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# MECHANISM OF INDUCTION OF HEPATIC DRUG-METABOLIZING ENZYMES BY ETHANOL—I.

## LIMITED ROLE OF MICROSOMAL PHOSPHOLIPIDS*

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**Abstract**—Two groups of female Sprague–Dawley rats were pair-fed nutritionally adequate liquid diets containing 36 per cent of total calories as ethanol or additional carbohydrate (controls). One group was fed a high fat diet (35 per cent of total calories as fat); the other group was fed a low fat diet (2 per cent of total calories as linoleate as the only source of fat). When given with the high fat diet, ethanol increased cytochrome P-450 and microsomal phospholipid content per g of liver. When given with a low fat diet, it increased cytochrome P-450 to a lesser extent and did not alter the microsomal phospholipid content when expressed per g of liver or per 100 g of body weight. Phosphatidylcholine accounted for the greater porportion of phospholipids and was increased by ethanol only with the high fat diet. L-Methionine-methyl[$^3$H] and [$^{14}$C]choline incorporation into phosphatidylcholine was unaltered by ethanol in either model. The fatty acid composition of phosphatidylcholine was altered by ethanol more significantly with the high fat diet. Since ethanol similarly enhances the activity of benzphetamine demethylation in both dietary models, quantitative and qualitative differences in microsomal phospholipids produced by ethanol are probably not responsible for the induction by ethanol of this drug-metabolizing enzyme activity. Further evidence to that effect was obtained from the measurement of benzphetamine demethylation *in vitro* by partially purified cytochrome P-450. reductase and lipid fractions of ethanol-fed and control rats fed a high fat diet. The stimulation of benzphetamine demethylation by the lipid fraction was identical whether using lipids from microsomes of ethanol-fed animals or control animals. Dietary fat plays a role in the induction by ethanol of cytochrome P-450 and NADPH-cytochrome P-450 reductase and on microsomal phospholipid content and composition. The effects of ethanol on microsomal phospholipids are probably not related to the induction of benzphetamine demethylation activity.

Lipids are involved in hepatic microsomal drug metabolism [1–6] and the induction by drugs of drug-metabolizing enzymes [7–9]. Phospholipids are essential for drug metabolism *in vitro* [10, 11], and it has been suggested that the differences in microsomal phospholipid composition due either to dietary manipulations [8, 12] or to induction by drugs [13–15], or related to sex [16], might account for differences in drug metabolism. In a microsomal reconstituted system, phospholipids of different fatty acid composition display different stimulatory effects on drug oxidation activities [11, 17]. Prolonged ethanol intake modifies hepatic phospholipid metabolism [18–22] and increases the activity of many hepatic drug-metabolizing enzymes [23–27]. Therefore, in considering the relationship between ethanol, hepatic microsomal phospholipid and microsomal drug-metabolizing enzymes, we wondered whether alterations in phospholipids could be responsible for the induction by ethanol of hepatic microsomal drug-metabolizing enzymes.

We have dissociated the ethanol effects on microsomal phospholipids from those on benzphetamine demethylation activity by using two dietary models different by their lipid content. The phospholipid composition of hepatic microsomes, the fatty acid composition of microsomal phosphatidylcholine and phosphatidylethanolamine and the incorporation *in vivo* of phosphatidylcholine precursors were studied.

We have also tested the effect of replacement of microsomal lipids of control rats by those obtained from microsomes of ethanol-fed rats in the reconstituted system for benzphetamine demethylation.

### MATERIALS AND METHODS

*Animals and diets.* Female Sprague–Dawley rats were purchased from Canadian Breeding Laboratories (Saint-Constant, Québec) in groups of weanling littermates and fed laboratory chow and water *ad lib.* until the start of the experiment. Upon reaching a weight of 120–150 g, they were housed in individual wire-bottom cages and pair-fed nutritionally adequate liquid diets [28] (Table 1) for 21 days. Diets containing 35 per cent of total calories as fat will be referred to as the high fat diets (HFD); diets containing 2 per cent of total calories as linoleate as the only source of dietary lipid will be referred to as the low fat diets (LFD). The average daily intake of ethanol with the HFD and the LFD was comparable to that which has been reported previously [27]. Diets were available until sacrifice.

