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In vitro modification of cholesterol content of rat liver microsomes. Effects upon membrane 'fluidity' and activities of glucose-6-phosphatase and fatty acid desaturation systems

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The cholesterol content of rat liver microsomal membranes was modified in vitro by incubating microsomes and cytosol with liposomes prepared by sonication of microsomal lipids and cholesterol. In this way, the cholesterol to phospholipid molar ratio was increased from 0.11–0.13 in untreated microsomes to a maximal of 0.8 in treated ones. Cholesterol incorporation in microsomes produced an increase in the diphenylhexatriene steady-state fluorescence anisotropy and a decrease in the efficiency of pyrene-excimer formation which indicated a decrease in the rotational and translational mobility, respectively, of these probes in the membranes lipid phase. Cholesterol incorporation in microsomes did not affect significantly the glucose-6-phosphatase activity in 0.1% Triton X-100 totally disrupted microsomes, but diminished the glucose-6-phosphatase activity of 'intact' microsomes. This indicates that possibly the glucose 6-phosphate translocation accross the microsomal membrane is impeded by an increase in the membrane apparent 'microviscosity'. Cholesterol incorporation in microsomes decreased NADH-cytochrome *c* reductase without affecting NADH-ferricyanide reductase activity. The Δ^9 desaturation reaction rate was enhanced by cholesterol incorporation at low but not at high palmitic acid substrate concentration. Δ^5 and Δ^6 desaturase reaction-rates were increased both at low and high fatty acid substrate concentrations. These results suggest that a mechanism involving fatty acid desaturase enzymes, might exist to self-regulate the microsomal membrane lipid phase 'fluidity' in the rat liver.

Introduction

It has been largely suggested that the physical dynamic properties of membrane lipid phase can regulate several membrane associated biological functions [1] such as substrate transport [2–4] or enzyme activities [5–7]. In this respect, it is important to investigate the effect of physical properties of liver microsomal membrane upon glucose-6-phosphatase and fatty acid desaturation systems. The effect of microsomal membrane fluidity on the fatty acid desaturases is specially important since these enzymes may modify the degree of unsaturation of the lipid bilayer and the fatty acid unsaturation is one of the factors that modifies the fluidity of the membrane [8]. Therefore, fluidity-desaturase activities relationship could play the role of a membrane fluidity self-regulator.

In a previous report [9], we have shown that microsomal membrane fluidification by means of short-chain aliphatic alcohols incorporation increases glucose-6-phosphatase activity of intact

microsomes but has no effect on the detergent-disrupted membrane. The same agents also evoked an increase of the microsomal electron transport in the binary system NADH-cytochrome b_5 reductase, cytochrome b_5 but had no effect on the single enzyme NADH-cytochrome b_5 reductase and they decreased the Δ^6 and Δ^9 desaturation of fatty acids.

To get further evidence that the aforementioned modifications in the enzyme acitivities were evoked by fluidity changes of the microsomal bilayer we investigated in the present experiments the effects produced by a decrease in the membrane fluidity. It has been recognized that cholesterol has a condensing effect in model [10,11] and biological [3,12] membranes when they are in the liquid-crystalline state. Besides, it may be transferred from cholesterol-rich liposomes to cholesterol-deficient membranes [12–14]. Therefore, cholesterol was used in the present experiments as an antifluidizing agent and the 'in vitro' incorporation in microsomes provoked exactly the opposite effects on the glucose-6-phosphatase and fatty acid desaturation systems than short-chain alcohols.

Materials and Methods

Animals and diets. 2-months-old male Wistar rats were used. They were fed on a standard diet (Purina chow) and directly used in the experiments except for the fatty acid desaturation experiments. In these cases, animals were starved for 48 h and refed for 24 h on a pure casein diet for Δ^5 and Δ^6 saturation experiments and on a pure sucrose diet for Δ^9 desaturation experiments. This was made in order to increase the starting level of fatty acid desaturase activities to facilitate the determinations since some inactivation occurs during the treatment to incorporate cholesterol into the microsomal membrane.

Microsomal lipid separation and liposomes vesicles preparation. Microsomes were prepared as was elsewhere described [15] and total microsomal lipids were obtained by the Folch's procedure [16]. In order to obtain microsomal phospholipids free of cholesterol, total lipids in chloroform (0.5 mg/ml) were vigorously shaken in the presence of silicic acid (20 mg/mg of lipid) and filtered. After two washes, with chloroform (1 ml/mg of lipid) to eliminate the neutral lipids, phospholipids were eluted three times with 1 ml of methanol per mg of lipid and methanol eliminated in a rotatory evaporator. In this way the cholesterol/phospholipid molar ratio was lowered from 0.13 in the total lipid fraction to 0.0005 in the phospholipid fraction.

