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EFFECTS OF CHRONIC ETHANOL ADMINISTRATION ON THE COMPOSITION OF MEMBRANE LIPIDS IN THE MOUSE

DAVID R. WING, DAVID J. HARVEY, JANET HUGHES, P. GRAHAM DUNBAR, KATHRYN A. MCPHERSON and WILLIAM D. M. PATON
University Department of Pharmacology, South Parks Road, Oxford OX1 3QT, U.K.

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Abstract—The relative proportions of the phospholipid fatty acids of erythrocyte membranes in mice were changed by chronic ethanol treatment and were not related to effects of the drug on nutrition, body temperature or experimental stress. Similar changes were observed using two different routes of ethanol administration and they did not reflect the metabolic effects of ethanol seen in the phospholipid fatty acids of whole liver. The observed increased content of saturated fatty acids and decreased content of polyunsaturated acids support the concept of adaptive changes taking place in the membrane during tolerance development to compensate for an increased membrane fluidity caused by ethanol. However, an increased content of the mono-unsaturated acid, octadecenoic (oleic), was found and there was no change in the cholesterol/phospholipid ratio.

Other contrasting types of plasma membrane in mice showed different patterns of change in their phospholipid fatty acids during chronic ethanol administration. It is suggested that changes in membrane lipid composition could only partly account for an adaptation to ethanol-induced membrane disordering.

The actions of ethanol *in vivo* are known to be exerted in part at the membrane level [1]. Thus the anaesthetic potency of ethanol can be predicted from its lipid solubility [2] and the drug has also been shown to increase membrane fluidity *in vitro*. Further, the fact that tolerance develops to the "fluidizing" effect after chronic ethanol treatment [3, 4] suggests that the membrane may be the site involved in the general development of tolerance to alcohol. A number of studies have therefore investigated chronic effects of ethanol on membranes. It has been shown, for example, that lipids extracted from membranes of ethanol-tolerant mice produce more rigid bilayers than those from controls [5]. Littleton and John [6] provided evidence that membrane-lipid composition is altered during chronic ethanol administration to mice with a proportionate increase in the saturated fatty acid content of membrane phospholipids in their brain synaptosomes and a decrease in the unsaturated acid content. Such changes are consistent in direction with the formation of a more rigid membrane bilayer. Another mechanism whereby the membrane can become intrinsically less fluid at physiological temperatures was proposed by Chin *et al.* [7] who found that chronic ethanol treatment of mice produced an increased cholesterol content in erythrocyte membranes.

Membrane lipids are, however, also subject to change by a variety of factors *in vivo* so further evidence is required to substantiate the possibility that changes in lipid composition represent a mechanism by which an adaptation can occur to the increase in membrane fluidity induced by the ethanol. This is especially so since some evidence does not readily support this simple view of such an adaptive process. Sun and Sun [8], for example, found a decrease in the saturated fatty acids and an increase in the polyunsaturated acids of ethanolamine phosphoglycerides from synaptic plasma membranes after chronic ethanol treatment. These changes, in isolation, are consistent in direction with the formation of a less rigid membrane. Further, Koblin and Deady [9] found no influence on the sensitivity of the nervous system of mice to alcohol when several of the polyunsaturated fatty acid components of brain membranes had been altered by dietary means.

This paper reports the effects of chronic ethanol administration on four contrasting plasma membranes to test the generality of any such effect on lipid composition. Two routes and time courses of ethanol administration have been used and the roles of nutrition, stress and change of body temperature as influencing factors have also been assessed.

MATERIALS AND METHODS

Mice (Charles River CD-1, males 24–29 g) were maintained on Charles River diet no. 22RF with free access to water. Lighting was provided from 07.30 hr to 19.30 hr.

Two alternative routes of ethanol administration were used. One was by i.p. injection twice daily at 09.00 hr and 18.00 hr with 20% (w/v) ethanol in saline (0.85% w/v aq. NaCl) at 4.5 g ethanol/kg for seven days [5]. Controls were injected with saline only. Mice were housed at 22°, with free access to food except when controls were "pair-fed". "Pair-fed" controls were injected with saline containing glucose equicaloric to the ethanol dose. Tissues from at least four mice were combined for each membrane preparation. Individual tissues were used in the

experiment in which whole livers were directly extracted for lipids. Since the daily ethanol doses induced sleep, the sleeping times were measured after the morning injections in certain experiments in which tolerance development was being tested. The index used was the interval from the time of the injection to that at which the mouse regained its righting reflex (with ability to repeat this reflex action within 1 min).

The other route of ethanol administration was through continuous inhalation of vapour for two weeks at a concentration of 14.5 mg ethanol/l. air (days 1–3) rising to 17.0 mg/l. air (days 4–14). In these experiments 15 mice were housed in glass tanks of approximately 25 l. capacity. Air from a compressed air line was passed through a filter to remove oil vapour and then through a silica gel column to remove water. Absolute ethanol was evaporated by continuous injection into the air stream which flowed into the tank at 60 l./hr. Control animals received the air supply without added ethanol vapour. Ethanol administration commenced after a three-day acclimatisation period. The temperature of the tanks was 28–29°.

