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ADAPTIVE CHANGES IN LIPID COMPOSITION OF RAT LIVER PLASMA MEMBRANE DURING POSTNATAL DEVELOPMENT FOLLOWING MATERNAL ETHANOL INGESTION

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The fatty acid composition of constituent phospholipids and the cholesterol content of rat liver plasma membranes were determined subsequent to maternal alcohol ingestion during pregnancy and lactation. The alcoholic group was given a liquid Metrecal diet containing 37% ethanol-derived calories. The control group was pair-fed an isocaloric sucrose/Metrecal diet. Litters were killed for lipid analyses at days 5, 15 and 25 after birth. These studies revealed that the total phospholipid phosphorus was similar and increased significantly with age in both groups. Cholesterol also increased significantly with age in both groups but was greater in the alcoholic pups, resulting in a higher cholesterol/phospholipid molar ratio. While the phosphatidylethanolamine (PE) content increased with age in both groups, that of sphingomyelin decreased. Phosphatidylserine + phosphatidylinositol (PS + PI) was significantly higher in the control group at all ages studied. A consistent increase of C22:6 in phosphatidylcholine (PC), sphingomyelin, PS + PI and in the total phospholipid fraction from alcoholic pups was observed. Although other fatty acid changes were found in PC, PS + PI and sphingomyelin, PE was not affected. These results suggest that specific adaptive changes were induced in the liver plasma membrane lipids of the progeny from alcoholic rats.

Introduction

Ethanol, like other general anesthetics, is known to act primarily within the lipid bilayer of biological membranes [1,2], and cellular adaptation to its chronic presence is believed to be mediated by alterations in the physical properties of the lipid bilayer, which can presumably be determined, at least in part, from the membrane lipid composition [3–6].

A variety of studies from this [7,8] and other laboratories [9–12] have demonstrated that chronic ethanol consumption may lead to functional and structural alterations of rat liver cellular organelles. These have been generally interpreted to reflect an adaptation to the presence of ethanol, partially mediated through modifications of the physical and chemical properties of the cell membranes. In fact, several authors have shown that the lipid composition of cellular membranes from microorganisms [13,14] and different animal tissues [15–21] is altered in response to prolonged exposure to ethanol.

We [22] have recently observed that chronic maternal ethanol ingestion has a detrimental effect on the postnatal development of rat liver plasma membrane α_1-adrenergic receptors. Results obtained with the alcohol-fed pups showed a significant decrease (30–70%) in receptor density (B_{max}) but no changes in the binding affinity (K_D)

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Abbreviations: PE, phosphatidylethanolamine; PC, phosphatidylcholine; PS, phosphatidylserine; PI, phosphatidylinositol.

throughout postnatal development. Based on the above observations, these abnormalities can be envisioned as being partially modulated by changes in the lipid composition of the plasma membranes. We have, therefore, determined the effects of maternal ethanol ingestion on the fatty acid composition of individual liver plasma membrane phospholipids from newborn rats.

Materials and Methods

Materials

Metrecal was prepared by Mead Johnson, Canada. Vitamin Diet Fortification Mixture was obtained from ICN Nutritional Biochemicals. Sucrose, phenol reagent and all chromatography grade organic solvents were purchased from Fisher Scientific Inc. Boron trifluoride/methanol was from Chromatography Specialties. Silicic acid and lipid mixtures for thin-layer chromatography standards were obtained from Sigma Chemicals. Fatty acid methyl esters for gas chromatography reference standards were from Applied Sciences. Ethanol (100%) was purchased from Consolidated Alcohol Co. Ltd., Toronto, Ontario.

Animal treatment

Virgin female Sprague-Dawley rats weighing 200–225 g were purchased from Canadian Breeding Farms (St. Constance, Quebec) and given water and Purina Rat Chow ad libitum for 1 week. Rats were then mated overnight and the appearance of sperm in the vaginal washings established day 1 of pregnancy. The pregnant rats were randomly divided into two groups and had their diets changed to a nutritionally adequate liquid low-fat (5%) Metrecal diet providing 1 kcal/ml and supplemented with 3 g/l Vitamin Diet Fortification Mixture. The diet from the designated alcoholic group contained ethanol, which provided 37% of the total calories. The control group was pair-fed with the same diet, but with sucrose isocalorically substituted for ethanol. Both groups of rats were maintained on their respective diets throughout pregnancy and lactation. All pups were weaned at 21 days postnatally. Litters were killed between 8 and 10 a.m. on days 5, 15 and 25 after birth, and liver plasma membranes were then isolated for lipid composition analyses.

Plasma membrane preparation

Livers were rapidly excised and the hepatic plasma membranes were isolated as previously described [22], using essentially the fractionation scheme of Neville [23] described by Wolfe et al. [24]. Experiments measuring marker enzymes (Table I; [25,26]) demonstrated that the highly enriched plasma membranes were only minimally contaminated with other cell organelles.