*Differential centrifugation.* At the end of the experiment, 1 hr prior to killing, all animals were injected

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Table 1. Composition of liquid diets (per cent of total calories) for the various groups of animals studied

| | High fat diet | | Low fat diet | |
|---|---|---|---|---|
| | Control | Ethanol | Control | Ethanol |
| Protein | 18 | 18 | 18 | 18 |
| Carbohydrate* | 47 | 11 | 80 | 44 |
| Lipid | | | | |
| Corn oil olive oil mixture | 33 | 33 | | |
| Linoleate | 2 | 2 | 2 | 2 |
| Ethanol† | | 36 | | 36 |

* Dextri-maltose (Mead Johnson, Evansville, Ind.).
† Ethanol concentration of the diet is 5% (w/v).

intraperitoneally with choline[1,2-$^{14}$C]chloride, 30 $\mu$Ci (3.84 $\mu$moles), and L-methionine-methyl[$^{3}$H], 100 $\mu$Ci (0.10 $\mu$mole), in 0.7 ml of 0.9% NaCl. 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 the post-mitochondrial supernatant at 105,000 $g$ for 60 min; microsomes were washed once and resuspended in 0.15 M KCl.

Cytochrome P-450 was measured in homogenates by the method of Greim [29] as validated in our laboratory [30, 31] and in microsomal suspensions, according to Omura and Sato [32], 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.* [33]. 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 which was used to correct for microsomal losses as previously described [31, 34]. All values of microsomal protein and phospholipids per g of liver of this study are corrected values.

*Lipid extraction.* Resuspended microsomes (15 mg protein/ml) were promptly homogenized with 19 vol. chloroform–methanol (2:1, v/v) [35], containing 1 mg/liter of hydroquinone [36], in a glass homogenizer equipped with a Teflon pestle. After standing for 1 hr, the microsomal lipid extract was filtered and an aliquot was evaporated to dryness under a stream of nitrogen at a temperature of 15° or below. The crude extract (10–20 mg lipid) was then immediately resuspended in 1 ml chloroform–methanol (19:1, v/v) saturated with water, and stored overnight at −20° under nitrogen. Removal of the water-soluble nonlipid contaminants was done by Sephadex column chromatography according to Siakotos and Rouser [37]. The extract was applied to a 1.5-cm × 7-cm column packed with Sephadex G-25 (Pharmacia Fine Chemicals Inc., Montreal, Quebec), previously washed and equilibrated with methanol–water (1:1, v/v). The lipids were eluted with 35 ml chloroform–methanol (19:1, v/v) saturated with water at a flow rate of 0.5 ml/min. Samples of the washed lipid extract were then analyzed for total incorporation of radioactivity, and total phosphorus [38].

*Phospholipid analyses.* The washed microsomal extract was evaporated to dryness under nitrogen and resolubilized in a small volume of chloroform methanol (2:1, v/v). Separation of phospholipids was done by two-dimensional thin-layer chromatography (t.l.c.) according to Rouser *et al.* [39]. "Redi-Coats" t.l.c. plates (Supelco Inc., Bellefonte, Pa.) were activated by heating for 30 min at 110° and equilibrated for 20 min just before use in a nitrogen atmosphere of 50 per cent humidity under which samples (1 to 1.5 mg lipids) were then spotted. The plates, three for each rat, were developed in the first dimension with chloroform–methanol–28% aqueous ammonia (65:35:5, v/v), dried for 10 min under a stream of nitrogen and developed in the second dimension with chloroform–acetone–methanol–acetic acid–water (5:2:1:1:0.5, v/v). They were then air-dried for a few min. Spraying with 98% sulfuric acid was used on plates where phospholipid phosphorus distribution analysis was to be done. Phosphorus determination was done according to Rouser *et al.* [39], following direct aspiration of spots. Spots on plates used for analysis of radioactivity distribution, and on those used for fatty acid analysis of phosphatidylcholine and phosphatidylethanolamine, were detected by spraying with 1% iodine in methanol. The identity of phospholipids was established by cochromatography with known reference compounds (Serdary Res. Lab. Inc., London, Ontario, Canada).