Tissue samples and membrane isolation. At the end of the treatment period, mice were killed by cervical dislocation and bled from the neck. Blood was collected in heparinized tubes at 4°. Samples from at least four mice were combined. With the vapour-treated mice, the livers and brains in addition were rapidly dissected and rinsed in ice-cold saline.

Isolation of membrane preparations followed conventional procedures and were carried out below 4°. The method of Hanahan and Ekholm [10] was used for isolation of erythrocyte membranes, that of Emmelot *et al.* [11] for liver plasma membranes, and those of Sun and Sun [12], Jones and Matus [13] and Cotman [14] for myelin and synaptic plasma membranes. In brief the procedures were as follows.

For erythrocyte membranes, after removal of plasma and washing of erythrocytes, the cells were lysed in hypotonic Tris–HCl and centrifuged at 20,000 *g* for 40 min. The membranes were then washed three times in the buffer by resuspension and recentrifugation.

For liver plasma membranes, the livers, approximately 10 g in total, were minced in 1 mM $NaHCO_3$ pH 7.5 [40% (w/v) liver:$NaHCO_3$ solution] and gently homogenised, filtered and centrifuged at 4000 *g* for 15 min. The pellet was resuspended and the procedure repeated. The distinct "fluffy" upper layer of the pellet was collected and similarly washed and spun on a discontinuous sucrose gradient at 70,000 *g* for 90 min. The fraction at the d 1.16–d 1.18 interface was collected and washed.

For myelin and synaptosomal plasma membranes, brains were minced and homogenised in 0.32 M sucrose and centrifuged at 1000 *g* for 10 min. The supernatant was centrifuged at 15,000 *g* for 12 min and the pellet was resuspended in 0.32 M sucrose. It was layered onto 0.8 M sucrose and the crude myelin at the interface was washed and purified by relayering. The pellet was lysed in hypotonic Tris–HCl (5 mM), pH 8.1, lightly homogenised and centrifuged at 10,000 *g* for 15 min. The cloudy supernatant was collected and centrifuged on a discontinuous sucrose gradient at 60,000 *g* for 110 min, the synaptosomal plasma membrane-enriched fraction at the 28.5–34% (w/w) interface was collected and washed.

The quality of the membrane preparations was checked by electron microscopy and by assay of marker enzymes, namely acetylcholinesterase (for erythrocyte membranes), Na^+/K^+ATPase (synaptic plasma membranes), 5′nucleotidase (liver plasma membranes), 2′,3′-cyclic nucleotide-3-phosphohydrolase (myelin) and succinic dehydrogenase for mitochondrial contamination. A portion of each preparation was kept for assay of protein content by the method of Lowry *et al.* [15]; the remainder, usually 80% of the total preparation, was extracted for lipid analysis.

Lipid extractions and estimations. After initial uptake in methanol and two 40 sec periods of sonication in a standard Sonor device, membrane lipids were extracted into 2:1 (v/v) chloroform:methanol [16]. The lipid extraction procedure of Folch *et al.* [16] was also used in the whole-liver experiment. Lipids were then separated by silicic acid column chromatography [17] into neutral lipid (chloroform eluate), glycolipid (acetone eluate) and phospholipid (methanol eluate) fractions. Phospholipids were assayed by measurement of the phosphorus content according to the method of Morrison [18]. After alkaline hydrolysis of phospholipids, their fatty acids were methylated using methanol/HCl prepared from a mixture of acetyl chloride in anhydrous methanol (Applied Science Laboratories, Inc., State College, PA). The major constituent acids, hexadecanoic (16:0, palmitic), octadecanoic (18:0, stearic), octadecenoic (18:1, mainly oleic), octadecadienoic (18:2, linoleic), eicosaenoic (20:1), eicosatetraenoic (20:4, arachidonic) and docosahexaenoic acid (22:6) were identified by gas–liquid chromatography (GLC) and combined gas chromatography–mass spectrometry (GC–MS: V.G. Micromass 12B, 70 eV, 2 m × 2 mm glass column packed with 3% SE-30 on 100/120 Gas Chrom Q, Applied Science Laboratories, Inc., State College, PA) and quantified by GLC (Varian 2400 gas chromatograph fitted with dual flame ionization detectors) using a glass column (2 m × 2 mm) packed with 10% SP-2330 on 100/120 Chromosorb WAW (Supelco Inc., PA) connected to a Kent Chromalog. 3 integrator. This assay was carried out with a programmed oven temperature rise of 4°/min between 130 and 240°, and a carrier gas flow of N_2 at 30 ml/min. The flash heater and detector temperatures were 280 and 300° respectively. Eicosadienoic acid (20:2) was used as internal standard. The precision of the technique was estimated by taking standard fatty acids through the hydrolysis and GLC assay stages; values for the coefficient of variation ranged from 0.1 to 0.7%.