Lipid separation and phospholipid analyses

Lipids were extracted from plasma membranes immediately after their preparation, using the procedure of Bligh and Dyer [27], dried under nitrogen and redissolved in chloroform. Neutral lipids and phospholipids were separated by silicic acid column chromatography (Sil-LC, 325 mesh, 5 cm $\times$ 5.5 mm internal diameter). Neutral lipids were eluted with 6 column vol. of chloroform, and phospholipids were eluted with 3 column vol. of chloroform/methanol (1 : 2) followed by 3 column vol. of methanol. The neutral lipids were dried under nitrogen, redissolved in chloroform and analyzed for cholesterol content by the method of Zlatkis et al. [68] as described by Kates [28]. An aliquot of the total phospholipid fraction was digested according to the method of Duck-Chong [29] and the inorganic phosphorus assayed according to the method of Chen et al. [30]. Individual phospholipids were separated from a second aliquot fraction by two-dimensional thin-layer chromatography on Merck silica gel G plates (250 μm thick). The solvents used were chloroform/methanol/7 M ammonium hydroxide (65 : 35 : 5, v/v) and chloroform/methanol/acetone/acetic acid/water (50 : 10 : 20 : 10 : 5, v/v) for the first and second directions, respectively [31]. The separated phospholipids were visualized by exposure to iodine vapor, scraped from the plate and eluted with chloroform/methanol (1 : 1). Quantitation of each phospholipid class was done by determination of phospholipid phosphate using the same method described for the quantitation of the total phospholipid fraction.

Gas liquid chromatography

Methyl esters of fatty acids from total and individual phospholipids were prepared with boron trifluoride/methanol reagent by the method of

Morrison and Smith [32]. The fatty acid methyl esters were analyzed on a F&M model 402 gas liquid chromatograph equipped with a flame-ionization detector (detector temperature, 280°C) and a glass column (1.83 m × 4 mm internal diameter) packed with 3% OV-1 on 100–200 mesh Gas Chrome Q. The injector port temperature was 210°C while the initial column temperature was 150°C. The temperature of the oven was programmed from 150 to 250°C at a rate of 10°C/min. The flow rate of the carrier gas helium was adjusted to 40 ml/min under a head pressure of 40 lb/min under a head pressure of 40 lb/inch^2. Peak areas and retentions times were recorded with a Hewlett-Packard Model 3390A Integrator. Retention times were compared to those of known standards. Under these conditions in the lipid fraction of the Metrecal diet, 63% of the material present was identified as fatty acids. 25% of the unidentifiable material(s) was eluted after C22 : 6. Of the fatty acids identified in the diet, there were 22% of C16 : 0; 15% of C18 : 1, 18 : 2; 19% of C18 : 0; 6% of C14 : 1; 4% of C14 : 0; and 3% each of C20 : 4 and C22 : 6.

Protein determination

Protein was determined by the method of Lowry et al. [33], using recrystallized bovine serum albumin as standard.

Statistical analysis

All results are expressed as mean ± S.E.; the level of significance of the difference between mean values was assessed by the two-tailed Student's *t*-test.

Results

Chronic maternal ethanol administration

The intake of total nutrients and calories for both the maternal alcoholic and pair-fed control groups fulfill the nutritional requirements of laboratory rats [34]. The average food intake for animals in the maternal alcoholic group was 247 ± 17 (S.E., $n = 5$) kcal/kg body weight per day, and the average ethanol intake ranged from 10 to 15.7 g/kg body weight per day. Although the weight gain during pregnancy was similar in both maternal groups, the postnatal weight gain over the period studied of the newborn pups of alcohol-fed mothers was reduced by 33% when compared to the corresponding control pups. Blood ethanol concentrations (measured between 8 and 10 a.m.) in newborn pups of alcohol-fed mothers ranged between 4 mM at day 5 and 40 mM at day 25. In pregnant and lactating mothers, blood alcohol levels varied between 35 and 40 mM.

Isolation of rat liver plasma membranes

The yield of hepatic plasma membrane protein per g wet liver weight was constant, being independent of either age or diet treatment, and close to 2.5 mg/g. The specific activities of marker enzymes in liver plasma membranes from rats of different ages is shown in Table I. The relative specific activity of an enzyme, i.e., the ratio of the specific enzyme activity in the isolated membrane fraction to that in the homogenate, is an indication of the degree of purification. As shown in Table I, the isolated membrane fractions are highly enriched in 5'-nucleotidase, a specific plasma membrane marker. The extent of microsomal and mitochondrial contamination was assessed with the measurement of the relative specific activities of glucose-6-phosphatase and monoamine oxidase, respectively. Results shown demonstrate that the extent of such contamination is minimal. It is interesting to note that the relative specific activities of the marker enzymes are independent of either age or diet treatment, and the values obtained are in agreement with others [35,36].

