*Pharmacology Biochemistry & Behavior*, Vol. 35, pp. 75–84. © Pergamon Press plc, 1990. Printed in the U.S.A. 0091-3057/90 $3.00 + .00

# Effects of Chronic Ethanol Administration on Plasma-Membrane-Bound Glycosyltransferase Activities

ALMUDENA FERNANDEZ-BRIERA,* PIERRE LOUISOT AND RENÉE MORELIS

**Department of Biochemistry and Molecular Biology*
*University of León, 24007 León, Spain*
*Department of Biochemistry, University of Lyon, Lyon Sud Medical School*
*INSERM, U 189 BP 12, 69921 Oullins, Cedex, France*

Received 7 December 1988

FERNANDEZ-BRIERA, A., P. LOUISOT AND R. MORELIS. *Effects of chronic ethanol administration on plasma-membrane-bound glycosyltransferase activities.* PHARMACOL BIOCHEM BEHAV **35**(1) 75–84, 1990.—Physiological and membranous modifications induced by a 4-week ethanol administration in mouse liver plasma membrane were studied. Galactosyl- and glucosyltransferase activities were stimulated in the presence of dolichylphosphate alone or with phosphatidyl-choline. The galactosyltransferase activity was inhibited by chronic ethanol administration. These enzymes were modulated by different phospholipids. The phosphatidic acid was the most efficient activator. Ethanol provoked an inhibition of the galactosyltransferase activity whatever the phospholipid used, as well as an inhibition of the glucosyltransferase activity, chiefly in presence of phosphatidyl-inositol. The preincubation of control or treated mouse liver plasma membranes with liposomes loaded by dolichyl-phosphate and cholesterol greatly enhanced the enzymatic activities without removing the inhibition by ethanol treatment.

Plasma membrane Glycosyltransferase Phospholipidic effectors Glycolipid Chronic ethanol consumption

JUST like anaesthetic drugs, ethanol is known to bring about changes in neuronal membranes (1, 11, 27), liver cell organelles and blood serum enzymes (20). It is also known to modify membrane-bound enzymes' activities (2, 7, 22, 25).

However, different approaches exist for measuring the metabolic alcohol effect: acute ethanol ingestion prior to killing the animals (22), ethanol addition to enzymatic incubation mixture (18) or chronic alcohol administration either on short term or long term (10,22). From these different studies, several coinciding or opposite results are found. Nevertheless, they furnish evidence to indicate that ethanol interacts with the membrane lipid bilayer, provokes alteration of membrane structure and increases lipid fluidity (29,21). In acute administration experiments, Corazzi (2) found that plasma membranes appeared to be the most interesting subcellular organelle in the changes produced by ethanol; he suggested that alterations of enzymatic activities could be due to a change of plasma membrane lipid composition provoked by ethanol exposure.

In a previous report (4) we showed the existence of plasma-membrane-bound glycosyltransferase systems. In a subsequent study (5) we tried to establish how phospholipids affected the glycosylation process. We argued that an examination of two membrane-bound enzymes after a 4-week ethanol administration could serve two purposes. The first would be to determine whether other cell membrane enzymes (besides $Na^+K^+$ATPase, 5'-nucleotidase, glutaminyltransferase) are also affected (particularly the enzymes included in posttraductional processes). The second objective would be to determine whether the lipidic modulation of these enzymes remained the same in the control membranes and in those of alcohol-treated animals. Nishimura and Teschke's hypothesis (20) should be born in mind here, as they suggested that ethanol had different effects on the activities of liver plasma membrane enzymes.

## METHOD

Mice, strain OF1 (IFFA Credo, Les Oncins, France), one month old, were used. The recently weaned mice were allowed free access to solid food and water, which contained increasing amounts of ethanol (2.5 to 17.5%) for four weeks, the percentage of ethanol being increased every five days. Pair-fed control mice were given a similar diet for the same period, but without ethanol. During the treatment period, the mice were housed in plastic boxes, 6 or 8 animals per box, at 22°C on a 12-hr light/dark cycle. Once killed, treated and control mice underwent laparotomy, the livers being rapidly removed and placed in a solution containing 1 mM $NaHCO_3$ and 1 mM $CaCl_2$ at pH 7.5, at 4°C.

Plasma membranes were isolated by modifying the method described by Ray (24). Mouse livers were homogenized and fractionated by differential centrifugations in 1 mM $NaHCO_3$, 1 mM $CaCl_2$ pH 7.5, according to Fig. 1. The purification of plasma membranes was performed on a sucrose discontinuous gradient as described previously (4). The purity of plasma membrane fractions was assessed by several specific marker-enzymes and by electron

ABBREVIATIONS

| | |
|---|---|
| dol-P | dolichol monophosphate |
| PE | phosphatidyl-ethanolamine |
| PS | phosphatidyl-serine |
| PI | phosphatidyl-inositol |
| PC | phosphatidyl-choline |
| lyso-PC | lysophosphatidyl-choline |
| sphingo | sphingomyeline |
| PA | phosphatidic acid |

microscopy. Monoamine oxidase (EC 1.4.3.4.) as an outer mitochondrial membrane marker enzyme was assayed according to Tabor *et al.* (28), alkaline p-nitrophenyl phosphatase (EC 3.1.3.1.) as a plasma membrane marker enzyme, was assayed by modifying Ray's method (24), and by the spectrophotometrical determination of p-nitrophenol formed from p-nitrophenyl phosphate. Protein concentrations were determined by the method of Gornall (9).

UDP-[$^{14}$C]-glucose (11.2 GBq/mmol) and UDP-[$^{14}$C]-galactose (12.7 GBq/mmol) were supplied by New England Nuclear. Benzylamine, paranitrophenyl-phosphate, dolichyl monophosphate (grade III), phospholipids and cholesterol were purchased from Sigma.