Cholesterol was assayed in the neutral lipid fraction from the silicic acid column by GLC using stigmasterol as internal standard. Conditions were as described for the fatty acid assay except that the glass columns were packed with 3% SE-30 on 100/120 Gas Chrom Q, and the oven temperature was set at 280°. Cholesterol was quantified by peak height ratios.

Assay of ethanol. Blood ethanol concentrations

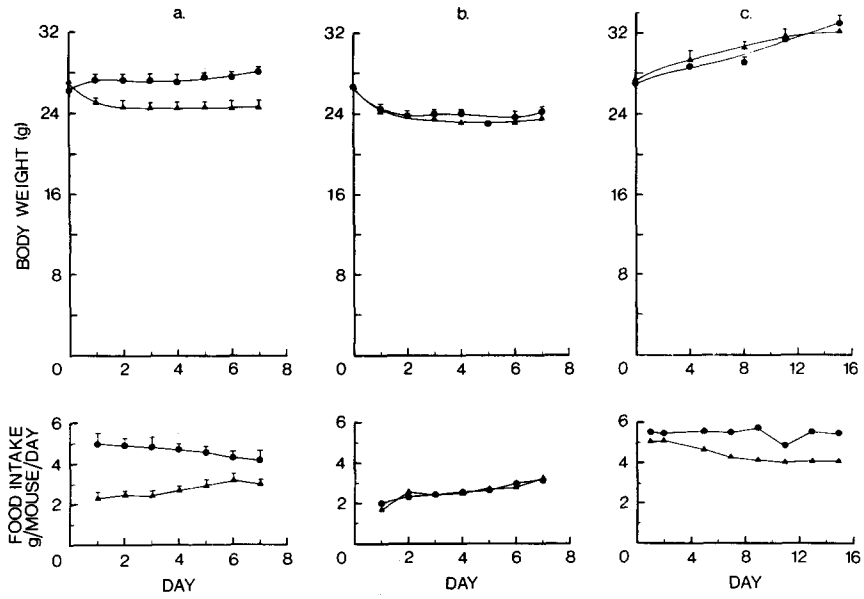

Fig. 1. Effects of ethanol on body weight and food intake. (a) Ethanol by i.p. injection: mice with free access to food were injected with either ethanol solution (▲) or saline (●) according to Materials and Methods. Mean values + S.E.M. are plotted for body weights of 43–47 mice, and for mean food intakes in four experiments. (b) Ethanol by i.p. injection and control mice "pair-fed": mice were injected with either ethanol solution (▲) or equicaloric glucose solution (●), both in saline, according to Materials and Methods. Control mice were restricted in their food intake to that of the ethanol-injected mice during the previous 24 hr. Over the week's course this averaged 2.6 g/mouse/day and mean values are plotted. Mean body weights + S.E.M. are plotted for 14 mice in one experiment. (c) Ethanol by inhalation of vapour: mice were provided with (▲) or without (●) ethanol vapour according to Materials and Methods. Mean values + S.E.M. are plotted for body weights of 19–25 mice in two experiments. Mean food intakes are plotted for one experiment.

were assayed by an alcohol dehydrogenase method using SIGMA kit 331-UV.

Ethanol vapour from the administration tank or from the expired air of the mouse was measured by GLC as above except that the glass column was packed with Porapak Q and the oven, injector and detector temperatures were 195, 240 and 260° respectively. Calibration was by peak heights and known concentrations of ethanol vapour. Samples for assay from the tank were obtained from its inlet tube. When expired air was assayed, the mouse was placed in an oxygenated, 60 ml vessel which was sealed and maintained at 28°. One ml samples were taken in a gas-tight syringe at 4 min and 5 min for gas chromatography; over this period equilibration in the vessel was approached. The method was therefore sensitive to the respiratory state of the mouse, so was not applicable to those made unconscious by ethanol or recovering from a period of unconsciousness.

Chemicals. All reagents were of 'AR' grade and obtained from B.D.H. Ltd. (Poole, U.K.) and Sigma London Chemical Co. (London, U.K.). Organic solvents were doubly distilled before use.

Statistics. Either the percentage change from the mean experimental control value due to ethanol treatment was calculated for each test group of mice and the probability of the change being significantly different from zero was tested, or Student's *t*-test was applied directly to the observed test and control values.

RESULTS

Ethanol treatment, body weight, food and water intake

(a) *By injection.* Figure 1a shows that although mouse body weights at the end of the week's treatment were significantly lower in ethanol-injected mice than in controls with unrestricted food, they were not significantly lower than after the first day of ethanol treatment. Food intake in the test mice was initially 50% or less than that in controls but rose to 80% of the control value by day 6. At this time the daily ethanol load constituted 12.5% of the combined caloric intake of ethanol and food leaving a total caloric deficit of only 12% compared with controls. Water consumption at that time was the same in both test and control groups.