Liposomes (5 mM in phospholipid) were prepared in 0.25 M sucrose/1 mM EDTA (pH 7.0) (medium A) except for the fatty acid desaturation experiments. In this case a solution of 0.25 M sucrose/0.15 M KCl/5 mM $MgCl_2$/1.4 mM *N*-acetylcysteine/0.1 mM EDTA/62 mM phosphate buffer (pH 7.4) (medium B) was used. Cholesterol-rich liposomes were prepared mixing the appropriate amount of cholesterol with the appropriate amount of the total lipid extract (Tl-Chol. liposomes) or of the phospholipid fraction (Pl-Chol. liposomes) to give a cholesterol/phospholipid molar ratio of 1 : 1 or 1 : 2 and evaporating the solvent with a N_2 flush. Microsomal phospholipid (Pl) or total lipids (Tl) liposomes were prepared evaporating directly the appropriate amount of the corresponding lipid extract. Suitable volumes of buffer were then added to the lipid mixture and after 1 h to permit hydration they were sonicated in an Ultrasonic sonicator at maximal potency for 10 (Pl liposomes), 15 (Tl liposomes) or 20 (Tl-Chol. or Pl-Chol. liposomes) minutes under a N_2 atmosphere. In order to eliminate big multilamellar liposomes, they were centrifuged for 2 h at $100\,000 \times g$ and the precipitate discarded.

Modification of the microsomal cholesterol content The transference of cholesterol from cholesterol-rich liposomes to microsomal membranes has been accomplished experimentally by the use of lipid transferring proteins [17]. In these experiments the tedious purification of cholesterol transferring protein has been obviated by the direct use of the supernatant of $12\,000 \times g$ that contains both, microsomes and cytosolic transferring proteins. In these mild conditions and at the low temperature used, good cholesterol incorporation is obtained with relatively little damage to the enzyme tested.

Livers were homogenized in medium A or B (2 ml/g of liver) and centrifuged for 20 min at $12\,000 \times g$. A volume of $12\,000 \times g$ supernatant was in-

cubated for 2 h at 5–7°C with 8 volumes of the corresponding liposome preparation and then centrifuged for 1 h at 100000 × *g*. In some cases, the precipitate obtained was reincubated for another 2 h at 5–7°C with the same amount of liposomes preparation and cytosol and then recentrifuged. For analytical and fluorescence determinations pellets were washed twice with medium A. For enzymatic measurements microsomal pellets were resuspended in medium A or B without washing. Modified microsomes were used immediately when fatty acid desaturation was investigated. In other cases they were stored at −80°C for no longer than a week.

Fluorescence anisotropy and pyrene excimer formation measurements. The labeling of the microsomes with 1,6-diphenyl-1,3,5-hexatriene (Aldrich Chemical Co.) and with pyrene (Fluka) as well as steady-state fluorescence anisotropy (r_s) measurements and apparent 'microviscosity' calculation were described elsewhere [9]. The efficiency of pyrene excimer formation was evaluated by the ratio of the excimer to monomer fluorescence intensity (I_e/I_m) and measured as was indicated [9].

Enzymatic determinations. Δ^5, Δ^6 and Δ^9 desaturation reactions were measured by the conversion of the (1-^{14}C)-labeled fatty acid 20 : 3 ($n-6$), 18 : 2 ($n-6$) and 16 : 0, respectively, to the corresponding product by gas-liquid radiochromatography as was already described [18]. NADH-ferricyanide reductase and NADH-cytochrome *c* reductase determinations were already described [18]. Glucose-6-phosphatase and mannose-6-phosphohydrolase activity measurements, 0.1% Triton X-100 treatment and mannose-6-phosphohydrolase latency calculation were described elsewhere [9,15]. Glucose-6-phosphatase activity of 'intact' microsomes was calculated as indicated by Arion et al. [19] using the equation:

$$\text{G-6-Pase}_{\text{Aim}} = \frac{\text{G-6-Pase}_{\text{Aum}} - \left[\text{G-6-Pase}_{\text{Adm}}\left(\frac{\text{M-6-Pase}_{\text{Aum}}}{\text{M-6-Pase}_{\text{Adm}}}\right)\right]}{\left(1 - \frac{\text{M-6-Pase}_{\text{Aum}}}{\text{M-6-Pase}_{\text{Adm}}}\right)}$$

where G-6-Pase$_{\text{Aum}}$ is the glucose-6-phosphatase activity of non-detergent treated microsomes, G-6-Pase$_{\text{Adm}}$ is the glucose-6-phosphatase activity of 0.1% Triton X-100 totally disrupted microsomes, M-6-Pase$_{\text{Aum}}$ is the mannose-6-phosphohydrolase activity of non-detergent treated microsomes, M-6-Pase$_{\text{Adm}}$ is the mannose-6-phosphohydrolase activity of 0.1% Triton X-100 totally disrupted microsomes.

Thus, G-6-Pase$_{\text{Aim}}$ is the theoretical activity for the system if 100% of the intrinsic enzyme was housed in 'intact' microsomal vesicles.

Analytical determinations. Proteins were measured by the method of Lowry et al. [20]. Phospholipids and cholesterol were measured by the methods of Chen et al. [21] and Allain et al. [22], respectively, after extracting total lipids by the procedure of Folch et al. [16].