Incubation mixtures used for the study of [$^{14}$C]-glucose incor-

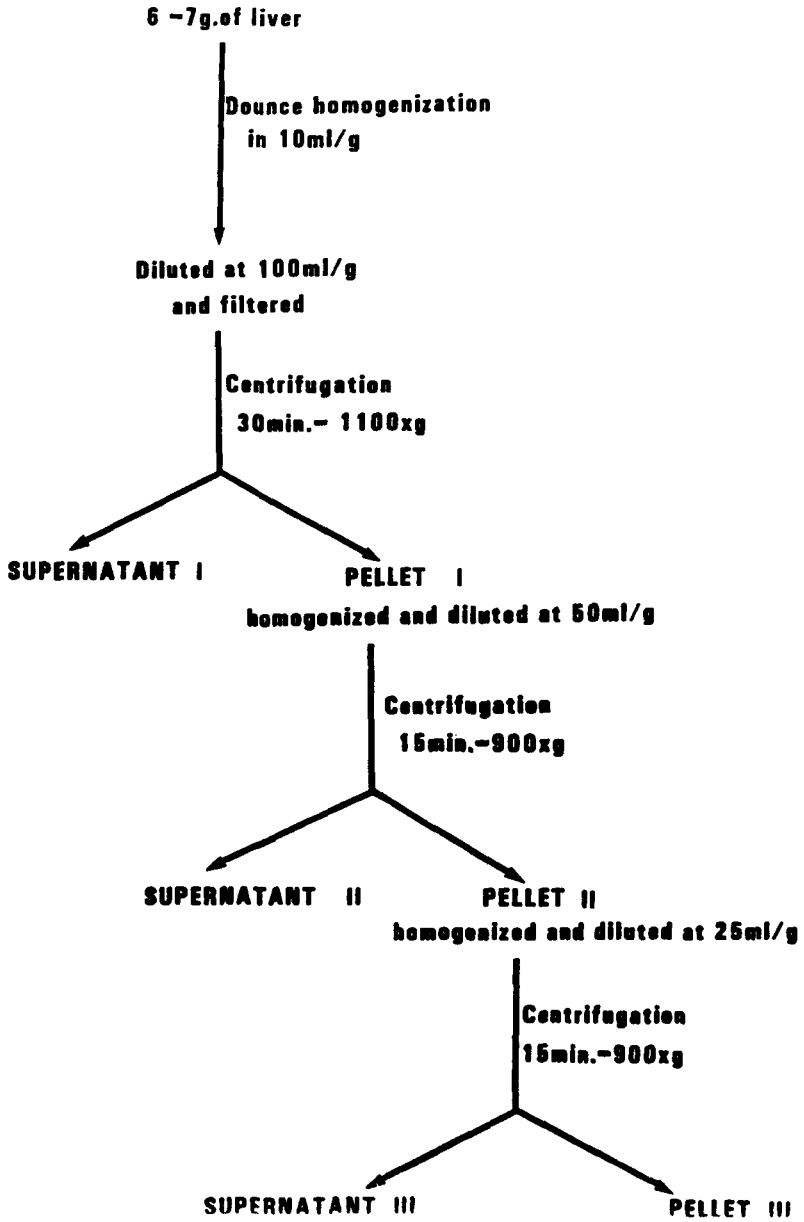


FIG. 1. Fractionating of mouse livers to isolate plasma membranes. In each step the supernatant was discarded and the pellet diluted in homogenizing solution (1 mM $NaHCO_3$, 1 mM $CaCl_2$, pH 7.5, 4°C) to half the previous volume.

TABLE 1

EFFECT OF DIETARY SUPPLEMENTATION WITH ETHANOL ON SOLID AND LIQUID DAILY INTAKE

| Percent of Ethanol in Water | Liquid Consumption (ml/day/mouse) Control | Treated | $p$ | Solid Consumption (g/day/mouse) Control | Treated | $p$ |
|---|---|---|---|---|---|---|
| 2.5% | 4.2 ± 0.4 (5) | 3.5 ± 0.3 (5) | N.S. | 3.9 ± 0.3 (3) | 4.0 ± 0.4 (4) | N.S. |
| 5.0% | 4.7 ± 0.4 (5) | 4.0 ± 0.2 (5) | N.S. | 4.8 ± 0.4 (4) | 4.6 ± 0.4 (4) | N.S. |
| 7.5% | 5.1 ± 0.2 (5) | 4.3 ± 0.1 (5) | $p<0.01$ | 4.5 ± 0.2 (4) | 4.1 ± 0.3 (4) | N.S. |
| 10.0% | 5.1 ± 0.1 (5) | 3.9 ± 0.1 (5) | $p<0.001$ | 4.3 ± 0.2 (3) | 3.8 ± 0.3 (3) | N.S. |
| 12.5% | 4.8 ± 0.1 (5) | 3.8 ± 0.1 (5) | $p<0.001$ | 4.6 ± 0.2 (3) | 3.6 ± 0.3 (3) | N.S. |
| 15.0% | 5.2 ± 0.2 (5) | 3.6 ± 0.1 (5) | $p<0.001$ | 4.2 ± 0.2 (4) | 3.9 ± 0.3 (4) | N.S. |
| 17.5% | 5.2 ± 0.5 (5) | 3.4 ± 0.1 (5) | $p<0.01$ | 4.4 ± 0.7 (4) | 3.5 ± 0.2 (4) | N.S. |

The water of the controls was ethanol-free. The values represent the mean of the number of experiments indicated between brackets, each one including 6 mice ± S.E.M. (standard error mean).
N.S. = not significant.

poration into lipidic endogenous acceptors of purified plasma membranes contained, unless otherwise indicated, the following components: 0.8 mg of plasma membrane preparation per one ml of incubation mixture; 5 mM $MgCl_2$; 4 mM NaF; 10 mM Tris-HCl pH 7.4; 8.0 μM UDP-[$^{14}$C]-glucose. Incubation mixtures used for the study of [$^{14}$C]-galactose incorporation into lipidic endogenous acceptors of purified plasma membrane contained, unless otherwise indicated, the following components: 0.8 mg of plasma membrane preparation per one ml of incubation mixture; 5 mM $MnCl_2$; 4 mM NaF; 10 mM Tris-HCl pH 7.4; 8.0 μM UDP-[$^{14}$C]-galactose. When dol-P (17 μM) and/or lipidic effectors (380 μM) were used for glycosylation reaction they were evaporated under a stream of nitrogen; Tris buffer solution was added and the lipids were sonically dispersed in an ultrasonic water bath (Bransonic bath-type sonicator 220) until the solution was clear or slighty opalescent by the procedure of Mitranic *et al.* (19). Incubations were performed at 37°C and stopped by the addition of 10 vol. of chloroform/methanol (2:1, v/v). The suspension was mixed vigorously, kept at room temperature for at least 10 min, and centrifuged after addition of 3 vol. of water; then the chloroform/methanol extract was collected as previously described (6). Radioactivity was measured in a Tri-Carb Packard liquid scintillation counting system using Packard Emulsifier Scintillator 299™ as the liquid scintillation counting mixture. Enzymatic activity was expressed as picomol of [$^{14}$C]-glucose or [$^{14}$C]-galactose incorporated into lipidic endogenous acceptors per mg of protein. Thin-layer chromatography (TLC) of the glycosylated lipidic products was performed on silica gel 60F-254 plates which were developed three times in chloroform/methanol/ammonium hydroxide/water (70:44:5:5, v/v/v/v), and once in chloroform/methanol/water (10:10:3, v/v/v). The radioactivity on the plates was located by liquid scintillation counting or by scanner (Packard Scanner 2022).

The statistical analyses for comparisons between control and treatment results were carried out using Student's $t$-test. Statistical significance was defined as $p<0.05$.

## RESULTS

The chronic ethanol administration had no effect, or caused a slight decrease in relative liver weight. Table 1 shows the consumption of drink and food by treated and control animals during the treatment process. Dietary supplementation with ethanol caused the animals to eat less; although the differences between control and treated mice were not statistically significant. Moreover, significant reductions of liquid intake were observed in the ethanol-treated mice (down to 75 to 80% of that of the control mice), which were exacerbated when the concentration of ethanol in the water was raised. These reductions are statistically significant up to 7.5% ($p<0.01$ or $p<0.001$). Drinking water supplementation with ethanol (15%) gave a mean daily intake of 28.12 g/kg body weight.