When control mice were restricted in their food intake to that of ethanol-treated mice ("pair-fed"), they were injected with glucose solution equicaloric to the ethanol solution. No significant differences in body weight were then observed (Fig. 1b). The final water consumptions were equal.

It was noted that if older male mice with an initial mean body weight of 32–33 g or more were used only a very small percentage would survive the week's administration of ethanol.

(b) *By inhalation.* No significant differences in body weight were observed between mice receiving ethanol vapour in their air and controls (Fig. 1c).

Table 1. Cholesterol and phospholipid content of erythrocyte membranes (lack of effect of chronic ethanol treatment by injection)

	mg Phospholipid/ mg protein	mg Cholesterol/ mg protein	mg Cholesterol/ mg phospholipid
Control	0.408 ± 0.012	0.142 ± 0.015	0.349 ± 0.036
	N.S.	N.S.	N.S.
Ethanol	0.400 ± 0.023	0.131 ± 0.009	0.327 ± 0.010

Mean values ± S.E.M. n = 4 or 5 groups of 6–8 mice in two experiments.
N.S. = not significantly different.

Both increased steadily during the two-week treatment. Overall food and water intake in mice receiving ethanol vapour were both about 80% of control values.

Tolerance development and assay of blood ethanol

This was assessed in the mice receiving ethanol by injection. Doses of 4.5 g ethanol/kg caused a loss of righting reflex. The sleeping time, measured as described in Materials and Methods, declined by 33% ($P < 0.02$) from 49 min (± 5, S.E.M.) to 33 min (± 3, S.E.M.) over the week's administration. The mean blood ethanol concentration at which the mice woke rose from 3.62 ± 0.18 (S.E.M.) to 4.0 ± 0.09 mg/ml over this period. The development of functional tolerance could not therefore be accounted for only by an alteration in the rate of metabolism of ethanol but required also some other adaptation presumably at the cellular level.

With a dose i.p. of 4.5 g ethanol/kg in naive mice a maximum drop in body temperature of 5° was observed 1–2 hr after the injection. When this hypothermia was prevented by elevating the cage temperature, the decline in sleeping time over the week's treatment was reduced by half but a similar rise in the blood ethanol concentration on waking was still observed.

When sequential estimates of blood alcohol concentrations were required from the same mice, expired-air vapour concentrations of ethanol were measured by gas chromatography and related to the blood concentration from a standard curve (see, for example, [19]). In this way the blood volume of the mouse was not disturbed. Under the conditions described in Materials and Methods, declines in estimates of the blood ethanol concentrations were obtained showing that in the naive mouse they had dropped to 0.1 mg/ml or less at the time of the second daily injection. This method was suitable for expired-air concentrations as low as 0.05–0.10 μg ethanol/ml air (with coefficients of variation in the range 4–8%).

For the vapour-treated mice, ethanol was detectable in the blood (but usually at much less than 1 mg/ml) on removal of the animals from the administration tank. It was found, however, that at only slightly higher concentrations of vapour input (22.6 mg/l.) a significant death rate occurred during the two week's administration.

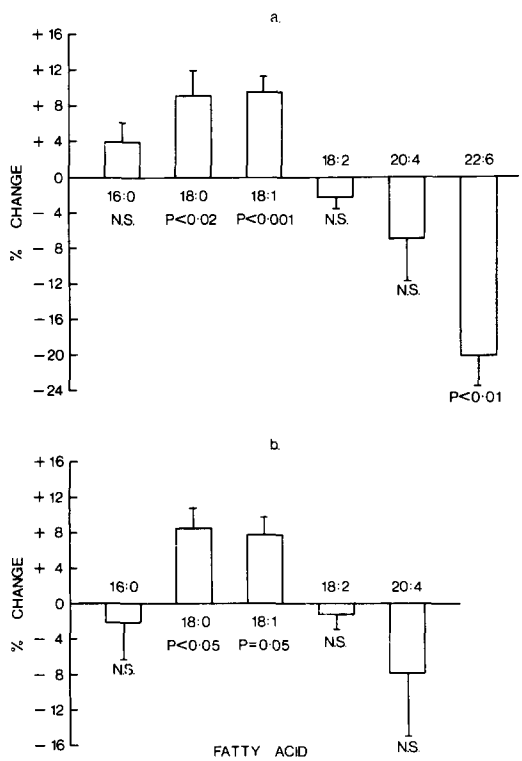

Fig. 2. Per cent changes in fatty acid content of erythrocyte membrane phospholipids after chronic ethanol treatment. Mice had free access to food and water. Mean values ± S.E.M. are given for the per cent changes (expressed as a percentage of each control fatty acid value taken as 100%). N.S. = not significantly different from zero change. (a) By i.p. injection: n = 8 groups of 5–8 mice in three experiments. (b) By inhalation: n = 4 groups of 5 mice in two experiments. (Limited data for docosahexaenoic acid include decreases of 18%.)