Total phospholipid and cholesterol content of plasma membranes

As indicated in Table II, total phospholipid content significantly ($P < 0.01$) increased from day 5 to day 25 after birth for both alcoholic and control pups. Cholesterol also increased significantly ($P < 0.01$) with age in both groups. Although total phospholipid content is independent of diet treatment, cholesterol was greater in the alcoholic pups ($P < 0.05$ at days 5 and 15; $P < 0.01$ at day 25), resulting in a significantly higher ($P < 0.05$) cholesterol/phospholipid molar ratio. The values obtained for the phospholipid and cholesterol content of hepatic plasma membranes from 25-day-old pair-fed control pups are similar to those reported by other investigators [37–39].

TABLE I

SPECIFIC ACTIVITIES OF MARKER ENZYMES IN LIVER PLASMA MEMBRANES FROM RATS OF DIFFERENT AGES

Specific enzyme activities are expressed as μmol P_i/mg protein per h for 5'-nucleotidase and glucose-6-phosphatase and as cpm·10^5/mg protein per 15 min for monoamine oxidase. The relative specific activity (in brackets) is the ratio of the enzyme activity in the isolated membrane fraction to that in the homogenate. Values represent the mean ± S.E. for the number of preparations (n) indicated.

Enzymes	5 days (n = 7)		15 days (n = 5)		25 days (n = 8)	
	Control	Alcoholic	Control	Alcoholic	Control	Alcoholic
5'-Nucleotidase						
Liver homogenate	4.0 ± 0.8	3.5 ± 0.4	3.7 ± 0.5	4.2 ± 1.0	3.8 ± 0.5	4.0 ± 0.7
Plasma membrane	34.2 ± 5.0	33.0 ± 2.7	34.0 ± 4.1	32.0 ± 6.0	31.0 ± 3.0	35.0 ± 4.0
	(8.5 ± 2.0)	(9.2 ± 1.5)	(9.2 ± 2.4)	(7.6 ± 3.0)	(8.1 ± 2.0)	(8.7 ± 2.3)
Glucose-6-phosphatase						
Liver homogenate	1.8 ± 0.3	1.6 ± 0.5	1.7 ± 0.5	1.6 ± 0.2	1.9 ± 0.7	1.8 ± 0.3
Plasma membrane	1.1 ± 0.2	1.2 ± 0.4	1.4 ± 0.2	1.4 ± 0.3	2.0 ± 0.5	1.4 ± 0.5
	(0.6 ± 0.2)	(0.7 ± 0.1)	(0.8 ± 0.2)	(0.9 ± 0.2)	(1.0 ± 0.1)	(0.7 ± 0.2)
Monoamine oxidase						
Liver homogenate	28.3 ± 1.7	22.0 ± 1.1	21.0 ± 6.3	25.0 ± 3.0	22.3 ± 5.0	31.0 ± 4.3
Plasma membrane	59.3 ± 3.1	41.0 ± 5.0	41.0 ± 5.4	40.0 ± 5.0	45.0 ± 8.3	58.0 ± 5.1
	(2.1 ± 0.3)	(1.9 ± 0.2)	(1.9 ± 0.3)	(1.6 ± 0.1)	(2.0 ± 0.3)	(1.9 ± 0.2)

TABLE II

LIPID COMPOSITION OF LIVER PLASMA MEMBRANES FROM RATS OF DIFFERENT AGES

Plasma membranes were extracted according to Bligh and Dyer [27]. Polar and non-polar lipids were fractionated by silicic acid column chromatography as described in Materials and Methods. The cholesterol content was measured by the method of Zlatkis et al. [68] as described by Kates [28]. Phospholipids were digested according to the method of Duck-Chong [29] and the inorganic phosphorus assayed according to the method of Chen et al. [30]. Values represent the mean ± S.E. for the number of preparations indicated in parentheses.

Lipid class	5 days (8)		15 days (6)		25 days (10)	
	Control	Alcoholic	Control	Alcoholic	Control	Alcoholic
Phospholipid phosphorus (μmol/mg protein)	0.33 ± 0.02 [a]	0.31 ± 0.01 [a]	0.39 ± 0.01	0.42 ± 0.02	0.41 ± 0.01	0.44 ± 0.02
Cholesterol (μmol/mg protein)	0.19 ± 0.02 [a]	0.25 ± 0.02 [a]	0.32 ± 0.02	0.38 ± 0.02 [c]	0.34 ± 0.03	0.41 ± 0.03 [c]
Molar ratio (cholesterol/phospholipid)	0.64 ± 0.03 [a]	0.74 ± 0.02 [a]	0.81 ± 0.03	0.93 ± 0.04 [c]	0.85 ± 0.03	0.92 ± 0.03 [b]

[a] Significantly different from the same animal group at 15 and 25 days after birth ($0.001 < P < 0.01$).
[b] Significantly different from the control ($0.02 < P < 0.05$).
[c] Significantly different from the control ($0.001 < P < 0.01$).