After treatment with ethanol, the isolation of plasma membranes was carried out as described in the Method section for both treated and control animals. After the fractionation process the homogenate and supernatants of the differential centrifugations obtained from ethanol-treated mice were markedly opalescent and milky. Table 2 shows the percentage of enzymatic markers recovered in the different fractions for the isolation and purification of plasma membranes. As this table shows, alkaline p-nitrophenyl phosphatase activity from ethanol-treated mouse plasma membrane was increased by 40% as compared with the value obtained from control mice. On the other hand, the rates of monoamine oxidase activity recovered in purified plasma membranes were not significantly affected by the ethanol treatment. These results indicated that the ethanol treatment did not increase the contamination of isolated plasma membranes but rather pro-

TABLE 2

EFFECT OF CHRONIC ETHANOL TREATMENT ON THE DETERMINATION OF ENZYMATIC MARKERS DURING THE PREPARATION AND PURIFICATION OF PLASMA MEMBRANE

| Fractions | Activity of Enzymatic Markers: Monoamine Oxidase (EC 1.4.3.4.) Ethanol | Control | Alkaline p-Nitrophenyl Phosphatase (EC 3.1.3.1.) Ethanol | Control |
|---|---|---|---|---|
| Homogenate | 100.0 | 100.0 | 100.0 | 100.0 |
| Pellet I | 49.0 | 48.5 | 44.5 | 38.0 |
| Pellet II | 18.7 | 20.0 | 35.0 | 33.6 |
| Pellet III | 6.9 | 9.9 | 33.0 | 30.0 |
| Purified membrane | 0.0 | 0.0 | 11.9 | 8.5 |

Enzymatic determinations were carried out as described in the Method section. The results are expressed as a percentage of activity with regard to total homogenate activity, and they are the means of 5 alcohol treatments.

TABLE 3

GALACTOSYL- AND GLUCOSYLTRANSFERASE ACTIVITIES IN PLASMA MEMBRANE DERIVED FROM THE LIVERS OF BOTH CHRONICALLY ETHANOL-TREATED AND UNTREATED MICE

| | Without Exogenous Addition | | + Dolichylphosphate | | + Dolichylphosphate + Phosphatidyl-Choline | |
|---|---|---|---|---|---|---|
| Enzymatic Activity | Control | Ethanol | Control | Ethanol | Control | Ethanol |
| Galactosyltransferase activity | 6.15 | 4.51 | 40.75 | 13.95 | 74.73 | 33.36 |
| Glucosyltransferase activity | 40.50 | 40.25 | 227.50 | 248.50 | 345.00 | 348.75 |

Enzymatic determinations were carried out at 37°C as described in the Method section; incubation times were 8 min for galactosyltransferase assays and 5 min for glucosyltransferase assays. When indicated, 17 μM dolichylphosphate and 380 μM phosphatidyl-choline were added to the incubation mixture. Results (means of 4 determinations) are expressed as picomol of sugar incorporated into endogenous lipidic acceptors per mg of protein of plasma membrane.

voked the recovery of a high amount of plasma membrane marker activity. The increase of alkaline p-nitrophenyl phosphate activity was due to an increased specific activity (per mg of membrane protein) of the enzyme (data not shown).

*Glycosyltransferase Activities in Plasma Membranes From Control and Ethanol-Treated Mice*

After the incubation of control plasma membranes with UDP-galactose or UDP-glucose, a low but significant incorporation of galactose or glucose occurred in lipidic endogenous acceptors (Table 3). There was a substantial increase in these two glycosyltransferase activities whenever exogenous dolichyl monophosphate was added to the incubation mixture. The incubation of plasma membranes with phosphatidyl-choline liposomes loaded with dolichylphosphate greatly enhanced the incorporation of glucose or galactose into endogenous lipidic acceptors. Under these conditions, the level of activity of glucosyltransferase was very high. When plasma membranes were derived from treated mouse liver the galactosyltransferase was markedly affected, but there was no significant change in glucosyltransferase activity. The thin-layer chromatography of these biosynthesized products (extracted using chloroform/methanol mixture) in different solvent systems showed that the exogenous dolichyl monophosphate was not the acceptor of glucose or galactose in the plasma membranes isolated from both ethanol-fed mice and water-fed control mice. Figure 2 shows the kinetic study of transferring galactose and glucose into lipidic endogenous acceptors. The plasma membranes of control and alcohol-treated animals were preincubated with the phosphatidyl-choline liposomes loaded with dolichyl monophosphate. Here galactosyltransferase activity increased with respect to time for up to 5 minutes, but this activity is significantly lower in treated mice. Alcohol seemed to be responsible for its influence on this enzymatic activity (Fig. 2a). In the case of glucosyltransferase activity the enzyme from the livers of treated mice had the same activity in the first minutes of incubation with UDP-glucose as that obtained from control animals, but after 5 min of incubation its activity diminished, and decreased progressively in time (Fig. 2b). It is important to underline that the sole addition of dolichylphosphate to the assay mixture markedly affected the glycosyltransferase activities, bringing about activities 4 times greater (Table 3). In order to throw light on this last point, we studied the effect of dolichylphosphate addition at different concentrations as illustrated in Fig. 3; the addition of dolichylphosphate alone caused a pronounced increase in lipidic glycosylation. Galactosyltransferase activity (Fig. 3a) reached a plateau when 50 μM of dolichylphosphate was added to the assay mixture. The enzyme taken from treated animals was also affected, but less so and when dolichylphosphate reached 50 μM enzyme activity fell to 35% of that of the controls. Figure 3b shows that the glucosyltransferase activities of control and treated animals gradually increased when the amount of dolichylphosphate added to the assay mixture was also increased. Below 50 μM of dolichylphosphate the glucosyltransferase of treated animals did not differ significantly from that of control animals, but when the concentration of dolichylphosphate in the assay mixture rose above 50 μM, the activity level of the glucosyltransferase of treated mice was slightly but meaningfully higher than in that of the controls.

*Modulation of Galactosyltransferase Activity by Different Lipidic Effectors: Effect of Ethanol Treatment*

Several phospholipids (all tested at the concentration of 380 μM) were compared in their ability to modulate galactosyltransferase. These compounds differed from each other in the identity of the polar group. They were preincubated with plasma membrane galactosyltransferase in the presence of dolichylphosphate. The data comparing the relative abilities of these different phospholipids to stimulate enzymatic activity are summarized in Table 4. Phosphatidyl-inositol and phosphatidic acid had a very strong incentive effect in the case of control galactosyltransferase. Table 4 also shows that the enzyme included in the plasma membranes derived from the livers of treated mice was stimulated by the different phospholipids used; this stimulation was quite the same, whatever the phospholipid used, though it was slightly higher in the presence of phosphatidic acid. It should be stressed that this modulation was less efficient than that observed with control animals. The data obtained for all vesicles of phospholipid + Dol-P were significantly different in control and treated mice ($p<0.001$). This result suggested that ethanol treatment may inhibit the enzymatic activity and modify the membrane's structure.