*Radioactivity determinations.* Radioactivity of the lipid samples was measured in a Nuclear Chicago Isocap/300 liquid scintillation counter equipped with a PDS/3 data reduction system. Lipid spots scraped from t.l.c. plates were added directly to 15-ml polyethylene vials and counted in a solution of 0.7% 2,5-diphenyloxazole (PPO) and 0.03% 1,4-bis [2-(5-phenyloxazole)] benzene (POPOP) in scintillation grade dioxane containing 10% naphtalene and diluted with 0.2 vol. water [40]. Glacial acetic acid, 50 $\mu$l/vial, was also added to prevent adsorption of phospholipids to Silica gel particles [41]. Counting efficiency in this system was 80 per cent for $^{14}$C and 20 per cent for $^{3}$H.

*Fatty acid analysis.* The phosphatidylcholine and phosphatidylethanolamine spots on t.l.c. plates were scraped in screw-cap vials with Teflon-lined caps and subjected to direct transesterification on the adsorbent [42]. Fatty acid methyl esters were analyzed on a Fisher Victoreen gas chromatograph (model 4400) equipped with hydrogen flame detectors. A 6-ft × 0.125-in. O.D. stainless steel column packed with 15% diethylene glycol succinate (DEGS) on 80–100 mesh Chromosorb W AW (Chromatographic Specialties Ltd., Brockville, Ontario) was used. Samples were run isothermally at 185°. Helium was used as the carrier gas at a flow rate of 20 ml/min. An Autolab 6300 digital integrator was used for the calculation of peak areas. Fatty acid methyl esters were identified by comparison of retention times with standards of known composition (Hormel Institute, Austin, Minn.).

*Preparation of purified cytochrome P-450, NADPH-cytochrome c reductase and lipid fractions.* Microsomes were prepared from groups of rats pair-fed the high fat ethanol and control diets for 21 days. Liver microsomes were prepared as described by Lu and Levin [43]. Cytochrome P-450 was partially purified according to Levin *et al.* [44]; the step III P-450 frac-

Table 2. Effect of chronic ethanol administration on weight gain, liver weight, microsomal protein, cytochrome P-450 and phospholipid (mean ± S. E. M.)

| | High fat* | | | Low fat† | | |
|---|---|---|---|---|---|---|
| | Control | Ethanol | Ethanol / Control | Control | Ethanol | Ethanol / Control |
| Weight gain (g/day) | 1.92 ± 0.12 | 1.37 ± 0.11‡ | 0.71 | 2.24 ± 0.13 | 2.19 ± 0.13 | 0.98 |
| Liver wt (g/100 g body wt) | 3.91 ± 0.08 | 4.66 ± 0.11§ | 1.19 | 4.14 ± 0.13 | 4.34 ± 0.12 | 1.05 |
| Microsomal protein | | | | | | |
| (mg/g liver) | 46.49 ± 1.36 | 47.05 ± 1.34 | 1.01 | 49.38 ± 1.96 | 46.10 ± 2.48 | 0.93 |
| (mg/100 g body wt) | 180.62 ± 5.15 | 220.08 ± 7.65§ | 1.22 | 202.81 ± 6.32 | 201.20 ± 12.09 | 0.99 |
| Cytochrome P-450 | | | | | | |
| (nmoles/mg protein) | 0.95 ± 0.03 | 1.70 ± 0.04§ | 1.79 | 0.80 ± 0.03 | 1.05 ± 0.03§ | 1.31 |
| (nmoles/g liver) | 43.87 ± 1.79 | 78.02 ± 2.37§ | 1.78 | 37.48 ± 1.37 | 46.88 ± 2.30§ | 1.25 |
| Phospholipids | | | | | | |
| (μgPi/mg protein) | 17.00 ± 0.64 | 20.68 ± 0.49§ | 1.22 | 17.16 ± 0.41 | 18.52 ± 0.66• | 1.08 |
| (μgPi/g liver) | 782.8 ± 27.0 | 974.0 ± 39.4‡ | 1.24 | 843.6 ± 32.9 | 846.4 ± 41.1 | 1.00 |
| (μgPi/100 g body wt) | 3061.6 ± 167.2 | 4528.0 ± 249.8§ | 1.48 | 3465.6 ± 130.7 | 3677.6 ± 265.2 | 1.06 |