Results

Cholesterol effect on the microsomal membrane dynamic structure

In Fig. 1, the effect of cholesterol and microsomal proteins on the physical properties of the microsomal membrane lipid phase is graphically presented. The natural logarithm (ln) of the apparent microviscosity calculated from the diphenylhexatriene fluorescence anisotropy for complete microsomes, liposomes from sonicated lipids of microsomes and liposomes from sonicated phospholipids are presented as a function of the inverse of the absolute temperature. The bilayer containing both cholesterol and proteins showed higher apparent microviscosity than the phospholipid pure bilayer showing that both cholesterol and proteins evoke a condensing effect. While microsomal proteins elimination produced a stronger effect at high temperature, the cholesterol elimination resulted in a parallel line with the same effect in the total range of temperature tested. These parallel curves are typical of membranes differing only in the cholesterol content as was already shown in biological [12] and model [10] membranes.

Modificaton of the cholesterol content of rat liver microsomes

In a first intent to modify the microsomal cholesterol content, microsomes were incubated for 2 h at 5–7°C with Pl-Chol. liposomes. Under these conditions only a small modification of the cholesterol/phospholipid molar ratio was ob-

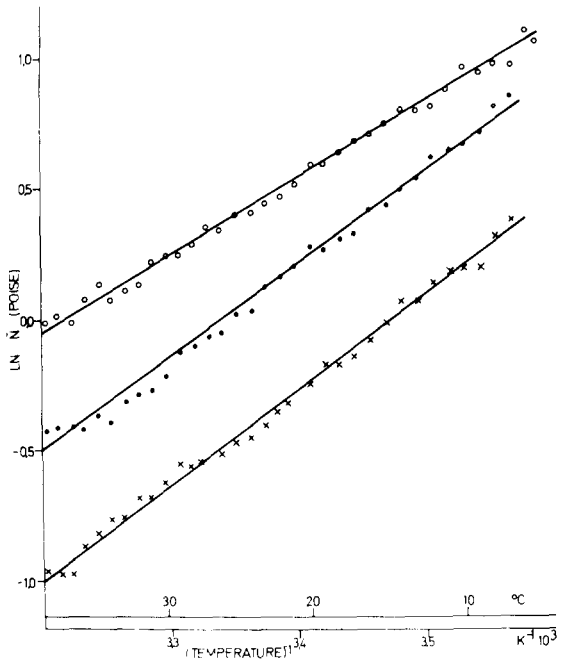

Fig. 1. Temperature dependence of apparent 'microviscosity' calculated from diphenylhexatriene fluorescence anisotropy data. ○———○, Complete microsomes 100 μg of protein/ml; ●———●, liposomes prepared from total microsomal lipids, 0.2 mg of lipid/ml; ×———×, liposomes prepared from microsomal phospholipids, 0.2 mg of lipid/ml. Samples were labeled with $2.5 \cdot 10^{-7}$ M of diphenylhexatriene.

tained (not shown data). However, when cytosol was present in the incubation media (by using the 12000 × *g* supernatant instead of the microsomal fraction) the increase in the cholesterol content of microsomes was evident (Table I). A higher efficiency in cholesterol enrichment was obtained when the microsomes were incubated with medium A in comparison with medium B. A second incubation of the so modified microsomes produced an additional cholesterol incorporation.

It is also observed in Table I that the phospholipid/protein ratio of microsomes is increased after the incubation with liposomes specially when total lipids (Tl) or Tl-Chol. liposomes and medium A were used. This might indicate that fusion or phospholipid transfer could occur in some extent. However, when phospholipid (Pl) liposomes were used the phospholipid/protein ratio increased without a corresponding decrease in the cholesterol/phospholipid ratio as would be expected when only phospholipid incorporation occurred in microsomes. Thus, it would seem that the increase in the phospholipid/protein ratio is at least in part due to a loss of microsomal proteins during the treatments. For this reason, microsomes incubated with Tl or Pl liposomes were used as 'control' microsomes in the experiments in which the effect of cholesterol on enzymatic activities was investigated.

Effect of cholesterol enrichment on the diphenylhexatriene fluorescence anisotropy and efficiency of pyrene excimer formation in the rat liver microsomal membrane

It can be observed in Fig. 2 that the diphenyl-

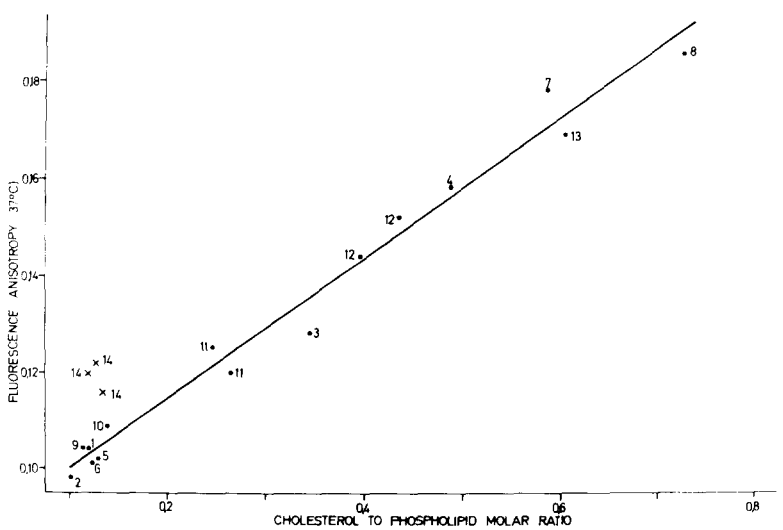

Fig. 2. Effect of cholesterol enrichment on the diphenylhexatriene steady-state fluorescence anisotropy in the rat liver microsomal membrane. Membranes (approx. 40 μg of lipid/ml) were labeled with $2.5 \cdot 10^{-7}$ M of diphenylhexatriene. Numbers indicate the treatment (see Table I). ●, Treated microsomes; ×, original microsomes.