Effects on lipid composition of erythrocyte membranes

(a) *By injection.* No significant effects of ethanol treatment on the amounts of membrane phospholipid or cholesterol, or in their ratio, were detected when controls were allowed free access to food (Table 1). The composition of the major fatty acids obtained from phospholipid hydrolysis are shown later in Fig. 4. Changes resulting from ethanol administration, expressed as a percentage of each control fatty acid value (taken as 100%) are shown in Fig. 2a. Significant increases in stearic acid and oleic acid were seen, while there was a decrease in docosahexaenoic acid.

When the control mice were restricted in their food intake to that of the ethanol-treated mice, the

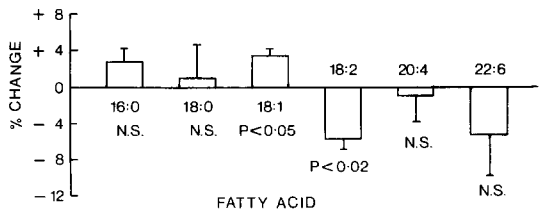

Fig. 3. Per cent changes in fatty acid content of erythrocyte membrane phospholipids after diet restriction ("pair-feeding") and replacement of injected ethanol with equicaloric glucose. Test mice were restricted in their food intake to that of ethanol-injected mice run concurrently (e.g. Fig. 1a) and equicaloric glucose was substituted for the injected ethanol during the week's administration. Control mice had free access to food and were injected with saline only. Mean values ± S.E.M. are given for the per cent changes (expressed as in Fig. 2). N.S. = not significantly different from zero change. n = 3 groups of 5 mice in one experiment.

changes in fatty acid composition (again calculated on a percentage basis) shown in Fig. 3 were obtained. Although a significant increase in oleic acid and a decrease in linoleic acid were observed, the effects due to ethanol in Fig. 2a remained significant for the former and not significant for the latter. Again, no effects of the drug on the content of phospholipid or cholesterol, or in their ratio were found. Thus values for mg cholesterol/mg phospholipid were 0.346 ± 0.005 (S.E.M.) for the saline-injected controls with free access to food, 0.345 ± 0.008 for the food-restricted, glucose-injected controls and 0.366 ± 0.021 for their matched ethanol-injected mice.

The possibility was tested that factors related to the stress of the injection routine might influence the control values. Figure 4 shows that similar fatty acid profiles were obtained whether the mice were left undisturbed, were sham-injected or were injected with saline or with an isotonic glucose load instead of saline. No differences were observed in the contents of membrane cholesterol and phospholipid.

(b) *By inhalation.* The percentage changes in phospholipid fatty acid composition resulting from vapour treatment are shown in Fig. 2b. These show similarities to those obtained after administration of ethanol by injection in that significant increases in stearic and oleic acids were seen and a decrease was indicated in arachidonic acid (limited data for docosahexaenoic acid include decreases of 18%). No significant effects on the amount of membrane phospholipid, cholesterol or their ratio were found (Table 2), although values were generally higher than in the shorter experiment when ethanol was given by injection (Table 1).

Phospholipid fatty acids of whole liver

The composition and percentage changes, due to injected ethanol treatment, of the phospholipid fatty acids of whole liver are shown in Fig. 5. The fatty acid profile is different from that observed in erythrocyte membranes (Fig. 4) and although the treatment caused a significant rise in stearic acid, as in the erythrocyte membranes, the overall pattern of change in profile was also different from that in the membranes.

Effects of ethanol treatment on lipid composition of liver plasma membranes, myelin and synaptic plasma membranes

The membrane composition of the quantitatively significant phospholipid fatty acids and their per-