* Thirty-five per cent of total calories as fat: 15 pairs.
† Two per cent of total calories as linoleate (only source of fat): 13 pairs.
‡ $P < 0.005$.
§ $P < 0.001$.
‖ Corrected for microsomal losses.
• $P < 0.05$.

tion was used. The reductase fraction was solubilized with Triton N-101 and separated by chromatography on DEAE cellulose; Triton N-101 was removed by a second chromatography on DEAE cellulose. Lipids were extracted with Emulgen 911 (Kao Atlas Co. Ltd., Nagoya, Japan) and the detergent in the pooled eluate was partially removed with Bio-Beads SM-2 (Bio-Rad Laboratories, Richmond, Calif.) as described by Lu *et al.* [45]. The crude lipid fraction was isolated from solubilized microsomes on a DEAE cellulose column according to Lu *et al.* [46] and contained deoxycholate, neutral lipids and phospholipids [11].

Benzphetamine* demethylation activity was measured by the rate of NADPH oxidation [10] in the presence of fixed amounts of cytochrome P-450, NADPH-cytochrome *c* reductase and variable amounts (0–56 μg) of the lipid fraction from ethanol-treated and control animals fed the HFD.

*Statistical methods.* The mean of the individual differences was tested by Student's *t*-test for pairs.

## RESULTS

Animals given ethanol with a HFD gained less weight than their controls but their livers weighed more per 100 g of body weight; microsomal protein per g of liver did not change but increased significantly when expressed per 100 g of body weight. Cytochrome P-450 and microsomal phospholipids per mg of protein were increased 79 and 22 per cent respectively. In rats fed ethanol with the LFD, there were no significant differences in weight gain, liver weight, microsomal protein and microsomal phospholipids per g of liver or per 110 g of body weight, when compared to controls. Cytochrome P-450 was significantly increased but less so than with the HFD (Table 2).

The composition of microsomal phospholipids is given in Table 3. Phosphatidylcholine (PC) accounted for about 60 per cent of microsomal phospholipids in both dietary models. The next component in importance was phosphatidylethanolamine (PE), 20 per cent of total microsomal phospholipids, followed by phosphatidylinositol (PI) and lysophosphatidylcholine (LPC) together, 10 per cent of the total microsomal phospholipids. Sphingomyelin (SP), phosphatidylserine (PS) and phosphatidic acid (PA) were present in smaller proportions. Per cent changes from controls after ethanol are given in Table 3. When the absolute amounts are calculated from the values of Tables 2 and 3 in rats fed ethanol and the HFD, PC increases 22 per cent ($P < 0.001$) and 25 per cent ($P < 0.005$) per mg of protein and per g of liver, respectively; PE does not change significantly and SP decreases 22 per cent ($P < 0.005$) per mg of protein and 21 per cent ($P < 0.02$) per g of liver. Ethanol with the LFD does not change the absolute content value of PC or PE but decreases SP 20 per cent ($P < 0.05$) per mg of protein and 25 per cent ($P < 0.005$) per g of liver.

The fatty acid composition of PC is presented in Table 4. The present study reveals slight differences between the two dietary models: significant differences in palmitic ($P < 0.001$), stearic ($P < 0.05$), oleic ($P < 0.001$), linoleic ($P < 0.02$) and arachidonic ($P < 0.005$) acids are observed between the HFD and LFD in controls as well as in ethanol-fed animals.

In rats fed the HFD, saturated fatty acids (SFA) account for more than 50 per cent of all fatty acids present in PC, mainly in the form of stearic acid. The remaining fatty acids are unsaturated fatty acids (UFA) with arachidonic acid accounting for twice the amount of oleic and linoleic acids. After ethanol administration, SFA decreased slightly ($P < 0.05$), mainly because of the change in palmitic acid concentrations; the 4 per cent (P:NS) increase in UFA is due to significant increases in oleic and linoleic acids by 28 and 18 per cent, respectively ($P < 0.005$ and $P < 0.025$). The increases in oleic and linoleic acids are associated with a slight decrease ($P < 0.05$) in arachidonic acid, quantitatively the most important change when absolute values are calculated. The net increase in UFA is therefore reduced to 4 per cent.