TABLE I

MODIFICATION OF THE CHOLESTEROL CONTENT OF RAT LIVER MICROSOMES

The supernatant of liver homogenate 12000 × *g* for 20 min (approx. 40 mg of protein/ml corresponding to approx. 10 mg of microsomal protein/ml) was incubated for 2 h at 5–7°C with 8 volumes of the liposomal preparations (5 mM in phospholipid) in the indicated medium. The suspension was then centrifuged at 100000 × *g* for 1 h. In the single-incubation samples pellets were washed twice and centrifuged again in the same conditions and resuspended in the buffer. In the double-incubation the pellet was reincubated with the same volume of liposomal preparation and cytosol and then washed and centrifuged again. Pl, phospholipid; Tl, total lipids; Chol., cholesterol. For preparation of the liposomes see Materials and Methods.

Sample Number	Liposomes	Medium	Number of incubations	Phospholipid/protein (μmol/mg)	Cholesterol/phospholipid (mol/mol)
1	Pl	A	1	0.58	0.119
2	Pl	A	2	0.49	0.101
3	Pl-Chol. (1 : 1)	A	1	0.54	0.344
4	Pl-Chol. (1 : 1)	A	2	0.57	0.487
5	Tl	A	1	0.68	0.130
6	Tl	A	2	0.76	0.125
7	Tl-Chol. (1 : 1)	A	1	0.74	0.584
8	Tl-Chol. (1 : 1)	A	2	0.79	0.724
9	Tl	B	1	0.54	0.115
10	Tl	B	2	0.51	0.140
11	Tl-Chol. (2 : 1)	B	1	0.50	0.247
12	Tl-Chol. (1 : 1)	B	1	0.47	0.394
13	Tl-Chol. (1 : 1)	B	2	0.44	0.602
14	Original microsomes			0.40	0.120

hexatriene fluorescence anisotropy increases when the cholesterol content of microsomes is enhanced. When microsomes were treated with liposomes not enriched in cholesterol such as Pl and Tl liposomes, a decrease in diphenylhexatriene fluorescence anisotropy without modification of the cholesterol/phospholipid molar ratio was observed. This might be due to the enhanced phospholipid/protein ratio.

The effect of cholesterol incorporation in microsomes upon the efficiency of pyrene excimer formation is shown in Fig. 3. In Fig. 3A the I_e/I_m

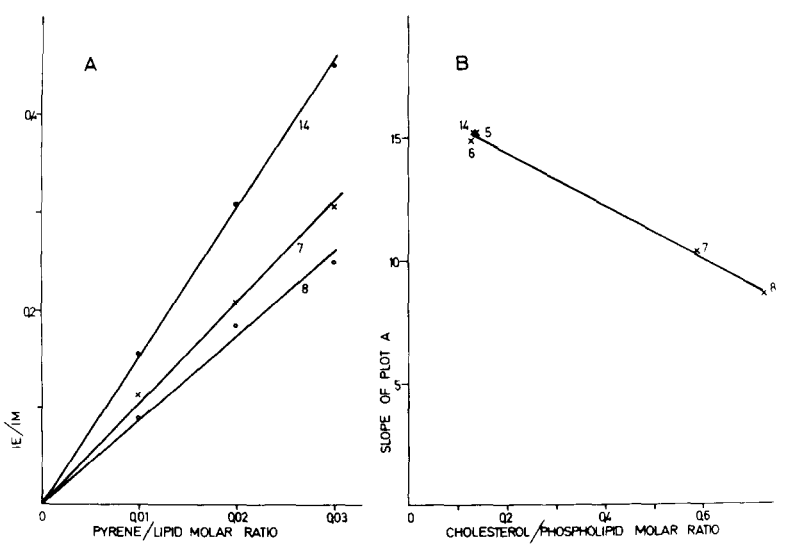

Fig. 3. Effect of cholesterol enrichment on the pyrene excimer formation in rat liver microsomes. Samples at a concentration of 0.2 mM in lipid (only cholesterol and phospholipids were considered) were labeled with the indicated pyrene concentration. I_e/I_m ratio was obtained from the ratio of fluorescence intensities at 472 nm and 392 nm and plotted versus the pyrene concentration (A). The slopes of I_e/I_m versus pyrene concentration curves were plotted versus the cholesterol phospholipid molar ratio (B). In A: ●———●, original microsomes (sample 14, see Table I); ×———×, cholesterol-enriched microsomes (sample 7, see Table I); ○———○, cholesterol-enriched microsomes (sample 8, see Table I). In B: Numbers indicate the sample treatment (see Table I).

