mg/hr)	10·0 ± 2·1	10·3 ± 2·9	23·3 ± 6·5†	15·0 ± 3·6§
Cytochrome P-450 (nmoles/mg)	1·03 ± 0·17	1·17 ± 0·13	1·90 ± 0·18†	1·69 ± 0·20‡
Cytochrome b_5 (nmoles/mg)	0·28 ± 0·08	0·32 ± 0·16	0·55 ± 0·12‖	0·36 ± 0·10
Cytochrome c reductase (nmoles/mg/min)	131·6 ± 33·9	106·0 ± 14·1	172·1 ± 32·7¶	149·1 ± 24·8‡
Glucose 6-phosphatase (nmoles Pi/mg/min)	208·0 ± 32·0	189·1 ± 44·3	139·3 ± 51·2¶	162·4 ± 55·0
MEOS (nmoles/mg/min)	10·4 ± 2·6	12·9 ± 3·6	17·7 ± 3·4**	18·7 ± 3·4§
Alcohol dehydrogenase (μmoles/mg/hr)	0·84 ± 0·18	0·86 ± 0·22	0·72 ± 0·18	0·88 ± 0·18
Ethanol disappearance (mg/100 ml/hr)	33·7 ± 5·4	35·6 ± 4·9	43·1 ± 5·5¶	41·8 ± 5·3§

* CS, choline-supplemented, CD, choline-deficient; CS + P, choline-supplemented plus phenobarbital; CD + P, choline-deficient plus phenobarbital. All values are expressed as means ±S.D.
† CS vs. CS + P, $P < 0.001$; CS + P vs. CD + P, $P < 0.05$.
‡ CD vs. CD + P, $P < 0.01$.
§ CD vs. CD + P, $P < 0.05$.
‖ CS vs. CS + P, $P < 0.01$; CS + P vs CD + P, $P < 0.001$.
¶ CS vs. CS + P, $P < 0.05$.
** CS vs. CS + P, $P < 0.001$.

As found in the previous experiment, choline deficiency by itself had no effect on parameters of ethanol metabolism. Phenobarbital administration resulted in similar increases in MEOS activity and in the rates of ethanol disappearance from the blood in the choline-supplemented and choline-deficient animals. The activity of alcohol dehydrogenase remained unchanged.

DISCUSSION

The microsomal fraction of liver cell homogenates contains the highest amount of hepatocellular phospholipid [2] as well as the principal pathways of phospholipid synthesis [28]. The phospholipids are an integral part of the membranes of the endoplasmic reticulum, and their association with components of the microsomal electron chain was first suggested by the observation that treatment of microsomes with phospholipase A resulted in conversion of cytochrome P-450 to the inactive hemoprotein cytochrome P-420 [19, 29]. More recently, phosphatidylcholine was shown to be an essential component for the transfer of electrons to cytochrome P-450 in a reconstituted microsomal enzyme system that hydroxylates drugs [10]. In the present study, feeding of a choline-deficient diet resulted, as reported previously [11, 12], in an increase in liver weight, total liver lipids and triglycerides, and in a decrease in phospholipids, the latter change due mostly to a fall in phosphatidylcholine. The decrease in liver phosphatidylcholine resulting from the feeding of a choline-deficient diet is due to decreased synthesis [30], which is not surprising, since phosphatidylcholine is the major microsomal component containing the choline moiety [31]. Both ethanol [5] and phenobarbital [4, 13] administration have been shown to result in an increase in hepatic phospholipids due principally to an increase in phosphatidylcholine synthesis [4, 5]. In the present study neither ethanol nor phenobarbital resulted in an increase in hepatic phospholipids or in the phosphatidylcholine fraction in the choline-supplemented rats. However, they prevented a decrease in total liver phospholipids in the choline-deficient rats. The lack of any significant increases in hepatic phospholipids after ethanol and phenobarbital in the choline-supplemented rats may be related to the time intervals between the last dose of the inducer and the time of sacrifice of the animals. In the ethanol experiment, the animals were fasted for a period of 14 hr prior to sacrifice, while no period of fasting was employed in the studies demonstrating increases in phospholipids secondary to ethanol [5, 32]. Fasting has been shown to result in an increase in microsomal phospholipids in control animals [33] and therefore could have masked any effect of ethanol on phospholipids in this study. With phenobarbital there was a 60-hr interval, which included 14 hr of fasting, between its last administration and sacrifice of the animals. Besides a possible effect of fasting on the control animals, the long 60-hr interval could have resulted in a fall in hepatic phospholipids from peak induced levels, since microsomal phospholipids have been shown to have a faster turnover rate than microsomal protein [34] with an average biological half-life of 28 hr [33].