*Kindly supplied by Upjohn International Inc., Kalamazoo, Mich.

Table 3. Effect of ethanol and dietary fat on per cent composition of hepatic microsomal phospholipids*

| | High fat† | | | Low fat‡ | | |
|---|---|---|---|---|---|---|
| | Control | Ethanol | Ethanol / Control | Control | Ethanol | Ethanol / Control |
| Origin | 0.43 ± 0.13 | 0.39 ± 0.09 | 0.91 | 0.95 ± 0.21 | 1.20 ± 0.63 | 1.26 |
| Phosphatidic acid | 0.80 ± 0.22 | 0.81 ± 0.14 | 1.01 | 0.62 ± 0.14 | 0.59 ± 0.19 | 0.95 |
| G§ | 0.26 ± 0.11 | 0.21 ± 0.07 | 0.81 | 0.25 ± 0.08 | 0.23 ± 0.09 | 0.92 |
| Phosphatidylserine | 3.64 ± 0.39 | 3.09 ± 0.18 | 0.85 | 2.93 ± 0.21 | 2.73 ± 0.16 | 0.93 |
| Sphingomyelin | 3.45 ± 0.18 | 2.12 ± 0.15 | 0.61 | 3.78 ± 0.29 | 2.84 ± 0.24• | 0.75 |
| Phosphatidylinositol and lysophosphatidylcholine | 10.20 ± 1.76 | 10.82 ± 0.30 | 1.06 | 10.30 ± 0.36 | 10.48 ± 0.29 | 1.02 |
| Phosphatidylcholine | 59.80 ± 1.20 | 62.88 ± 1.11** | 1.05 | 59.03 ± 0.74 | 59.52 ± 0.40 | 1.01 |
| Phosphatidylethanolamine | 21.09 ± 1.09 | 19.57 ± 0.73 | 0.93 | 21.36 ± 0.82 | 21.72 ± 0.57 | 1.02 |

* Values are presented as mean per cent of total phospholipid phosphorus ± S. E. M.
† Thirty-five per cent of total calories as fat: 15 pairs.
‡ Two per cent of total calories as linoleate (only source of fat): 13 pairs.
§ Unidentified spot.
‖ $P < 0.001$.
• $P < 0.005$.
**$P < 0.01$.

In animals fed the LFD the proportions of SFA and UFA in PC are similar to those found in the high fat model. SFA of control animals contained slightly less stearic acid. Arachidonic and linoleic acids account for a slightly lower proportion of the UFA, the oleic acid percentage being higher than in the control rats given the HFD. Changes brought about by ethanol administration show similar trends as in the high fat model, and a slight (3.6 per cent) but significant decrease ($P < 0.02$) in SFA is also observed with the low fat model. The arachidonic/linoleic acid ratio in PC is similar in control animals fed a HFD (3.81) and those fed a LFD (3.87). After ethanol, these values decreased 15–17 per cent (P:NS)

Table 4. Effect of ethanol and dietary fat on fatty acid composition of hepatic microsomal phosphatidylcholine*

| | High fat† | | | Low fat‡ | | |
|---|---|---|---|---|---|---|
| Fatty acid | Control | Ethanol | Ethanol / Control | Control | Ethanol | Ethanol / Control |
| 14:0 (Myristic) | 1.16 ± 0.17 | 1.25 ± 0.18 | 1.08 | 1.59 ± 0.16 | 1.17 ± 0.11§ | 0.74 |
| 16:0 (Palmitic) | 17.66 ± 0.55 | 15.86 ± 0.50‖ | 0.90 | 21.08 ± 0.69 | 21.30 ± 1.07 | 1.01 |
| 16:1 (Palmitoleic) | 0.57 ± 0.12 | 0.77 ± 0.09 | 1.35 | 1.24 ± 0.42 | 1.38 ± 0.39 | 1.11 |
| 18:0 (Stearic) | 34.64 ± 0.87 | 33.97 ± 0.91 | 0.98 | 31.37 ± 1.19 | 29.69 ± 1.32 | 0.95 |
| 18:1 (Oleic) | 6.65 ± 0.51 | 8.49 ± 0.45• | 1.28 | 11.05 ± 0.78 | 12.02 ± 0.63 | 1.09 |
| 18:2 (Linoleic) | 7.63 ± 0.59 | 9.02 ± 0.67** | 1.18 | 5.87 ± 0.26 | 6.69 ± 0.36 | 1.14 |
| 18:3 (Linolenic) | | | | 0.43 ± 0.07 | 0.65 ± 0.08§ | 1.51 |
| 20:4 (Arachidonic) | 26.11 ± 0.64 | 25.11 ± 0.62§ | 0.96 | 22.19 ± 0.87 | 21.27 ± 1.02 | 0.96 |
| 22:6 (Docosahexaenoic) | 4.03 ± 0.51 | 3.34 ± 0.38 | 0.83 | 3.32 ± 0.21 | 3.49 ± 0.18 | 1.05 |