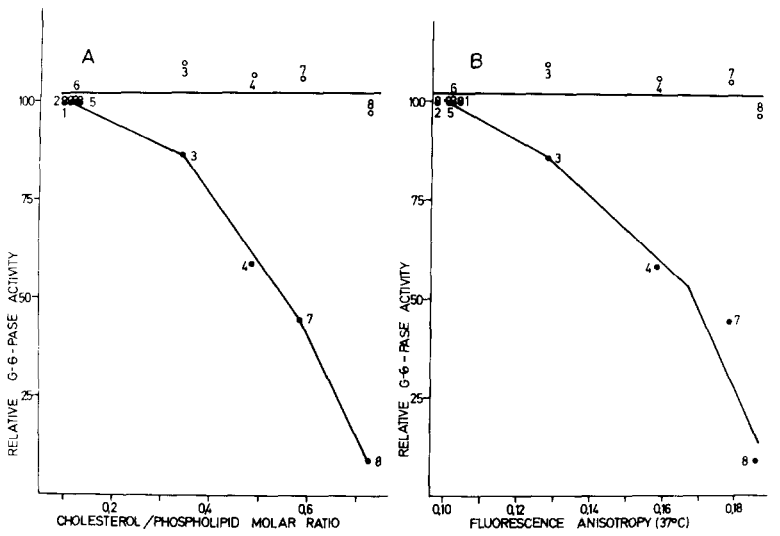

Fig. 4. Effect of cholesterol enrichment upon the activity of glucose-6-phosphatase microsomal systems. The activities are expressed as percent of the corresponding controls and they are plotted versus the cholesterol/phospholipid molar ratio (A) and versus the diphenylhexatriene fluorescense anisotropy (B). Numbers indicate the treatment (see Table I). ●———●, Glucose-6-phosphatase activity of 'intact' microsomes (calculated as indicated in Materials and Methods). ○———○, Glucose-6-phosphatase activity of Triton X-100 disrupted microsomes. The values for the mannose-6-phosphohydrolase latency in these samples were: in samples 1, 2, 3, 4, 5, 6, 7, and 8, and in original microsomes, 54, 50, 67, 63, 60, 54, 55 and 47, and 88%, respectively.

ratio was plotted versus pyrene concentration while the slopes of these curves were plotted versus the cholesterol/phospholipid molar ratio in Fig. 3B. In this last figure it can be observed that the efficiency of pyrene excimer formation is decreased by the cholesterol incorporation in microsomes. Thus, the enhancement of the cholesterol content of microsomes produced a decrease of both the rotational mobility of diphenylhexatriene and translational mobility of pyrene molecules in the membrane lipid phase.

Effect of cholesterol enrichment on the activity of Glucose-6-phosphatase system

The cholesterol enrichment of microsomes did not alter significantly the glucose-6-posphatase activity of microsomes disrupted by treatment with 0.1% Triton X-100 as is shown in Fig. 4. These results would indicate that the physical properties of the membrane lipid phase do not alter the hydrolytic activity of the enzyme.

The procedure followed to incorporate cholesterol in the membrane lipid phase produced a measurable disruption of the permeability barrier of microsomal vesicles as it was indicated by the decrease in the latency of mannose-6-phosphohydrolase activity (see legend to Fig. 4). However, if the G-6-Pase activity of non-detergent treated microsomes is corrected for the activity corresponding to the fraction of disrupted microsomal vesicles, we can obtain the activity corresponding to the 'intact' microsomal vesicle frac-

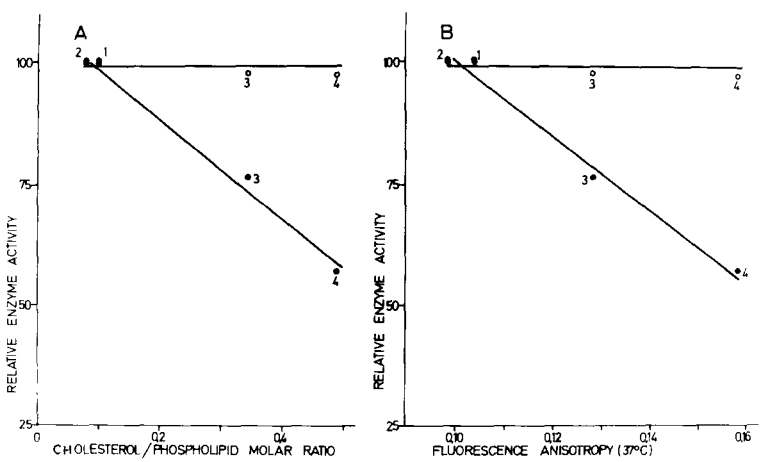

Fig. 5. Effect of cholesterol enrichment upon the electron transport chain activity in rat liver microsomes. The activities are expressed as percent of the corresponding controls and they are plotted versus the cholesterol/phospholipid molar ratio (A) and versus the diphenylhexatriene fluorescence anisotropy (B). Numbers indicate the treatment (see Table I). ○———○, NADH-ferricyanide reductase activity; ●———●, NADH-cytochrome *c* reductase activity.