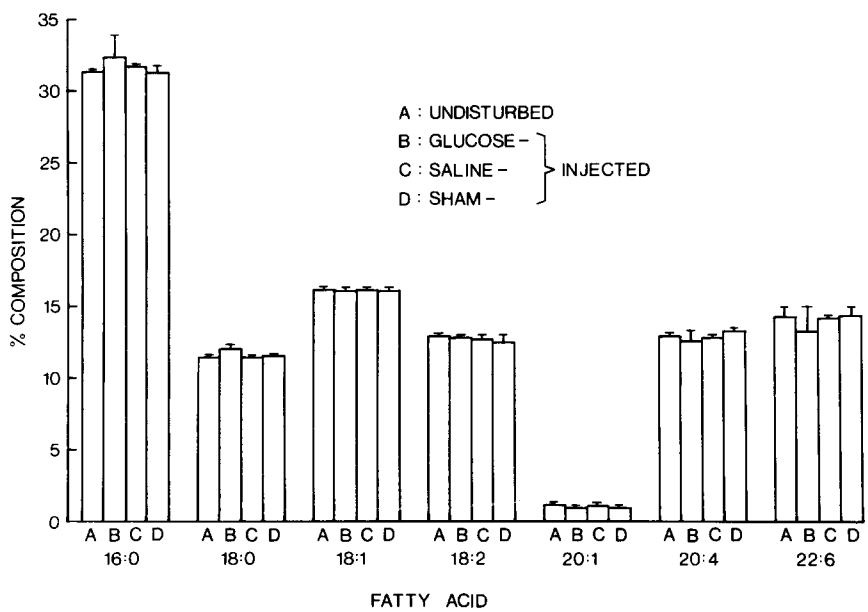

Fig. 4. Lack of effect of sham and control injections on phospholipid fatty acids of erythrocyte membranes. Mice were either left undisturbed or were sham-injected or were injected i.p. with saline or isotonic glucose solution during the week's administration. Mean values (+ range) of two groups of four mice (one experiment) are given for the percentage composition of the major phospholipid fatty acids.

Table 2. Cholesterol and phospholipid content of erythrocyte membranes (lack of effect of chronic ethanol treatment by inhalation)

	mg Phospholipid/ mg protein	mg Cholesterol/ mg protein	mg Cholesterol/ mg phospholipid
Control	0.521 ± 0.038	0.203 ± 0.031	0.386 ± 0.030
	N.S.	N.S.	N.S.
Ethanol	0.569 ± 0.057	0.240 ± 0.036	0.416 ± 0.024

Mean values ± S.E.M.
n = 3 or 4 groups of 5 mice in two experiments.
N.S. = not significantly different.

centage changes due to treatment by vapour inhalation are shown in Figs. 6–8 for liver plasma membranes, myelin and synaptic plasma membranes respectively. In the hepatic plasma membranes there was a significant decrease in stearic acid content and an increase in oleic acid. Myelin showed a significant decrease in docosahexaenoic acid and an increase of marginal significance in oleic acid. No significant changes were detected in the phospholipid fatty acids of the synaptic plasma membrane fraction but a trend towards an increased cholesterol/phospholipid ratio was indicated though this also was not significant (Table 3). This table also shows the lack of effect of ethanol treatment on the cholesterol and phospholipid content of liver plasma membranes and myelin.

DISCUSSION

Changes in erythrocyte membrane lipid composition were observed after chronic ethanol administration, consisting of significant increases in stearic and oleic acids and a decrease in docosahexaenoic acid. The question therefore arises as to whether these effects were caused directly by the ethanol or were the result of an indirect mechanism causing changes in the nutritional state or body temperature or to various stress-associated factors.

Evidence suggesting a direct effect is as follows.

First, the characteristic changes in fatty acid composition were found whether ethanol was given by injection or by inhalation. This indicates that while

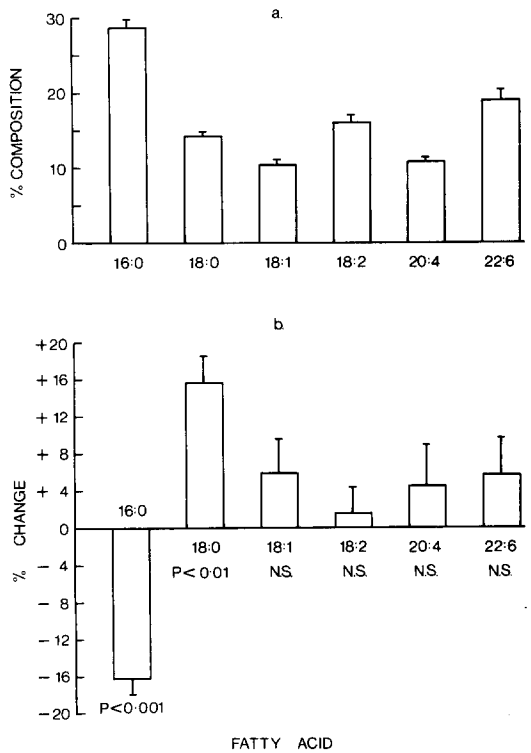

Fig. 5. Fatty acid content of whole liver phospholipids. Mean values ± S.E.M. are given for 5 individual mice in one experiment. (a) Per cent composition of major fatty acids in control mice. (b) Per cent changes (expressed as in Fig. 2) after chronic ethanol administration by i.p. injection according to Materials and Methods. N.S. = not significantly different from zero change.