Cholesterol was added with phosphatidyl-choline which had been evaporated and sonically dispersed in the buffer as described in the Method section. The addition of cholesterol to phosphatidyl-choline vesicles did not bring about significant modifications of the galactosyltransferase activity of the plasma membranes from control or treated mice (Table 4). When equimolar amounts of

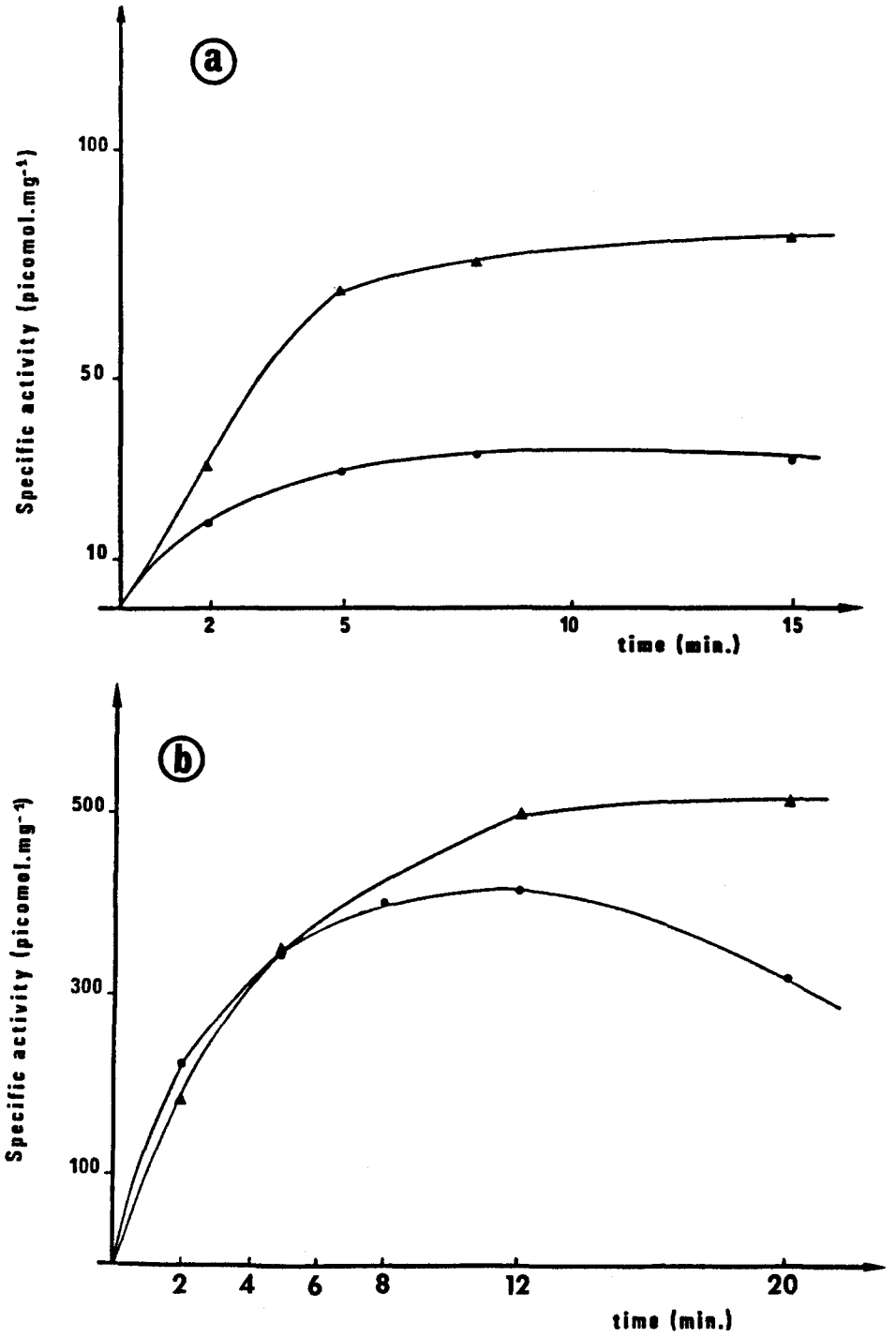


FIG. 2. Kinetic study of glycosyltransferase activities. Assays were performed as described in the Method section. The reaction mixture contained 17 μM dolichylphosphate and 380 μM phosphatidyl-choline. The results are expressed in picomole of galactose (a) or picomole of glucose (b) incorporated into endogenous lipidic acceptors per mg of plasma membrane protein. ●: Ethanol-treated mice; ▲: control mice.

cholesterol and dolichylphosphate were added to phosphatidyl-choline vesicles, the activity of the enzyme (from control and treated mice) increased substantially over the level observed in the absence of dolichylphosphate. It is important to underline that, in this case, ethanol treatment provoked an inhibition of the enzymatic activity. The fact that cholesterol, in the presence of dolichylphosphate and phosphatidyl-choline, affected differently and significantly the level of enzymatic activity whether the plasma membranes came from control or treated mice, is of particular interest. Indeed, cholesterol inhibited the controls'