The feeding of ethanol to the choline-supplemented animals did not result in any increase in hepatic lipids and triglycerides. These findings are in agreement with prior studies showing that large amounts of choline protect against the development of fatty infiltration of the liver produced by ethanol feeding. Lieber and DeCarli [35] found that the addition of choline to the diet in amounts between 2·5 and 5·0 mg/ml of diet resulted in a reduction but not in an elimination of the deposition of hepatic lipids and triglycerides produced by ethanol fed in the diet as 36 per cent of the calories. The addition of even larger amounts of choline to the diet, 18 mg/g of diet by Porta *et al.* [36] and 20 mg/g of diet by Dobbins *et al.* [37], resulted in complete prevention of the accumulation of hepatic lipids and triglycerides induced by feeding ethanol in the water as 26 and 30 per cent of daily dietary calories respectively. Similarly, in the present study no hepatic lipid accumulation was produced by feeding ethanol as 36 per cent of the dietary calories in the presence of 20 mg choline per g of diet.

The decrease in the hepatic content of phospholipids in choline deficiency has been found to be accompanied by a reduction in the amount of endoplasmic reticulum as well as by other alterations in ultrastructure [38]. It was therefore surprising that choline deficiency, with the exception of producing a decrease in microsomal protein concentration in one of the experiments, was not otherwise associated with changes in the components of microsomal electron transport or in the activities of the microsomal enzymes measured. On the other hand, choline deficiency resulted in a reduction of the optimal induction of aniline hydroxylase activity and cytochrome P-450 by both ethanol and phenobarbital, and of microsomal protein concentration and cytochrome b_5 by phenobarbital. It has been shown previously that dietary lipid is necessary for optimal induction by phenobarbital of cytochrome P-450 [39] and of aniline hydroxylase activity [39, 40]; however, the hepatic lipids, and in particular the phospholipid composition, were not determined in those studies. In a more recent study, the induction by phenobarbital of coumarin 3-hydroxylase was shown to be reduced by choline deficiency [41]. In addition, the observation by Dobbins *et al.* [37] that aniline hydroxylase was increased 2-fold above control in rats given ethanol in the drinking water and fed Purina chow, and 3-fold when the ethanol-treated rats were fed Purina chow supplemented with choline, is also in agreement with the findings of our study. Our results differ from those of Ariyoshi *et al.* [42], who found no reduction in the optimal induction of microsomal enzymes produced by ethanol in choline-deficient rats. Their experiment differed from ours, however, in that ethanol was given in the drinking water, the animals were not pair-fed, those receiving ethanol did not gain weight, and most significantly, their choline-deficient animals did not demonstrate a decrease in liver phospholipids.

The lack of change in the activity of glucose 6-phosphatase after ethanol feeding in the present study agrees with the findings of Carter and Isselbacher [43], but differs from those of Ishii *et al.* [44], who found an increased activity and suggested that the

difference in results may be related to the type of carbohydrate given in the diet. The dietary source of carbohydrate used in the control animals by Carter and Isselbacher [43] and in the present study was sucrose, which has been shown to increase the activity of the enzyme [45], while Ishii *et al.* [44] used Dextrimaltose, which is broken down to glucose and has no effect on the enzyme. The observation of a paradoxic decrease in the specific activity of glucose 6-phosphatase after phenobarbital administration corresponds to the findings of other investigators [46, 47]. This effect is due, most likely, to induction of microsomal enzyme proteins principally located in the smooth subfraction of the microsomes, with no concomitant change in glucose 6-phosphatase, whose activity is higher in the rough subfraction [47, 48], resulting in an apparent decrease in activity when expressed per mg of total microsomal protein.

The observed increases in the activity of the microsomal ethanol-oxidizing system and in the rates of ethanol disappearance from the blood, but no changes in alcohol dehydrogenase activity after the administration of ethanol and phenobarbital in the choline-supplemented animals, confirm the findings of previous studies [8, 15, 49]. Choline deficiency prevented the ethanol-induced but not the phenobarbital-induced increases in the activity of the microsomal ethanol-oxidizing system and in the rate of ethanol disappearance from the blood. Since initial publication of these observations in an abstract [50], the effect of choline deficiency in preventing induction by ethanol of the microsomal ethanol-oxidizing enzyme has been confirmed [51]. In the latter study, however, the rates of ethanol disappearance from the blood were not determined, nor were inducers other than ethanol studied. The differences in the effect of ethanol as compared with phenobarbital may be due to the fact that ethanol is a weaker inducer of microsomal enzymes than is phenobarbital, or the differences could be related to the nature of the enzymatic mechanism of ethanol oxidation by liver microsomes, which has been a matter of controversy [52–56].

In conclusion, this study demonstrates that dietary choline is necessary for optimal induction of microsomal enzymes by ethanol and phenobarbital, and for increases in the microsomal ethanol-oxidizing system and in the rates of ethanol disappearance from the blood after ethanol administration. A decrease in the availability of hepatic phosphatidylcholine is a possible cause whereby choline deficiency prevents the optimal induction of the microsomal enzymes.

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