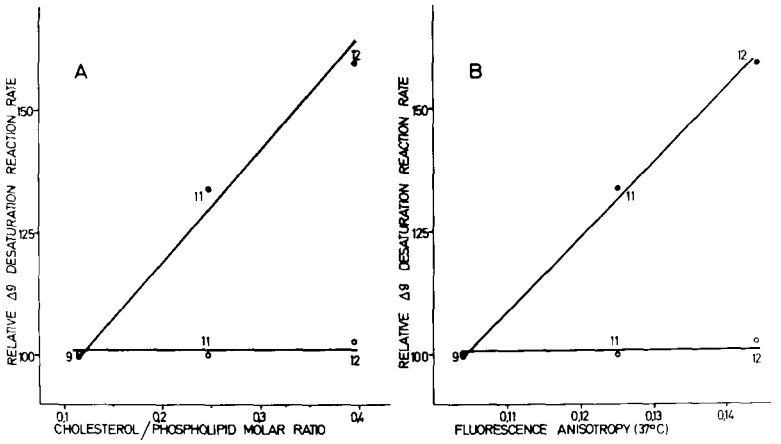

Fig. 6. Effect of cholesterol enrichment of rat liver microsomes on palmitic acid Δ^9 desaturation. Reaction rates are expressed as percent of the corresponding controls and are plotted versus the cholesterol/phospholipid molar ratio (A) and versus the diphenylhexatriene fluorescence anisotropy (B). Numbers indicate the treatment (see Table I). ○——○, 66 μM of palmitic acid in the incubation medium; ●——●, 2 μM of palmitic acid in the incubation medium. Absolute reaction rates for control microsomes were 142 and 5.4 pmol/min per mg of protein at high and low substrate concentration, respectively.

tion (see Materials and Methods). It can be seen in the Fig. 4 that in this case the glucose-6-phosphatase activity of 'intact' microsomes is notoriously decreased by the enhancement of cholesterol content of the membrane.

Effect of cholesterol enrichment on the activity of fatty acid desaturation system and its associated electron transport chain

Fig. 5 shows that NADH-ferricyanide reductase activity is not modified by the microsomal cholesterol content modification. On the contrary, when the interaction of both NADH-cytochrome b_5 reductase and cytochrome b_5 is measured by cytochrome c reduction, the activity is decreased when the cholesterol/phospholipid molar ratio of the microsomal membrane is increased.

The effect of microsomal cholesterol content enhancement on the fatty acid desaturation reactions is shown in Figs. 6, 7 and 8. In Fig. 6 it can be observed that the Δ^9 desaturase reaction rate is increased by cholesterol enrichment of microsomes when the palmitic acid substrate concentration is non-saturating, but the enzyme activity is not significantly modified when a higher substrate concentration is used.

The response of Δ^6 and Δ^5 desaturases to the microsomal cholesterol content modification is shown in Figs. 7 and 8, respectively. Differently to Δ^9 desaturase, Δ^6 and Δ^5 desaturation reaction rates are increased by cholesterol enrichment of microsomes both at low and high fatty acid substrate concentration.

Fatty acid desaturases (specially Δ^6 and Δ^9 de-

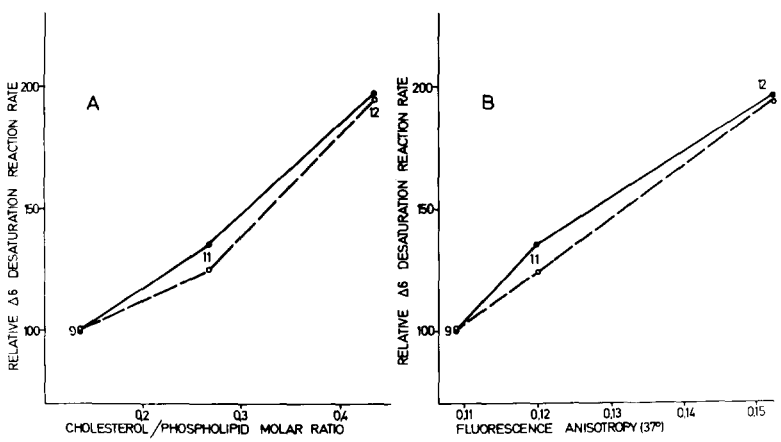

Fig. 7. Effect of cholesterol enrichment of rat liver microsomes on linoleic acid Δ^6 desaturation. Reaction rates are expressed as percent of the corresponding controls and are plotted versus the cholesterol/phospholipid molar ratio (A) and versus the diphenylhexatriene fluorescence anisotropy (B). Numbers indicate the treatment (see Table I). ○——○, 66 μM of linoleic acid in the incubation medium; ●——●, 2 μM of linoleic acid in the incubation medium. Absolute reaction rates for control microsomes were 78 and 3.3 pmol/min per mg of protein at high and low substrate concentration, respectively.