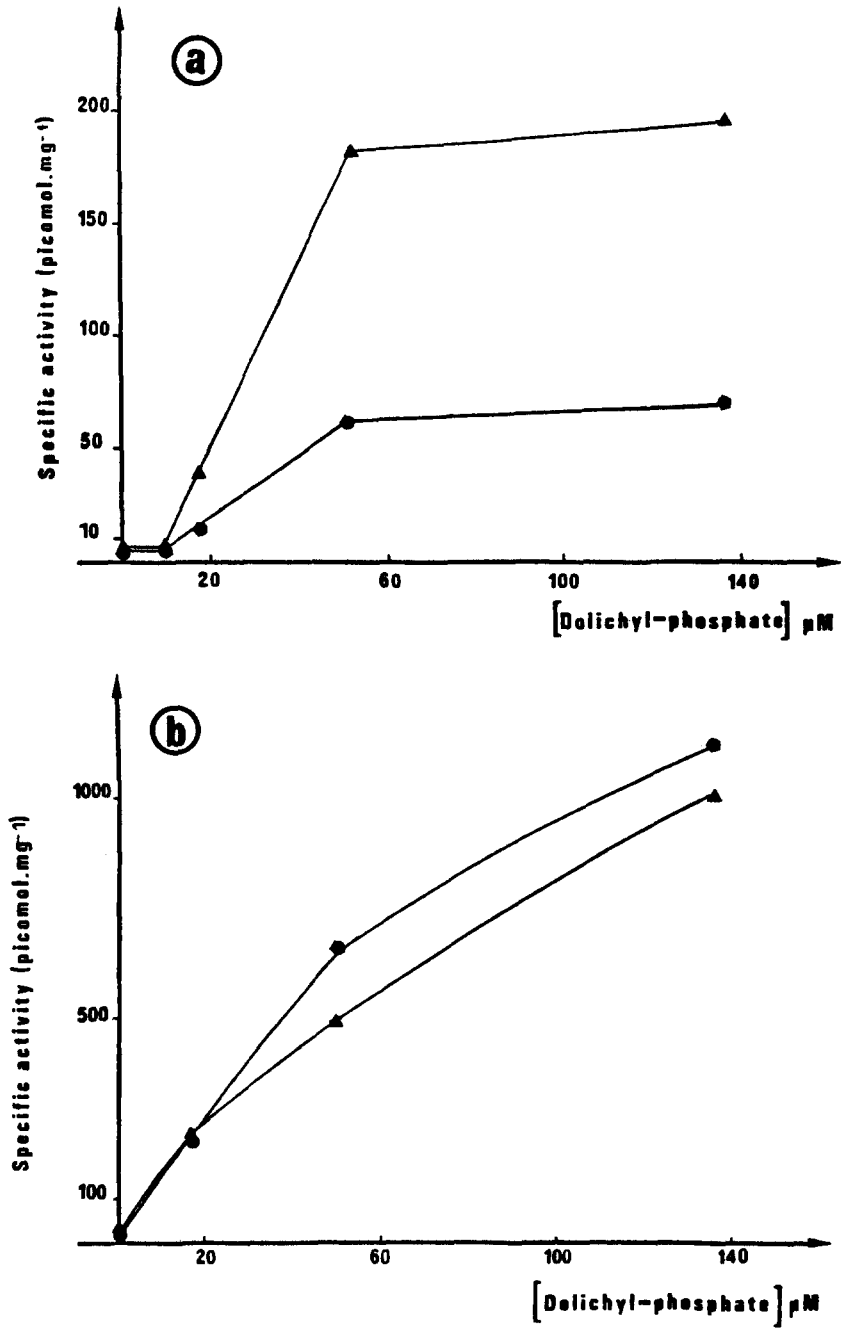


FIG. 3. Effect of dolichylphosphate concentrations on glycosyltransferase activities. Enzymatic determinations were carried out as described in the Method section. Incubations were performed for 8 min for galactosyltransferase and 5 min for glucosyltransferase assays. Results are expressed as picomole of galactose (a) or picomole of glucose (b) incorporated into endogenous lipidic acceptors per mg of plasma membrane protein. ●: Ethanol-treated mice; ▲: control mice.

membrane galactosyltransferase activity (80% of the level found without cholesterol). On the contrary, the addition of cholesterol increased the specific activity of treated membrane enzyme to 33%.

*Effects of Ethanol on Glucosyltransferase Activity After a Pretreatment of the Membrane With Different Liposomes*

Table 5 shows that the glucosyltransferase of control animals may be modulated by different phospholipids, as we reported in an earlier publication (5). Neutral lipids, such as phosphatidylcholine, stimulated enzymatic activity, but the activation was more efficient when the plasma membrane was preincubated with vesicles constituted by phosphatidic acid or phosphatidyl-inositol. The glucosyltransferase of plasma membranes prepared from the livers of treated mice was also modulated by phospholipids. This enzyme showed a preferential affinity for phosphatidic acid. The

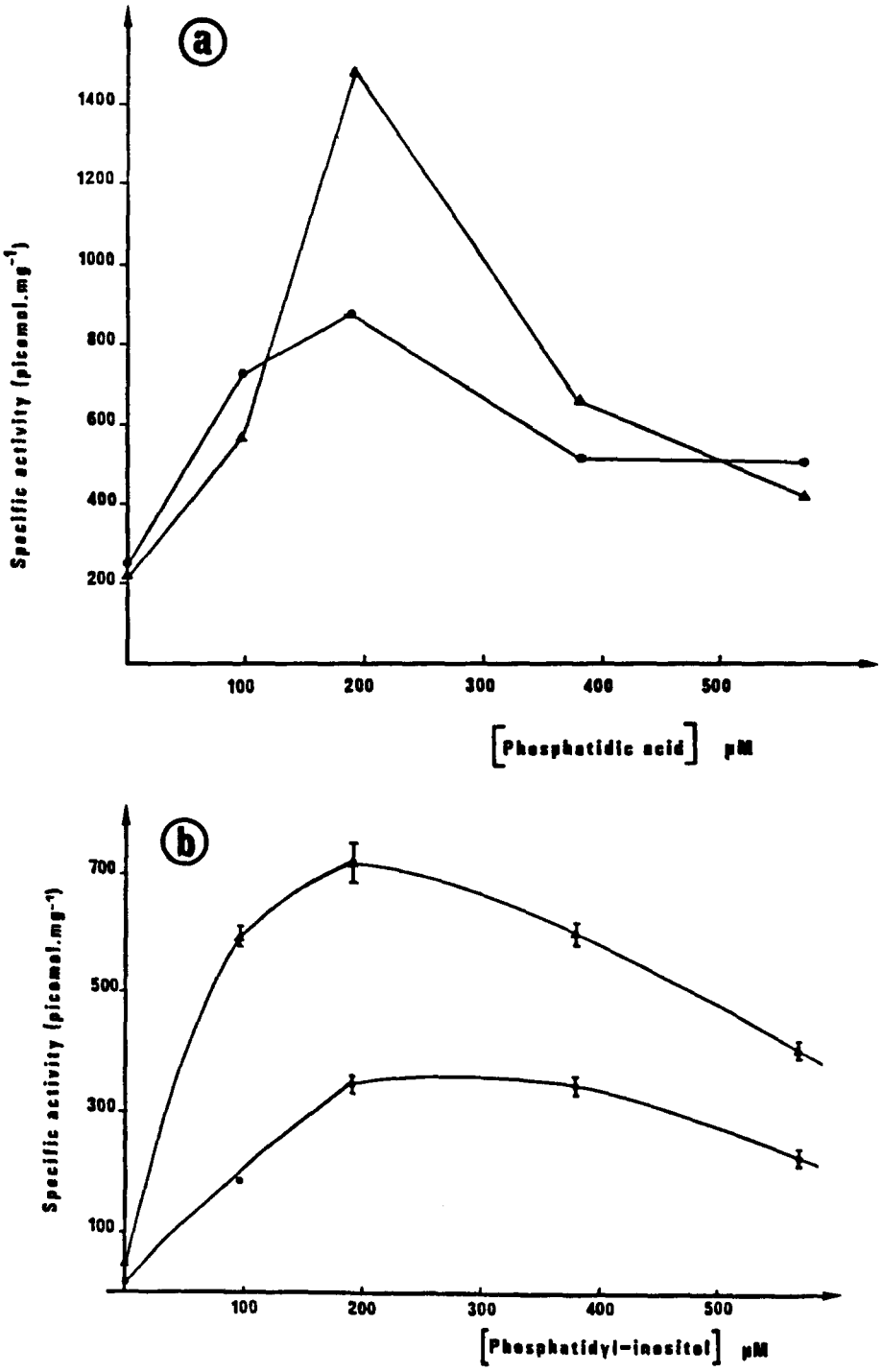


FIG. 4. Effect of varying phosphatidic acid (a) and phosphatidyl-inositol (b) concentrations on glucosyltransferase activities. The reaction mixture contained 17 µM dolichylphosphate. Assays were performed for 5 min as described in the Method section. ▲: Control mice; ●: ethanol-treated mice.