Phospholipid distribution in plasma membranes

As shown in Table III, the major individual constituent phospholipids in rat liver plasma membranes isolated from both alcoholic and control pups are PC, sphingomyelin and PE. These results are in agreement with those obtained by other investigators [35,37,38]. Moreover, the phospholipid distribution in plasma membranes from 25-day-old pair-fed control pups was quite similar to that found in adult rat liver plasma membranes in other studies [35,39–44].

The developmental pattern of the phospholipid distribution was characterized by an increase in PE and a decrease in sphingomyelin for both animal groups. The content of PC did not vary with age or diet treatment. Although the content of PS + PI was independent of age for both groups, it was significantly ($P < 0.01$) reduced in the

TABLE III

PHOSPHOLIPID DISTRIBUTION IN LIVER PLASMA MEMBRANES FROM RATS OF DIFFERENT AGES

Plasma membrane phospholipids were extracted as described in Table II. The different classes of phospholipids were separated by two-dimensional thin-layer chromatography using the solvents described by Kates [31]. The separated phospholipids were quantitated as μmol of phospholipid phosphate. The data are expressed as % of total phospholipids. Values represent the mean ± S.E. for the number of preparations indicated in parentheses.

Phospholipids	5 days (6)	15 days (5)	25 days (6)
Phosphatidylethanolamine			
Control	13.9 ± 1.1 [b]	23.2 ± 2.7	24.0 ± 3.7
Alcoholic	15.8 ± 1.5 [b]	27.0 ± 1.7	27.5 ± 2.1
Phosphatidylcholine			
Control	36.1 ± 3.4	34.7 ± 2.0	37.9 ± 3.8
Alcoholic	40.2 ± 2.1	37.0 ± 2.5	42.0 ± 2.6
Sphingomyelin			
Control	31.6 ± 2.0 [b]	24.8 ± 2.3	21.4 ± 4.0
Alcoholic	30.0 ± 2.4 [b]	22.2 ± 2.1	17.1 ± 2.3
Phosphatidylserine + phosphatidylinositol			
Control	18.4 ± 0.7	17.3 ± 1.0	16.7 ± 0.8
Alcoholic	14.0 ± 1.0 [a]	13.8 ± 0.9 [a]	13.0 ± 1.1 [a]

[a] Significantly different from the control ($0.001 < P < 0.01$).
[b] Significantly different from the same animal group at 15 and 25 days after birth ($0.001 < P < 0.01$).

plasma membranes from alcoholic pups at all ages studied.

Fatty acyl composition of total phospholipids

The fatty acid acomposition of liver plasma membrane total phospholipids from rats of different ages is shown in Table IV. From a developmental point of view, only one significant change was observed in the fatty acid content of total phospholipids. There was an increase of 39% in the level of C14 : 0 for both animal groups between days 5 and 15. The levels of the other fatty acid components of total phospholipids remained quite stable between days 5 and 25. There was also a significant increase ($P < 0.05$) in the level of C22 : 6 as a result of ethanol feeding at all ages studied. However, the level of unsaturation was not significantly modified by these changes.

Fatty acyl composition of liver plasma membrane individual phospholipids

The fatty acid composition of liver plasma membrane PE, PC, sphingomyelin and PS + PI is reported in Tables V–VIII. Fatty acids C16 : 0, C18 : 1, 18 : 2 and C18 : 0 are the major components of individual plasma membrane phospholipids at all ages studied.

TABLE IV

FATTY ACID COMPOSITION OF LIVER PLASMA MEMBRANE TOTAL PHOSPHOLIPIDS FROM RATS OF DIFFERENT AGES

Plasma membrane phospholipids were extracted as described in Table II. Samples were then transmethylated using the boron trifluoride/methanol reagent as described by Morrison and Smith [32]. The methylated fatty acid esters were separated by gas liquid chromatography as described in Materials and Methods. Peak areas and retention times were recorded on a Hewlet-Packard Model No. 3390A Integrator. Data are presented as percent of total phospholipid fatty acid, and values represent the mean ± S.E. for the number of preparations indicated in parentheses.

Fatty acids	5 days (6)		15 days (4)		25 days (7)	
	Control	Alcoholic	Control	Alcoholic	Control	Alcoholic
14 : 0	4.2 ± 0.7 [b]	3.4 ± 0.5 [b]	6.5 ± 0.6	6.1 ± 1.0	6.9 ± 1.0	5.5 ± 0.8
16 : 1	6.0 ± 0.9	6.4 ± 1.1	6.0 ± 0.8	7.0 ± 0.8	5.0 ± 0.8	5.6 ± 0.9
16 : 0	22.0 ± 1.3	21.2 ± 1.0	21.5 ± 1.1	18.5 ± 1.3	22.9 ± 0.9	22.5 ± 1.4
18 : 1, 18 : 2	18.0 ± 1.2	17.7 ± 1.0	19.3 ± 1.0	16.6 ± 2.0	16.6 ± 1.2	15.7 ± 0.8
18 : 0	24.0 ± 1.5	22.8 ± 1.3	21.2 ± 1.1	23.3 ± 1.6	24.4 ± 1.1	24.0 ± 1.4
20 : 4	14.1 ± 0.8	13.6 ± 1.3	13.5 ± 0.9	13.2 ± 1.3	13.9 ± 1.1	13.0 ± 0.5
22 : 6	11.7 ± 1.0	14.9 ± 1.1 [a]	12.0 ± 0.8	15.3 ± 0.5 [a]	10.3 ± 0.9	13.7 ± 0.8 [a]
Unsaturated	49.8 ± 0.9	52.6 ± 1.3	50.8 ± 1.1	52.1 ± 0.9	45.8 ± 1.1	48.0 ± 1.2