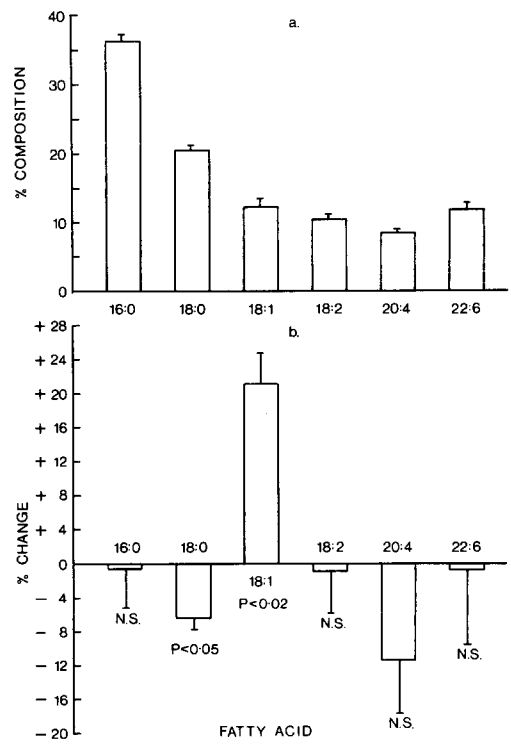

Fig. 6. Fatty acid content of liver plasma membrane phospholipids. Mean values ± S.E.M. are given for 4 groups of 5 mice in two experiments. (a) Per cent composition of major fatty acids in control mice. (b) Per cent changes (expressed as in Fig. 2) after chronic ethanol administration by inhalation according to Materials and Methods. N.S. = not significantly different from zero change.

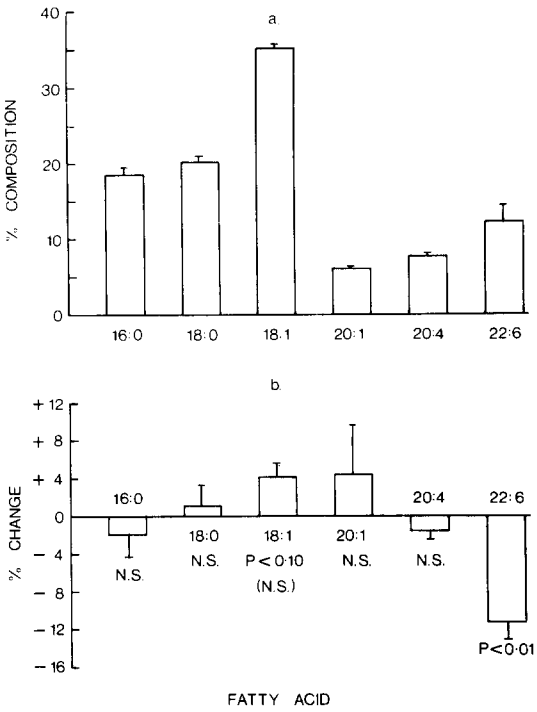

Fig. 7. Fatty acid content of myelin phospholipids. Mean values ± S.E.M. are given for 4 groups of 5 mice in two experiments. (a) Per cent composition of major fatty acids in control mice. (b) Per cent change (expressed as in Fig. 2) after chronic ethanol administration by inhalation according to Materials and Methods. N.S. = not significantly different from zero change.

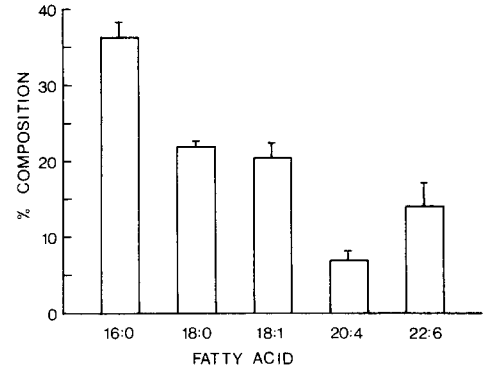

Fig. 8. Fatty acid content of synaptic plasma membrane phospholipids. Per cent composition of major fatty acids in control mice are given. Mean values + S.E.M. are shown for 4 groups of 5 mice in two experiments. Per cent changes after chronic ethanol administration by inhalation were all not significantly different from zero.

the experiments could not entirely exclude some effect due to change in body temperature, such a change could not account for the characteristic pattern, since the vapour-treated mice kept at 28–29° would not experience a significant hypothermia [20].

Secondly, nutritional factors were eliminated by pair-feeding experiments. The characteristic pattern of change was unaltered whether compared with controls that were pair-fed or with those that had an unlimited dietary supply. Diet restriction itself was found to produce a small (3%) rise in oleic acid and a 6% fall in linoleic acid.

Thirdly, no gross changes in phospholipid fatty acid composition were found in the erythrocyte membrane when control saline-injected mice were compared with undisturbed or sham-injected mice or with ones in which the carrier load of saline had been replaced by an isotonic glucose solution. In this way a variety of stress-related factors were eliminated as possible causes of compositional changes in the membrane lipids.

Finally, there is the possibility that the characteristic changes simply mirrored a general metabolic change such as might be reflected in the total phospholipid fatty acids of whole liver. This, however, was not the case; in the liver palmitic acid declined, stearic acid increased and there were no other significant changes.