effect of ethanol consumption on the glucosyltransferase activity occurred because of the nature of the phospholipid used. Its inhibitory effect was very strong and statistically significant when the plasma membranes were incubated with phosphatidyl-inositol or phosphatidic acid vesicles. In order to elucidate this effect induced by ethanol, we studied the modulation of phosphatidic acid and phosphatidyl-inositol at different concentrations comparatively on the control and ethanol-treated mice glucosyltransferases. The results reported in Fig. 4a show the effect of increasing concentrations of the two phospholipids on glucosyltransferase activities. It clearly appeared that the enzyme activity of the controls was very strongly increased by using phosphatidic acid at concentrations between 100 and 400 µM. The glucosyltransferase of ethanol-treated animals was also increased, but to a

TABLE 4

COMPARATIVE EFFECTS OF VARIOUS PHOSPHOLIPIDS, CHOLESTEROL AND/OR DOLICHYLPHOSPHATE ON GALACTOSYLTRANSFERASE ACTIVITIES IN LIVER PLASMA MEMBRANE FROM TREATED AND CONTROL MICE

| Exogenous Addition | Galactosyltransferse Activity Control Mice | Treated Mice | $p$ |
|---|---|---|---|
| None | 6.2 ± 0.1 (4) | 4.5 ± 0.4 (4) | $p<0.01$ |
| Dol-P | 40.8 ± 1.5 (3) | 14.0 ± 0.4 (3) | $p<0.001$ |
| PC | 7.5 ± 0.3 (3) | 6.8 ± 0.3 (3) | N.S. |
| PC + Dol-P | 74.7 ± 0.5 (4) | 33.4 ± 1.4 (6) | $p<0.001$ |
| PI + Dol-P | 82.0 ± 7.4 (4) | 35.0 ± 1.8 (4) | $p<0.001$ |
| PS + Dol-P | 56.4 ± 3.0 (4) | 37.6 ± 0.8 (4) | $p<0.001$ |
| PA + Dol-P | 159.0 ± 4.8 (4) | 48.8 ± 2.1 (4) | $p<0.001$ |
| PE + Dol-P | 64.5 ± 5.6 (4) | 28.5 ± 4.2 (4) | $p<0.001$ |
| PC + Chol | 3.8 ± 0.1 (3) | 3.6 ± 0.4 (3) | N.S. |
| PC + Chol + Dol-P | 60.6 ± 0.3 (3) | 44.5 ± 0.7 (3) | $p<0.001$ |

Assays were performed as described in the Method section for 8 min at 37°C. When indicated, 17 μM dolichylphosphate, 17 μM cholesterol and 380 μM phospholipid were added to the incubation mixture.

Results are expressed as picomol/mg plasma membrane protein, and are mean of the number of experiments in brackets ± S.E.M. (standard error mean).

N.S. = not significant.

TABLE 5

COMPARATIVE EFFECTS OF VARIOUS PHOSPHOLIPIDS, CHOLESTEROL AND/OR DOLICHYLPHOSPHATE ON GLUCOSYLTRANSFERASE ACTIVITIES IN LIVER PLASMA MEMBRANES FROM TREATED AND CONTROL MICE

| Exogenous Addition | Glucosyltransferase Activity Control Mice | Treated Mice | $p$ |
|---|---|---|---|
| None | 40.5 ± 2.7 (4) | 40.2 ± 1.5 (4) | N.S. |
| Dol-P | 227.5 ± 4.4 (4) | 248.5 ± 9.0 (4) | N.S. |
| PC + Dol-P | 345.0 ± 12.6 (5) | 348.8 ± 13.9 (4) | N.S. |
| PE + Dol-P | 123.3 ± 17.4 (3) | 150.0 ± 7.5 (3) | N.S. |
| PA + Dol-P | 641.0 ± 24.8 (5) | 546.2 ± 21.0 (4) | $p=0.01$ |
| PS + Dol-P | 469.0 ± 2.3 (4) | 414.0 ± 7.7 (4) | $p<0.001$ |
| PI + Dol-P | 603.8 ± 17.6 (6) | 343.4 ± 29.6 (5) | $p<0.001$ |
| Sphingo + Dol-P | 581.7 ± 7.7 (3) | 433.3 ± 6.8 (3) | $p<0.001$ |
| Dol-P + Chol | 363.0 ± 7.0 (2) | 286.5 ± 4.5 (2) | N.S. |
| PC + Dol-P + Chol | 397.7 ± 24.3 (3) | 380.3 ± 5.8 (3) | N.S. |
| PI + Dol-P + Chol | 602.3 ± 9.0 (3) | 426.3 ± 14.5 (3) | $p<0.001$ |
| PA + Dol-P + Chol | 1605.7 ± 26.0 (3) | 591.0 ± 18.0 (3) | $p<0.001$ |

Assays were performed as described in the Method section for 5 min at 37°C. When indicated, 17 μM dolichylphosphate, 17 μM cholesterol and 380 μM phospholipid were added to the incubation mixture.

Results are expressed as picomol/mg plasma membrane protein, they are the mean of the number of experiments in brackets ± S.E.M. (standard error mean).

N.S. = not significant.

lesser extent. The inhibition effect of ethanol was strongest when phosphatidic acid was used at 200 μM. Figure 4b shows that the incentive effect of phosphatidyl-inositol depended on the amount of phospholipid added into the vesicles and reached its peak when the concentration reached 200 μM. The glucosyltransferase of treated plasma membranes was also enhanced. The inhibitory effect of the ethanol intoxication occurred at all concentrations used, but it was maximal at 200 μM of phosphatidyl-inositol.

When equimolar amounts of cholesterol and dolichylphosphate were added to the assay mixture, the glucosyltransferase activity of the control mice was greatly enhanced as is shown in Table 5. Considering the first data with cholesterol, and in view of these findings on the phosphatidyl-inositol and phosphatidic acid modulations, it seemed interesting to study the changes in the glucosyltransferase activity when phosphatidic acid and phosphatidyl-inositol vesicles were loaded with cholesterol and dolichylphosphate. The enzyme activity in control animals still increased when cholesterol and dolichylphosphate were mixed with only phosphatidic acid; there was no change with phosphatidyl-inositol vesicles. But in the presence of phosphatidyl-choline the activation obtained by cholesterol diminished. It is also noteworthy that all vesicles of mixed lipid composition stimulated the glucosyltransferase activity of treated mice, but to a lesser extent, whatever the composition of these mixed lipid vesicles was. The only significant differences between control and ethanol-treated glucosyltransferase ($p<0.001$) are obtained in the presence of phosphatidic acid or phosphatidyl-inositol vesicles. The results show that ethanol treatment provoked an inhibition of enzymatic activity, its peak occurring when phosphatidic acid vesicles were used.