* Values are presented as mean per cent of total weight of fatty acids ± S. E. M.
† Thirty-five per cent of total calories as fat: 14 pairs.
‡ Two per cent of total calories as linoleate (only source of fat): 13 pairs.
§ $P < 0.05$.
‖ $P < 0.01$.
• $P < 0.005$.
**$P < 0.025$.

Table 5. Effect of ethanol and dietary fat on fatty acid composition of hepatic microsomal phosphatidylethanolamine*

| | High fat† | | | Low fat‡ | | |
|---|---|---|---|---|---|---|
| Fatty acid | Control | Ethanol | Ethanol / Control | Control | Ethanol | Ethanol / Control |
| 14:0 (Myristic) | 1.10 ± 0.13 | 1.42 ± 0.27 | 1.29 | 1.35 ± 0.18 | 1.28 ± 0.18 | 0.95 |
| 16:0 (Palmitic) | 17.88 ± 0.56 | 16.85 ± 0.75 | 0.94 | 19.55 ± 0.55 | 19.90 ± 0.66 | 1.02 |
| 16:1 (Palmitoleic) | 1.05 ± 0.11 | 1.28 ± 0.14 | 1.22 | 0.41 ± 0.12 | 0.48 ± 0.09 | 1.17 |
| 18:0 (Stearic) | 34.56 ± 0.56 | 34.61 ± 0.67 | 1.00 | 32.12 ± 0.39 | 31.70 ± 0.96 | 0.99 |
| 18:1 (Oleic) | 6.41 ± 0.26 | 6.79 ± 0.24 | 1.06 | 6.95 ± 0.31 | 5.45 ± 0.34§ | 0.78 |
| 18:2 (Linoleic) | 4.57 ± 0.36 | 5.03 ± 0.37 | 1.10 | 3.71 ± 0.25 | 3.75 ± 0.38 | 1.01 |
| 20:4 (Arachidonic) | 23.70 ± 0.51 | 23.27 ± 0.76 | 0.97 | 24.49 ± 0.41 | 25.74 ± 0.55 | 1.05 |
| 22:6 (Docosahexaenoic) | 9.05 ± 0.55 | 8.83 ± 0.46 | 0.98 | 9.12 ± 0.68 | 9.87 ± 0.46 | 1.08 |

* Values are presented as mean per cent of total weight of fatty acids ± S. E. M.
† Thirty-five per cent of total calories a fat: 15 pairs.
‡ Two per cent of total calories as linoleate (only source of fat): 12 pairs.
§ $P < 0.01$.

because of a decrease in arachidonic acid and of an increase in linoleic acid. Expression of these proportionality changes in terms of microsomal content in PC fatty acids shows that ethanol administration increases the linoleic and oleic acid content of microsomal membranes when it is given with a HFD. Qualitatively similar changes are seen after ethanol with the LFD, but fail to achieve statistical significance.

The composition of PE is summarized in Table 5. SFA and UFA are present in almost the same proportion. In the high fat-fed group, the SFA composition of PE of control animals is the same as that of PC. Unsaturated fatty acids show a greater proportion of docosahexaenoic acid, the percentage of which is also greater than that of linoleic and oleic acids. Chronic ethanol administration with a HFD produces no significant change in the fatty acid composition of PE. Controls fed the LFD have more palmitic ($P < 0.05$) and less stearic ($P < 0.005$) acid in their SFA than the controls fed the HFD. After ethanol, unsaturated fatty acids show a decrease in oleic acid ($P < 0.01$). Rats fed ethanol with the LFD when compared to the HFD show significant differences in palmitic ($P < 0.01$), stearic ($P < 0.02$), oleic ($P < 0.005$), linoleic ($P < 0.025$) and arachidonic ($P < 0.02$) acids.