[a] Significantly different from the control ($0.02 < P < 0.05$).
[b] Significantly different from the same animal group at 15 and 25 days after birth ($0.001 < P < 0.01$).

TABLE V

FATTY ACID COMPOSITION OF LIVER PLASMA MEMBRANE PHOSPHATIDYLETHANOLAMINE FROM RATS OF DIFFERENT AGES

Phosphatidylethanolamine was separated from other plasma membrane phospholipids by two-dimensional thin-layer chromatography as described by Kates [31]. After elution from the silica gel, the phosphatidylethanolamine was transmethylated and analysed for fatty acyl composition as described in Table IV. Data are presented as percent of total phospholipid fatty acid, and values represent the mean ± S.E. for the number of preparations indicated in parentheses.

Fatty acids	5 days (6)		15 days (4)		25 days (7)	
	Control	Alcoholic	Control	Alcoholic	Control	Alcoholic
14:0	11.2±1.4	11.7±1.0	10.7±0.9	11.7±1.1	9.7±0.7	9.0±0.9
16:1	11.0±1.4	13.5±1.1	10.0±1.3	12.0±1.5	10.8±1.4	12.8±1.1
16:0	18.9±1.3	17.6±1.3	16.7±0.8	17.5±1.5	20.4±0.8	18.3±1.0
18:1, 18:2	15.7±1.0	14.0±1.1	16.6±1.6	15.3±1.1	17.4±1.3	16.0±1.0
18:0	18.6±1.3	16.8±1.4	19.6±1.5	17.6±1.2	18.8±1.3	18.6±1.3
20:4	11.0±1.1	13.2±1.3	11.2±0.7	11.4±1.4	10.3±0.9	11.1±1.0
22:6	13.6±0.9	13.2±1.0	15.2±1.0	14.5±0.8	12.6±1.2	14.2±1.5
Unsaturated	51.3±1.4	53.9±1.1	53.0±1.6	53.2±1.1	51.1±1.4	54.1±1.2

Significant changes during development can be observed in the fatty acid composition of PC and sphingomyelin. As shown in Table VI, the level of C14:0 in PC increased 38% by day 15 and was up 55% by day 25 in both groups of animals. A slight decrease in the levels of C18:1, 18:2 in PC was also observed in the control group. The changes in sphingomyelin during development are summarized in Table VII. A significant increase ($P < 0.01$) in the levels of C18:1, 18:2 in both animal groups can be observed between day 5 and 25. This change was responsible for the significant increase in unsaturated fatty acids in the control and alcoholic group. The fatty acid composition of PE and PS + PI in both animal groups did not change between days 5 and 25, as shown in Table V and VIII, respectively.

The changes in fatty acid composition brought

TABLE VI

FATTY ACID COMPOSITION OF LIVER PLASMA MEMBRANE PHOSPHATIDYLCHOLINE FROM RATS OF DIFFERENT AGES

All conditions were the same as described in Table V.

Fatty acids	5 days (6)		15 days (4)		25 days (7)	
	Control	Alcoholic	Control	Alcoholic	Control	Alcoholic
14:0	5.0±0.4 [c]	5.2±0.7 [c]	8.2±0.7	8.0±0.6	12.1±0.9	10.0±1.1
16:1	8.3±0.9	9.5±0.4	7.9±1.3	9.3±1.0	7.6±0.9	9.1±0.8
16:0	19.7±0.7	17.8±0.3	19.5±0.5	17.1±1.1	19.0±0.4	17.7±1.3
18:1, 18:2	20.7±1.1	19.9±0.7	19.0±0.5	17.5±1.0	16.7±0.7	18.4±0.8
18:0	23.1±1.4	20.7±0.7	21.9±1.3	20.7±0.9	21.5±2.0	19.5±1.3
20:4	11.0±1.0	10.4±1.0	12.4±0.4	12.0±0.3	12.0±1.1	10.3±0.7
22:6	12.2±0.8	16.5±0.6 [b]	11.1±0.5	15.4±1.0 [b]	11.1±0.4	15.0±0.6 [b]
Unsaturated	52.2±0.7	56.3±1.0 [a]	50.4±1.0	54.2±0.7 [a]	48.4±0.5	52.8±0.6 [a]

[a] Significantly different from the control ($0.02 < P < 0.05$).
[b] Significantly different from the control ($0.001 < P < 0.01$).
[c] Significantly different from the same animal group at 15 and 25 days after birth ($0.001 < P < 0.01$).