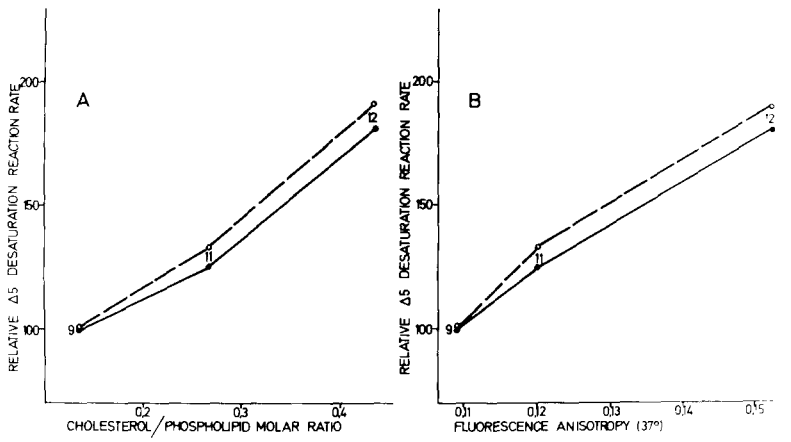

Fig. 8. Effect of cholesterol enrichment of rat liver microsomes on eicosa-8, 11, 14-trienoic acid Δ^5 desaturation. Reaction rates are expressed as percent of the corresponding controls and are plotted versus the cholesterol/phospholipid molar ratio (A) and versus the diphenylhexatriene fluorescence anisotropy (B). Numbers indicate the treatment (see Table I). ○——○, 66 μM of eicosatrienoic acid; ●——●, 2 μM of eicosatrienoic acid. Absolute reaction rates for control microsomes were 213 and 14.1 pmol/min per mg of protein at high and low substrate concentration, respectively.

saturases) are unstable and their activity is rapidly decreased at temperatures above 0°C. The Δ^9, Δ^6 and Δ^5 desaturase activities of control microsomes (sample treatment No. 9 in Figs. 6, 7 and 8 and in Table I) were 49%, 54% and 73%, respectively, from the corresponding activities in original microsomes, and these inactivations also occurred when microsomes were maintained for the same time at the same temperature in the absence of liposomes. Thus, it could be possible that the higher activities of cholesterol-enriched microsomes with respect to control microsomes shown in Figs. 6, 7 and 8 were due to a protective effect of cholesterol against the temperature inactivation. However, this is not very probable in the case of Δ^9 and Δ^5 desaturases, since with the first enzyme the effect was not shown at high substrate concentration, and in the second case the activity of the microsomes with the highest cholesterol content was greater than original microsomes activity. Notwithstanding it could be possible in the case of Δ^6 desaturase. However, the possibility of a protective effect of cholesterol was completely discarded by measuring the rate of temperature inactivation of desaturase enzymes in the different microsomes. After 30 min at 20°C, the percent inactivation of desaturase enzymes was the same for control and cholesterol-enriched microsomes. These were for Δ^9, Δ^6 and Δ^5 desaturases, respectively, 70, 72 and 38% for control microsomes and 73, 68 and 37% for cholesterol-enriched microsomes.

Discussion

The experiments described show that cholesterol is readily transferred from cholesterol-rich liposomes to microsomes if cytosol is present in the incubation medium. The enhancement of the microsomal cholesterol content results in a decrease in the rotational mobility of diphenylhexatriene molecules and in the rate of the lateral diffusion of pyrene molecules incorporated in the membrane lipid phase. These results are in accord with the condensing effect of cholesterol already described for model and biological membranes that are above the phase transition temperature in the liquid-crystalline state [10,12].

In spite of some objections [23] the microsomal glucose-6-phosphatase system according to Arion seems to be formed by at least three protein components [24–26]: (a) a relatively non-specific phosphohydrolase-phosphotransferase component which is bound to the luminal surface of microsomal vesicles and whose activity can be measured after the disruption of the permeability barrier of the microsomes by detergents or other agents [27]; (b) a specific glucose-6-phosphate translocase [28] whose activity is rate-limiting in 'intact' microsomal vesicles [24–26] and (c) a second translocase that mediates efflux of phosphate [19]. Cholesterol incorporation in microsomes does not affect notoriously the glucose-6-phosphatase activity if the microsomal vesicles are permeabilized by 0.1% Tri-

ton X-100 treatment. This activity was also not modified by membrane fluidification using short-chain aliphatic alcohols [9]. Moreover, this activity is not affected if the membrane lipid phase undergoes a liquid-crystalline to crystalline phase transition as was shown in microsomes modified by exogenous saturated phosphatidylcholine incorporation [29]. On the contrary, the glucose-6-phosphatase activity in 'intact' microsomal vesicles was decreased by cholesterol incorporation. Correspondingly, short-chain aliphatic alcohol fluidification of the microsomal membrane resulted in an enhancement of this activity without affecting the mannose-6-phosphohydrolase latency [9]. Therefore, in the light of Arion's model [24–26] it seems possible that the luminal glucose-6-phosphate phosphohydrolase catalytic component is not affected by the physical properties of the membrane lipid phase. The reaction occurs with water-soluble substrates which have free access to the catalytic center directly from the aqueous phase in the disrupted microsomes. On the contrary, the activity of the glucose-6-phosphate translocase seems to be dependent on the physical dynamic properties of the membrane lipid phase modified by cholesterol or aliphatic alcohol incorporation. Such type of effects have been also proposed to another membrane bound enzyme and transport system. β-Glucosides and β-galactosides transport rate across *Escherichia coli* membranes [4], or glucose transport rate across fibroblast membranes [3] are affected by the membrane lipid phase fluidity.