## DISCUSSION

Chronic ethanol consumption originates considerable metabolic disorders as well as changes affecting the ultrastructure of hepatic plasma membranes. In the present study, the effect of a 4-week chronic ethanol consumption on the liver plasma membrane isolation together with the alterations of the glycosylation process in this fraction are analyzed.

Following chronic alcohol consumption, liver plasma membrane alkaline p-nitrophenyl phosphatase activity was increased as a result of a greater specific activity. However, recent studies by Nishimura and Teschke (20) showed that similar intoxication has no effect on the alkaline phosphatase activity. It is important to underline that contradictory results are obtained for different plasma-membrane-bound enzymes after acute or chronic ethanol ingestion (2, 7, 10, 22). The different results obtained by several authors in chronic-ethanol-fed animals could be explained by the distinct ethanol intake achieved. In this study, we found that the daily dose of ethanol ingested was 28 g of alcohol/kg body weight (water + 15% of ethanol). In similar conditions, González-Calvin *et al.* (7) found a daily dose of ingested ethanol of 15 g/kg; these doses are considered sufficient to find fat infiltrated livers.

The data presented in this study demonstrate the effect of chronic ethanol ingestion on enzymatic activities of two highly important glycosyltransferases. To our knowledge, this is the first report describing the localization of lipidic-UDP-galactosyltransferase in plasma membranes. In a previous study, we showed that UDP-glucosyltransferase transferring glucose into lipidic endogenous acceptors was present in liver plasma membranes. These enzymatic activities were low (particularly the galactosyltransferase activity), when they came from plasma membranes which had just been prepared. A highly noteworthy point is that when dolichyl monophosphate is added to the incubation mixture, the enzymatic activities are greatly enhanced. Considering these data, it should be born in mind that dolichyl monophosphate is neither the acceptor of galactose nor that of glucose in these two glycosylation systems. It is well known that dolichol exists in liver plasma membranes as a free dolichol, dolichol phosphate or fatty acyl dolichyl ester (26). Exogenous dolichols can have profound

effects on the structure of a biological membrane. Schroeder (26) showed that dolichol increased the fluidity in synaptic plasma membranes.

It is well known that the membrane lipid composition and thus the physical properties of the membrane have a direct influence on membrane-bound enzyme activity (17). It is so well established that enzymes, and particularly different glycosyltransferases, are influenced by phospholipids (12,23). Our report provides evidence that these two plasma membrane glycosyltransferases are modulated by several phospholipids. According to Mitranic's investigations (19) the galactosyltransferase activity of bovine milk was stimulated by egg-phosphatidyl-choline, but contrary to our results, he found that egg-phosphatidic acid strongly inhibited enzymatic activity. Other acidic lipids such as phosphatidyl-serine and phosphatidyl-inositol inhibited this activity, but they were much less efficient than phosphatidic acid. By examining the lipids which are effective in vitro, one may arrive at some understanding of how glycosyltransferases are functioning, but the most obvious conclusion arrived at from the study of phospholipid activation is that there is no specific chemical structure of the lipid which can be associated with transferase stimulation. A wide range of lipids (of differing structures and differing head groups) serve as successful activators. Nevertheless, it should be stressed that phosphatidic acid is the most efficient activator to stimulate both activities. Phosphatidyl-inositol is also able to greatly enhance the level of these activities.

Among the many effects produced by ethanol, it has been suggested that membrane glycoconjugates may act as potential mediators of these effects of alcohol. The changes wrought by ethanol in the membrane may affect the function of the glycoconjugates as surface receptors and the associated messenger activities, and also the synthesis and degradation of the macromolecules themselves (16). Therefore, we must stress that the effect of phospholipid addition on glycosylation processes depends on whether the enzymes are from the livers of control mice or treated ones. For galactosyltransferase the activation by phospholipids seems to be the same, whatever the phospholipid used. This modulation is weaker than those observed in the control animals. Nevertheless, we must point out that the inhibition by ethanol treatment is strong and significant when phosphatidic acid is used. It should be noted especially that phosphatidic acid does not provoke the same increase in galactosyltransferase activity when the enzyme is from a control animal as when it is from a treated one. Ethanol inhibition, therefore, seems very strong in this case. The decrease in galactosyltransferase activities after chronic ingestion is the result of a diminished specific activity (per mg of membrane protein) of the enzyme, since no significant changes occurred in the yield of plasma membrane proteins or in the recovery of any plasma membrane marker enzyme activity.

In the case of glucosyltransferase activities, and at the phospholipid concentration used, ethanol provoked significant differences in the activity modulation by phosphatidic acid, phosphatidyl-inositol, phosphatidyl-serine and sphingomyeline. Thus, in order to elucidate the effect of ethanol, two phospholipids are retained for a further study: phosphatidic acid and phosphatidyl-inositol. So, it clearly appears that the effect of ethanol depends on the concentration of phospholipid. When the concentration used was 200 μM, a strong inhibition occurred.

This differential modulation by phospholipids of glycosyltransferase activities brought about by ethanol becomes interesting when it is considered that phospholipids cause membrane tolerance to ethanol (14). On these lines it has been suggested that specific anionic phospholipid classes are involved in adaptation to chronic ethanol consumption: phosphatidyl-inositol in microsomal membranes and cardiolipin in mitochondrial membranes (14). Furthermore, it has been shown that ethanol, in common with calcium-mobilizing hormones, activates hormone-sensitive phosphoinositide-specific phospholipase C, linking the phosphoinositide-linked signalling system with the effects of ethanol (13,15).

Our results provide evidence that ethanol does not have the same inhibitory effect on glucosyl- and galactosyltransferases. As reported by Gordon (8), benzylalcohol also profoundly affects the activities of a number of intrinsic liver plasma membrane enzymes. $Mg^{2+}$-ATPase is particularly inhibited in both the membrane-bound and soluble states. Our results would appear to be consistent with these published findings. In the light of these first data, alcohol may be considered to affect glycosyltransferase activities.

The last point to elucidate is the simultaneous effect of lipids and cholesterol. Cholesterol has been shown to be involved in the modulation of a variety of plasma membrane functions (30): Cholesterol has a special ordering effect on the membrane and this molecular ordering may affect the activity of membrane-bound enzymes. An increase in phospholipids and cholesterol may cause a rigidity which can compensate for the disordering effect of ethanol.

The present study demonstrates that chronic ethanol consumption has profound effects on the activities of two plasma-membrane-bound glycosyltransferases. It is very difficult to discriminate between the direct action of alcohol on enzymatic protein and the effects mediated by an increase in bilayer fluidity. As reported by Curran (3), chronic ethanol consumption alters the lipid structure of the membrane. Since glycosyltransferases are embedded in the plasma membranes, their activities may be altered indirectly by the interaction of ethanol with lipid portions of the membrane, thus disturbing the microenvironment in which these two enzymes are located. This fact could explain the different effect of ethanol on these two glycosyltransferases, but our results are also in good agreement with a specific action of ethanol on the glycosylation systems.