TABLE VII

FATTY ACID COMPOSITION OF LIVER PLASMA MEMBRANE SPHINGOMYELIN FROM RATS OF DIFFERENT AGES

All conditions were the same as described in Table V.

Fatty acids	5 days (6)		15 days (4)		25 days (7)	
	Control	Alcoholic	Control	Alcoholic	Control	Alcoholic
14:0	7.4±0.4	7.6±0.8	9.6±0.6	10.5±1.1	7.5±0.7	7.8±1.1
16:1	8.2±1.0	7.4±0.7	10.2±1.1	7.8±0.8	8.8±1.0	8.7±1.0
16:0	23.1±0.7	24.3±0.9 [b]	21.8±0.7	19.8±0.5	22.0±1.4	20.0±0.7
18:1, 18:2	15.5±0.5 [b]	14.6±1.1 [b]	19.2±1.1	18.8±0.7	21.5±0.5	20.1±0.9
18:0	21.8±1.3	20.8±0.8	16.4±0.8	16.7±1.1	18.9±0.7	17.4±1.0
20:4	12.4±0.6	10.5±1.0	11.1±0.6	11.5±1.2	10.0±0.5	10.6±0.5
22:6	11.6±0.7	14.8±0.8 [a]	11.7±1.0	14.9±0.7 [a]	11.3±0.6	15.4±0.9 [a]
Unsaturated	47.7±0.7 [b]	47.3±0.9 [b]	52.2±0.5	53.0±0.8	51.6±0.5	54.8±1.1 [a]

[a] Significantly different from the control ($0.02 < P < 0.05$).

[b] Significantly different from the same animal group at 15 and 25 days after birth ($0.001 < P < 0.01$).

about in individual plasma membrane phospholipids by the presence of ethanol in the diet are reported in Tables V–VIII. As shown in Table V, no major changes were induced by ethanol feeding in the fatty acid composition of PE. However, a consistent and significant increase ($P < 0.05$) in the level of C22:6 is observed in PC, sphingomyelin and PS + PI in the plasma membranes from alcoholic pups. This ethanol-induced increase in C22:6 was found at all ages studied and it is responsible for the significant increase in the unsaturation levels at days 5, 15 and 25 in PC and PS + PI. An ethanol-induced increase in the unsaturation of sphingomyelin was only observed 25 days after birth.

The postnatal developmental pattern of the fatty acids in rat liver plasma membrane phospholipids is, therefore, mainly characterized by significant changes in C14:0 and C18:1, 18:2 of PC and sphingomyelin, respectively. Maternal ethanol administration induced a significant increase in C22:6 of PC, sphingomyelin and PS + PI, which resulted in an increase in the level of membrane fatty acid unsaturation. The composition of fatty acids in PE was not affected by the ethanol administration.

TABLE VIII

FATTY ACID COMPOSITION OF LIVER PLASMA MEMBRANE PHOSPHATIDYLSERINE + PHOSPHATIDYLINOSITOL FROM RATS OF DIFFERENT AGES

All conditions were the same as described in Table V.

Fatty acids	5 days (6)		15 days (4)		25 days (7)	
	Control	Alcoholic	Control	Alcoholic	Control	Alcoholic
14:0	13.4±0.7	12.0±0.5	11.0±0.7	10.0±0.6	11.1±0.2	10.7±0.9
16:1	9.4±0.5	9.1±0.6	8.9±0.9	9.7±0.4	8.1±0.8	9.2±0.5
16:0	18.3±0.7	16.4±1.0	18.9±1.0	17.8±1.5	19.0±1.1	16.9±1.2
18:1, 18:2	17.1±0.7	18.0±1.3	19.4±0.5	18.8±0.7	17.4±0.7	19.6±1.0
18:0	19.0±1.0	18.7±0.2	19.1±1.7	18.0±1.1	21.4±2.0	18.4±1.3
20:4	11.4±1.7	10.3±0.7	11.7±0.5	11.2±0.4	10.9±1.0	10.1±0.9
22:6	11.4±0.6	15.5±0.5 [a]	11.0±0.4	14.5±0.8 [a]	12.1±0.9	15.1±1.1 [a]
Unsaturated	50.3±0.5	53.9±0.8 [a]	51.0±0.7	54.2±0.3 [a]	48.5±0.9	54.0±1.0 [a]

[a] Significantly different from the control ($0.02 < P < 0.05$).

Discussion

The overall focus of this investigation was to study the lipid composition of the rat liver plasma membrane during the postnatal development following maternal ethanol ingestion during pregnancy and lactation. The results reported suggest that specific adaptive changes were induced in the liver plasma membrane lipids of the progeny from alcoholic rats.