Table 3. Cholesterol and phospholipid contents of liver plasma membranes, myelin and synaptic plasma membranes (lack of effect of chronic ethanol treatment by inhalation)

	mg Phospholipid/ mg protein	mg Cholesterol/ mg protein	mg Cholesterol/ mg phospholipid
Liver plasma membranes			
Control	0.311 ± 0.008	0.119 ± 0.012	0.386 ± 0.047
	N.S.	N.S.	N.S.
Ethanol	0.326 ± 0.018	0.133 ± 0.023	0.403 ± 0.057
Myelin			
Control	0.698 ± 0.079	0.482 ± 0.014	0.677 ± 0.013
	N.S.	N.S.	N.S.
Ethanol	0.744 ± 0.037	0.489 ± 0.051	0.660 ± 0.068
Synaptic plasma membranes			
Control	0.424 ± 0.031	0.122 ± 0.011	0.288 ± 0.008
	N.S.	N.S.	N.S.
Ethanol	0.448 ± 0.035	0.143 ± 0.019	0.316 ± 0.017

Mean values ± S.E.M.
n = 3 or 4 groups of 5 mice in two experiments.
N.S. = not significantly different.

We have concluded, therefore, that the effects of ethanol on erythrocyte membrane composition cannot be accounted for by indirect effects through changes in nutrition, body temperature, stress of handling or fluid or solute load, so that they may reasonably be supposed to express a direct action of ethanol on the membrane.

The further question then arises as to how far the changes seen can be regarded as an adaptation calculated to reduce an increased membrane "fluidity" due to the drug. Compatible with this concept is the observed increase in content of saturated fatty acids and decreased content of polyunsaturated acids seen in erythrocyte membranes, but the increase in the mono-unsaturated fatty acid, oleic, and the lack of an increase in cholesterol content (in relation to either erythrocyte membrane protein or phospholipid) do not fit so readily. The increased cholesterol content of erythrocyte membranes in ethanol-tolerant mice observed by Chin *et al.* [7] may reflect the different strain of mice used in that study. The primary role of an altered lipid composition need not be to cause a general change in the intrinsic fluidity of the membrane but rather to alter the ability of the membrane to fluidize to ethanol [5] or even to alter the functional activity of membrane proteins without a change in fluidity [21]. A feature of membranes that had become resistant to disordering by ethanol after long-term administration of the drug was that they showed reduced "binding" of the alcohol [22].

It might be expected that adaptive processes involving membrane lipids would show themselves generally in membranes. However, the differences in the patterns of change of phospholipid fatty acids between membrane types were more striking than the similarities. This could be due to several factors, for example, the varying patterns of change may be associated with specific differences in the composition of each membrane and in the different rates of turnover of the membrane lipids. Thus in those membranes with a high content of cholesterol relative to protein, namely myelin and erythrocyte membranes, significant decreases in docosahexaenoic acid were seen. Although increases in oleic acid were more general, the synaptic plasma membrane failed to show this effect. It is by no means established whether changes are expressed over the whole membrane or whether they occur at specifically important regions. Also, the contribution to fluidity of different fatty acids with unsaturated bonds at varying depths in the membrane is poorly understood. Furthermore, adaptive processes may not be confined to the plasma membrane but could well involve intracellular organelles. Thus a recent study suggested that an important action of alcohol was to increase the concentration or effectiveness of calcium within the cell [23].

Detailed mechanisms for direct actions of ethanol in changing membrane lipid compositions are speculative. The general observation with the membranes used in this study was that the significant effects of ethanol administration on phospholipid fatty acids of chain length longer than 18 C units were to decrease their percentage composition. An inhibitory effect on elongase enzymes to prevent normal rates of production of such long chain fatty acids might be compatible with the observation. However, the desaturase enzymes, by virtue of being rate-limiting, assume a greater importance and ethanol has been shown to inhibit the activities of the Δ^6 and Δ^5 desaturases [24] which might be expected to reduce the formation of such long-chain polyunsaturated fatty acids as arachidonic and docosahexaenoic. The possibility that fatty acid desaturase activities might themselves be dependent on membrane fluidity (at least for microsomes), being inactive in the more fluid state, has been raised by Kates and Pugh [25]. This would constitute a model of self-regulation for the membrane and would indicate an inhibitory action by ethanol on desaturase activity through increasing membrane fluidity.

In conclusion, the results of this study suggest that changes found in membrane lipid composition in ethanol-tolerant mice can partly account for an adaptation to ethanol-induced membrane disordering, but that even with animals of the same strain, age and sex, the responses are not similar in contrasting membrane types. Study of the timing of the responses in different membranes in relation to tolerance development might indicate the main features of the adaptive changes. Until a more precise localization of the changes in membrane lipids can be established, together with fluidity measurements in more discrete areas, the significance of the effects on the functioning of the membrane cannot be specified in detail.

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