ACKNOWLEDGEMENTS

This work was supported by the Institut National de la Santé et de la Recherche Médicale U. 189 and the University of Lyon (Lyon-Sud Medical School). A. Fernandez-Briera was a post-doctoral researcher. She was supported by I.N.S.E.R.M. (International Relation Service).

## REFERENCES

1. Beauge, F.; Gleuret, C.; Barin, F.; Nordmann, R. Brain membrane disordering by alcohols: comparative effects of acute administration of ethanol, isopropanol or t-butanol. Alcohol.: Clin. Exp. Res. 8:82(A); 1984.
2. Corazzi, L.; Arienti, G.; Porcellati, G. The effect of actue ethanol administration on the activity of membrane bound enzymes of rat liver. Pharmacol. Res. Commun. 12:739–749; 1980.
3. Currant, M.; Seeman, P. Alcohol tolerance in a cholinergic nerve terminal: relation to the membrane expansion-fluidization theory of ethanol action. Science 197:910–911; 1977.
4. Fernández-Briera, A.; Louisot, P.; Morélis, R. Purification of liver plasma membranes and localization of a protein galactosyltransferase. Arch. Int. Physiol. Biochim. 97:221–230; 1989.
5. Fernández-Briera, A.; Louisot, P.; Morélis, R. Characterization of a glucosyltransferase activity in liver plasma membrane: modulation by cations and lipidic effectors. Int. J. Biochem. 20:951–958; 1988.
6. Gateau, O.; Morélis, R.; Louisot, P. Biosynthèse de mannosyl-phosphoryl-dolichol dans la membrane externe des mitochondries. Eur. J. Biochem. 88:613–622; 1978.
7. González-Calvin, J. L.; Saunders, J. B.; Crossley, I. R.; Dickenson, C. J.; Smith, H. M.; Tredger, J. M.; Williams, R. Effects of ethanol administration on rat liver plasma membrane-bound enzymes. Bio-

chem. Pharmacol. 34:2685–2689; 1985.
8. Gordon, L. M.; Sauerheber, R. D.; Esgate, J. A.; Dipple, I.; Marchmont, R. J.; Houslay, M. D. The increase in bilayer fluidity of rat liver plasma membranes achieved by the local anesthetic benzyl alcohol affects the activity of intrinsic membrane enzymes. J. Biol. Chem. 255:4519–4527; 1980.
9. Gornall, A. G.; Bardawill, C. J.; David, H. M. Determination of serum proteins by means of Biuret reaction. J. Biol. Chem. 177: 751–766; 1949.
10. Guerri, C.; Grisolia, S. Chronic ethanol treatment affects synaptosomal membrane-bound enzymes. Pharmacol. Biochem. Behav. 18: 45–50; 1983.
11. Guerri, C.; Wallace, R.; Grisolia, S. The influence of prolonged ethanol intake on the levels and turnover of alcohol and aldehyde dehydrogenase and brain ($Na^+$,$K^+$)ATPase of rat. Eur. J. Biochem. 86:581–587; 1978.
12. Hochman, Y.; Zakim, O.; Vessey, D. A. A kinetic mechanism for modulation of the activity of microsomal UDP-glucuronyltransferase by phospholipids. Effects of lysophosphatidyl-cholines. J. Biol. Chem. 256:4783–4788; 1981.
13. Hoek, J. B. Rubin, R.; Thomas, A. P. Ethanol-induced phospholipase C activation is inhibited by phorbol esters in isolated hepatocytes. Biochem. J. 251:865–871; 1988.
14. Hoek, J. B.; Taraschi, T. F. Cellular adaptation to ethanol. Trends Biochem. Sci. 13:269–274; 1988.
15. Hoek, J. B.; Thomas, A. P.; Rubin, R.; Rubin, E. Ethanol-induced mobilization of calcium by activation of phosphoinositide-specific phospholipase C in intact hepatocytes. J. Biol. Chem. 262:682–691; 1987.
16. Klemm, W. R. Membrane glycoconjugates as potential mediators of alcohol effects. Prog. Neuropsychopharmacol. Biol. Psychiatry 11: 633–658; 1987.
17. Lin, D. C. Involvement of the lipid and protein components of ($Na^+$,$K^+$) ATPase in the inhibitory action of alcohol. Biochem. Pharmacol. 29:771–775; 1980.
18. Mitranic, M. M.; Boggs, J. M.; Moscarello, M. A. The effect of linoleic acid and benzyl-alcohol on the activity of glycosyltransferases of rat liver golgi membranes and some soluble glycosyltransferases. Biochim. Biophys. Acta 693:75–84; 1982.
19. Mitranic, M. M.; Boggs, J. M.; Moscarello, M. A. Modulation of bovine milk galactosyltransferase activity by lipids. J. Biol. Chem. 258:8630–8636; 1983.
20. Nishimura, M.; Teschke, R. Effect of chronic alcohol consumption on the activities of liver plasma membrane enzymes: gamma-glutamyl-transferase, alkaline phosphatase and 5′-nucleotidase. Biochem. Pharmacol. 31:377–381; 1982.
21. Polokoff, M. A.; Simon, T. J.; Harris, R. A.; Simon, F. R.; Iwahashi, M. Chronic ethanol increases liver plasma membrane fluidity. Biochemistry 24:3114–3120; 1985.
22. Rao, P. A. Kumari, C. L.; Sadasivudu, B. Acute and short term effects of ethanol on membrane enzymes in rat brain. Neurochem. Res. 10:1577–1585; 1985.
23. Ratnam, S.; Fraser, I. H.; Mookerjea, S. Effect of phosphatidyl-inositol and phosphatidyl-serine on membrane-bound galactosyltransferase. Can. J. Biochem. 58:58–66; 1980.
24. Ray, T. K. A modified method for the isolation of the plasma membrane from rat liver. Biochem. Biophys. Acta 196:1–9; 1970.
25. Reyes, E. The effects of alcohol on γ-glutamyl-transpeptidase: a membrane-bound enzyme. Proc. West. Pharmacol. Soc. 23:433–436; 1980.
26. Schroeder, F.; Gorka, C.; Williamson, L. S.; Wood, W. D. The influence of dolichols on fluidiy of mouse synaptic plasma membranes. Biochim. Biophys. Acta 902:385–393; 1987.
27. Seeman, P. The membrane action of anaesthetics and tranquilizers. Pharmacol. Rev. 24:583–655; 1972.
28. Tabor, C. W.; Tabor, H.; Rosenthal, S. M. Methods in enzymology. vol. II. New York: Academic Press; 1955:390–393.
29. Wood, W. G.; Schroeder, F. Membrane effects of ethanol: bulk lipid versus lipid domains. Life Sci. 43:467–475; 1988.
30. Yeagle, P. L. Cholesterol and the cell membrane. Biochim. Biophys. Acta 822:267–287; 1985.