We have previously observed [22] that although the weight gain during pregnancy was similar in both the alcoholic and pair-fed control dams, the postnatal weight gain over the period studied of the newborn pups of alcohol-fed mothers was reduced by 33% compared with pair-fed controls. However, ragardless of diet treatment or age, both the liver wet weight to body weight ratio and the yield of hepatic plasma membrane protein per wet liver weight in both pair-fed groups were constant. Moreover, results shown in Table I indicate that the liver plasma membranes isolated from both alcoholic and pair-fed control pups were equally enriched in 5′-nucleotidase, and minimally contaminated with microsomes and mitochondria. Thus, chronic maternal ethanol ingestion does not affect either the yield or the degree of purification of the hepatic plasma membranes isolated from newborn pups.

The postnatal changes in the total phospholipid and cholesterol content of plasma membranes from alcoholic and pair-fed control pups are shown in Table II. Total phospholipid and cholesterol content increased by 30 and 70%, respectively, in both groups; adult values were attained 15 days after birth. Although the total phospholipid content was similar in both groups at all ages studied, the amount of cholesterol present in the hepatic plasma membranes from alcoholic pups was significantly greater than that of the pair-fed controls, resulting in a higher cholesterol/phospholipid molar ratio. An increase in the cholesterol/phospholipid molar ratio may, therefore, represent an adaptive response of the plasma membrane to the chronic presence of ethanol. Chin et al. [4] reported an increase in the cholesterol content of erythrocyte and brain membranes from mice physically dependent on ethanol, and they concluded that this alteration may be partially responsible for the development of tolerance to the presence of ethanol. Although we did not investigate whether the plasma membranes from the alcoholic pups were tolerant to ethanol, it is conceivable that an increase in the cholesterol content may well result in an enhancement of the degree of order in the membranes, which become more resistant to the fluidizing effects of ethanol [4].

While other investigators have reported an increase in the cholesterol/phospholipid ratio in synaptic plasma membranes from mice [19] and brain microsomal membranes from rats [45] physically dependent on ethanol, other studies using mice synaptosomal membranes [46] and rat liver plasma membranes, myelin and synaptosomes [47] have reported no changes in the cholesterol/phospholipid ratio subsequent to chronic ethanol administration. Such discrepancies might be due to a variety of reasons, including the strains of animals used and the routes, dosages and duration of ethanol administration. Nevertheless, the increase in the cholesterol/phospholipid ratio observed in our study most probably represents adaptive changes at the cellular level following chronic maternal ethanol ingestion.

The phospholipid distribution in plasma membranes isolated from alcoholic and pair-fed control pups is shown in Table III. In agreement with other data [35,39–44], our study indicates that the major individual constituent phospholipids in the plasma membrane are PE, PC and sphingomyelin. This pattern was independent of either age or the diet fed. Adult values for the phospholipid distribution in plasma membranes from both animal groups were seen in animals 15 days old, and it is interesting to point out that the phospholipid distribution in plasma membranes from 25-day-old pair-fed control pups was also quite similar to that found in adult rat liver plasma membranes [35,39–44].

The developmental pattern of the distribution of phospholipids was also characterized by an increase of 70% in PE and a concomitant decrease of 65% in sphingomyelin in both groups of animals. The content of PC did not vary with age or diet, and although that of PS + PI was independent of age in both animal groups, it was significantly ($P < 0.01$) reduced in the hepatic plasma membranes from alcoholic pups at all ages studied.

Hence, chronic maternal ethanol ingestion led to a decrease in the hepatic plasma membrane content of PS + PI in newborn alcoholic pups. Crane et al. [48] studied the influence of ethanol on lipid metabolism in various mouse tissues and they showed that ethanol ingestion resulted in an increased degradation of phospholipid fractions, which was most evident in the PS + PI fraction. They ascribed their results as being due to a marked stimulation of peroxisomal β-oxidation induced by ethanol ingestion in the alcoholic livers. Since alcohol feeding induces lipid peroxidation [49], we cannot rule out that possibility as a plausible explanation of our results.