The incorporation of labeled methyl groups from L-methionine-methyl[$^3$H] in liver microsomal phospholipids, 1 hr after its administration [38], is highest in PC, where 95–97 per cent of the total radioactivity is recovered. The incorporation of this precursor is not significantly altered by ethanol feeding with either a HFD or a LFD. The incorporation of [$^{14}$C]choline is also highest in PC, where 97 per cent of the total phospholipid radioactivity is found; it was also not altered by ethanol feeding with either a HFD or a LFD. Ethanol administration did not alter the relative rate of incorporation of these two precursors as seen by examination of the $^3H/^{14}C$ ratio of PC. This ratio is not valid for the other phospholipids, which contain very little radioactivity. Control values for PC are not significantly different from values in alcohol-fed rats. The ratio values are $2.48 \pm 0.53$ (control) and $2.62 \pm 0.40$ (ethanol) in rats fed the HFD and $2.07 \pm 0.25$ (control) and $2.01 \pm 0.28$ (ethanol) in the LFD models.

Benzphetamine demethylation measured in the P-450-containing reconstituted system was stimulated by the microsomal lipid fraction of control and ethanol-fed rats. At zero lipid concentration, there was a slight benzphetamine demethylation activity partly due to some lipid contamination (2.47 μg phospholipid/assay) from the purified step III cytochrome P-450 of ethanol rats (53.5 μg/mg of protein) and from the purified reductase of ethanol rats (2.4 μg/mg of protein). Maximal activity was obtained after adding 42 μg phospholipid/assay. The curves of benzphetamine demethylation activity, as a function of lipid content of the assay, are identical when using control and ethanol-fed rat microsomal lipids (Fig. 1). The use of cytochrome P-450 and reductase fractions from control animals yielded lower absolute values for benzphetamine demethylation activity but failed to disclose any differences between the different lipid fractions.

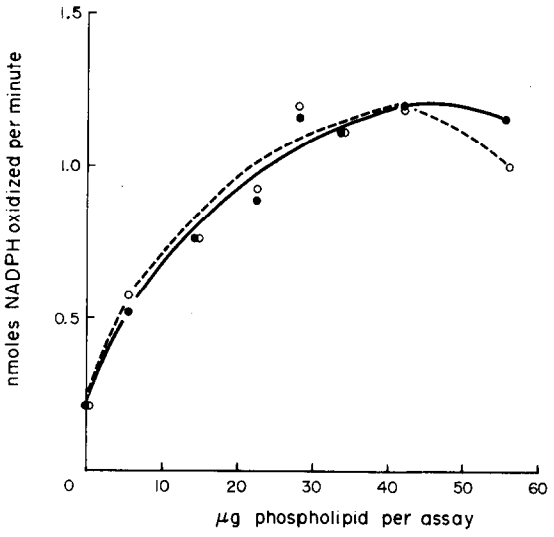


Fig. 1. Benzphetamine *N*-demethylation in the presence of fixed amounts of cytochrome P-450 and NADPH-cytochrome *c* reductase; the amount of phospholipids varied from 0 to 56 μg phospholipid/assay. The reaction mixture contained $MgCl_2$ 3 mM, EDTA 0.05 mM, benzphetamine 1 mM, NADPH 0.1 mM, step III cytochrome P-450 (from microsomes of rats fed ethanol with the HFD) 0.198 nmole/assay, and reductase (from microsomes of rats fed ethanol with the HFD) 165 units/assay, in a total volume of 1 ml. The activity was measured by following the rate of oxidation of NADPH. Closed circles: lipid fractions from microsomes of control rats; open circles: lipid fractions from microsomes of ethanol-fed rats.