Analysis of the fatty acids in the total and individual phospholipids of liver plasma membranes (Tables IV–VIII) during the postnatal development showed that C16:0, C18:1, 18:2 and C18:0 are the major components. These results are in agreement with those of Dobiasova et al. [50], who studied the fatty acid composition of rat liver phospholipids during postnatal development. While the fatty acid composition of PE and PS + PI did not vary in either group over the period studied, significant developmental changes took place in the fatty acid composition of PC, sphingomyelin and total phospholipids. An increase of 39% in C14:0 was observed in the fatty acid content of total phospholipids in both animal groups between days 5 and 15, which was compensated for by slight changes in the level of other fatty acids. The amount of C14:0 in PC (Table VI) was also increased by 38% in the 15-day-olds and up to 55% in the 25-day-olds from both groups of animals. A concomitant slight decrease in C18:1, 18:2 was observed in the 25-day-old animals. The developmental changes that took place in sphingomyelin (Table VII) between days 5 and 25 were characterized by a marked decrease in C16:0 in the alcoholic pups and a significant increase in C18:1, 18:2 in both groups which was responsible for the age-related increase in lipid unsaturation. Although not significant, a slight increase in C14:0 was also observed in sphingomyelin from the 15-day-olds in both groups. The developmental changes taking place in the fatty acid composition of total phospholipids, PC and sphingomyelin were, therefore, independent of diet treatment in most cases and they revealed changes mainly in the levels of C14:0 and C18:1, 18:2. Since the fatty acid composition of individual phospholipids in the plasma membranes from newborn pups fed both diets did not reflect the fatty acid content of the diet (refer to Materials and Methods), the increase in the relative amounts of C14:0 and C18:1, 18:2 might be partially explained by the induction of lipogenesis in response to the suckling-weaning transitional period [51] rather than by a change in the suckling pattern during lactation.

The effect of ethanol administration on membrane fatty acid composition has been studied in unicellular organisms, mammalian cells and rodents given ethanol either in their drinking fluid, via intraperitoneal injection or through continuous inhalation of ethanol vapor. Ingram et al. [52] found that ethanol induced an increase in unsaturated fatty acids in *Escherichia coli*, and a decrease in C18:0, with a concomitant increase in C16:0 in Chinese hamster ovary cells. Thompson and Reitz [18] found the content of C18:1 and C18:2 to be significantly increased while that of C20:4 was decreased significantly in total mitochondrial fatty acids from male rats fed a low-fat diet as opposed to those fed a high-fat, ethanol-containing diet. Cunningham et al. [20] reported major changes in C16:0 and C18:1 in microsomes, and in C16:0, C20:4, C18:1 and C18:2 in mitochondria from rats maintained on a liquid high-fat diet containing 36% of calories as ethanol. More recently, Shorey et al. [53] observed that muscle phospholipids from rats fed an ethanol-containing liquid diet did not appreciably differ in fatty acid composition from that in the pair-fed controls. Taken altogether, these results seem to indicate that no clear pattern was observed in the fatty acid composition changes in membranes from either cells or tissues chronically exposed to ethanol. This discrepancy may reflect not only variations in methodological approaches but also different responses to ethanol in various membranes.

Although several studies have been published on the fatty acid composition of rodent liver plasma membranes [37,43,54–57], there are no reports in the literature on the effects of ethanol administration on the fatty acid composition of such membranes. Results from our experiments

indicate that, at all ages studied, chronic maternal ethanol administration induced a significant increase in the levels of C22:6 in total phospholipids, PC, sphingomyelin and PS + PI (Tables IV, VI–VIII), which resulted in an increase in the level of unsaturation of the hepatic plasma membranes from the alcoholic pups. PE (Table V) was not affected by the ethanol treatment.

The increase in the phospholipid content of C22:6 in the alcoholic pups was observed during lactation (days 1–21) and after the pups had been weaned (days 21–25). Inasmuch as this change in C22:6 persists throughout the suckling-weaning period and since the fatty acid composition of the hepatic plasma membranes does not reflect the content of the diet, our results cannot be attributable either (i) to an indirect effect of ethanol via changes in the fatty acid content of the maternal milk which is delivered to the pups or (ii) to an effect of ethanol on the fatty acid content of the liquid diet that will by itself affect the liver plasma membrane composition of the alcoholic pups. The latter possibility was also considered by Cunningham et al. [20], who found that the liquid diet alone had no significant effects on the fatty acid content of the mitochondria.

The elevated levels of C22:6 in the hepatic plasma membranes from alcoholic pups resulted in a significant increase in phospholipid unsaturation. Sun and Sun [5] also reported an increase in the polyunsaturated acids of ethanolamine phosphoglycerides from synaptic plasma membranes after chronic ethanol treatment. Since ethanol causes disruption of biological membranes [1–3], it is possible that the changes in the degree of unsaturation observed here may cause generalized changes in the intrinsic 'fluidity' of the hepatic plasma membranes from these newborn alcoholic pups [58,59]. Further, these changes may, through adaptation, induce alterations in membrane-associated biochemical processes, such as modulation of the activities of membrane-bound enzymes [60,61] and transport systems [62,63], regulation of the binding properties of hormone receptors [64–66] and membrane phospholipid interactions with abused drugs [67]. Some of these possibilities are now being examined.

These results indicate that although chronic maternal ethanol administration did not appear to affect the postnatal development of the liver plasma membrane lipids, specific adaptive changes were induced in the membrane lipids of the progeny. Further work is being directed at elucidating (i) the precise structural and functional implications of the changes observed and (ii) the relative contribution of the in utero exposure to ethanol as opposed to its chronic administration during both pregnancy and lactation on these changes.

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