## DISCUSSION

Dietary lipids, mainly linoleic acid [4], are important for drug metabolism. Hepatic microsomal phospholipids, particularly phosphatidylcholine, are essential for drug metabolism *in vitro* [10, 11]. Changes in hepatic microsomal phospholipid may play a role in the increase in drug-metabolizing enzyme activities induced by various drugs [8, 13–15]. Most evidence to that effect is circumstantial, however, and other studies support the hypothesis that dietary lipids may play a role through other factors such as acting on heme synthesis [47] to increase cytochrome P-450 content [34, 48]. We have previously observed that, in the absence of caloric deficiency, the induction by ethanol of cytochrome P-450 and NADPH-cytochrome P-450 reductase activity is greater with a HFD and that microsomal phospholipids are increased only in presence of the HFD. By contrast, the ethanol increase in benzphetamine demethylation activity was found to be of the same magnitude whether rats were fed the HFD or the LFD [27].

Chronic ethanol administration alters the composition of total [20, 21] and microsomal [49] hepatic phospholipids, but more striking modifications of hepatic microsomal phospholipids have been reported after administration of phenobarbital [38]. Ethanol is reported to increase microsomal phospholipids [22] only if it is administered with a HFD [27]. The mechanisms for such an increase in phospholipid content may be an increased synthesis and/or a decreased phospholipid breakdown. In male rats, phenobarbital increases the synthesis and decreases the breakdown of phospholipids, but in females only phospholipid

catabolism is decreased [50]. Glycerolipid synthesis is increased by chronic ethanol administration [20, 34]. Kemp and Fallon [51] have suggested that ethanol administration increases the methylation of phosphatidylethanolamine into phosphatidylcholine. In the present study, we could find no evidence of increased formation of PC via the Kennedy pathway [52] or via phospholipid methylation [53] after chronic administration of ethanol.

Quantitative changes in microsomal phospholipids might be responsible for increased microsomal drug-metabolizing enzyme activities. However, in a previous study using the same models [27], the increased activities of benzphetamine demethylation and aniline hydroxylation in rats fed ethanol with the LFD were not associated with any increase in microsomal phospholipids. In the same animal and dietary models, in the absence of any change in microsomal phospholipid content per 100 g of body wt [27], hexobarbital plasma clearance was increased significantly after ethanol and the LFD [54].

Qualitative changes in microsomal phospholipids after prolonged ethanol intake are of two types: ethanol modifies the proportion of various phospholipids and also alters their fatty acid composition, particularly that of PC [21, 49]. Mendenhall *et al.* [21] reported a decrease in palmitic and linoleic acids and an increase in arachidonic acid after ethanol in male rats. By contrast, French *et al.* [49] reported a decrease in stearic and arachidonic acids and an increase in linoleic acid, but only after 45 weeks of ethanol consumption. Miceli and Ferrell [55] reported decreased palmitic, linoleic and arachidonic acids and an increase in stearic acid in male mice. Our data may be different because of differences in dietary models, sex, strain, and species of the animals. Changes due to ethanol in the high fat- or low fat-fed animals are similar but less striking and less significant with the LFD.

Phosphatidylcholine is the active component of the lipid fraction in the activation of microsomal drug-metabolizing enzymes [11]. Phosphatidylcholines of different structures show different stimulating activities on the enzyme systems using purified cytochrome P-450 and reductase from solubilized microsomes [11, 17]. Since qualitative differences in microsomal phospholipids and phosphatidylcholine were more striking in rats fed ethanol and the HFD, we have chosen to use the lipid fractions of these animals and their controls in the reconstituted system of benzphetamine demethylation from solubilized microsomes. The stimulatory activity of the lipid fraction on the demethylation of benzphetamine of control rats and ethanol-fed rats was not different. Therefore, it seems unlikely that the differences in the fatty acid composition of phospholipids are responsible for the activity and induction of benzphetamine demethylase.

The exact role of microsomal phospholipid in drug metabolism is as yet unknown. Phospholipids probably do not lead to the formation of vesicular structures [56]. The specificity of the role of phospholipids in drug metabolism has recently been challenged, since detergents may replace the lipid fraction in the reconstituted system [57]. Our initial hypothesis that quantitative and/or qualitative alterations by ethanol in microsomal phospholipids could account for the induction by ethanol of the microsomal drug-metabolizing enzyme activity of benzphetamine demethylation could not be substantiated by our studies.

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