The results here obtained show that NADH-ferricyanide reductase activity is not affected by the condensing effect evoked by cholesterol incorporation in microsomes. Therefore, these results are in agreement with previous experiments that showed that this activity was neither affected by membrane fluidification evoked by short-chain alcohol incorporation [9] and was independent of the lipid phase transition in dimiristoylphosphatidylcholine artificial membrane [30] and in microsomes which were modified by exogenous dimiristoyl- or dipalmitoylphosphatidylcholine incorporation [29]. This flavoprotein presents its catalytic center exposed to aqueous phase [31] and uses water-soluble substrates. Therefore, lipid phase properties do not seem to affect their catalytic properties.

Conversely, NADH-cytochrome *c* reductase activity was decreased by cholesterol incorporation in microsomes. This activity measures the rate of electron transfer from NADH to the exogenous electron acceptor cytochrome *c*, through both the flavoprotein and cytochrome b_5 which interaction is the rate-limiting step [30,32,33]. NADH-cytochrome *c* reductase activity was also suddenly decreased when the membrane lipids underwent a fluid to ordered transition in artifical lipid vesicles [30] and in microsomes enriched with exogenous saturated phosphatidylcholines [29]. Besides, the activity of this binary system was increased by membrane fluidification by means of short-chain alcohols [9]. It has been postulated [32–34] that flavoprotein and cytochrome b_5 are randomly distributed in the plane of the membrane and that their interaction would be limited by their diffusion rate [30,32,33]. Therefore, in spite that cholesterol effect and other mentioned results do not distinguish if the lateral or the rational diffusion of the protein components is the determinant event for the electron transference, they provide coincident evidence that this event is dependent on the dynamic properties of the membrane lipid phase.

Cholesterol enrichment of rat liver microsomes produces an increase in the fatty acid desaturation reaction rates. On palmitic acid Δ^9 desaturation the effect seems to be evoked on the enzyme affinity for the substrate since the effect was dependent on substrate concentration and it was not seen at high saturating palmitic acid concentration. On Δ^6 and Δ^5 desaturases the effect was shown both at high and low fatty acid substrate concentration.

In a previous report [9] it has been shown that microsomal membrane fluidification evoked by isoamyl alcohol incorporation correlated a decrease of Δ^9 and Δ^6 desaturation. In the present experiment cholesterol incorporation in microsomes evoked a decrease of membrane 'fluidity' and the corresponding increase of the desaturation of fatty acids. The similarity of the antagonic responses of the desaturases evoked by two opposite changes of the membrane dynamics suggests that desaturase reactions may function as a self-regulating mechanism of the unsaturated acyl chain content and through changes of membrane 'fluidity'. This could be the case if unsaturated acids

increase membrane 'fluidity'. In this way, a decrease in membrane 'fluidity' would increase the desaturation and this would adjust the membrane 'fluidity' increasing it and vice-versa. This type of viscotropic regulation has been already proposed for the *Tetrahymena pyriformis* [35–37]. With the present work an extension to mammals systems would be suggested. However, it is important to consider that the regulation of fatty acid desaturation systems in mammals is very complicated due to hormonal mechanisms that may mask simpler and more primitive fluidity-desaturase self-regulation in many circumstances [38]. Moreover, the self-regulatory mechanism can not be responsible for the Δ^9 desaturation increase when rats are fed on an essential fatty acid-deficient diet [38,39] since in that case, the effect was observed on the V_{max}, and here only at low substrate concentration.

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References

1 Sandermann, H.Jr. (1978) Biochim. Biophys. Acta 515, 209–237
2 Yuli, I., Incerpi, S., Luly, P. and Shinitzky, M. (1982) Experientia 38, 1114–1115
3 Yuli, I., Wilbrandt, w. and Shinitzky, M. (1981) Biochemistry 20, 4250–4256
4 Thilo, L., Träuble, H. and Overath, P. (1977) Biochemistry 16, 1283–1290
5 Castuma, C.E. and Brenner, R.R. (1983) Biochim. Biophys. Acta 729, 9–16
6 Sinensky, M., Minneman, K.P. and Molinoff, P.B. (1979) J. Biol. Chem. 254, 9135–9141
7 Scheel, H.G., Acevedo, E., Conzelmann, E., Nelnkorn, H. and Sandhoff, K. (1982) Eur. J. Biochem. 127, 245–253
8 Lentz, R.B., Barenholz, Y. and Thompson, T.E. (1976) Biochemistry 15, 4521–4528
9 Garda, H.A. and Brenner, R.R. (1984) Biochim. Biophys. Acta 769, 160–170
10 Cogan, U., Shinitzky, M., Weber, G. and Nishida, T. (1973) Biochemistry 12, 521–528
11 Vincent, M., De Foresta, B., Gallas, J. and Alfsen, A. (1982) Biochem. Biophys. Res. Commun. 107, 914–921
12 Shinitzky, M. and Inbar, M. (1974) J. Mol. Biol. 85, 603–615
13 Yeagle, P.L. (1983) Biochim. Biophys. Acta 727, 39–44
14 North, P. and Fleischer, S. (1983) J. Biol. Chem. 258, 1242–1253
15 Brenner, R.R., Garda, H.A. and Pezzano, H. (1981) Anal. Asoc. Quím. Arg. 69, 37–53
16 Folch, J., Lees, M. and Sloane-Stanley, G.H. (1957) J. Biol. Chem. 226, 497–509
17 Van Eusden, G.P.H. and Wirtz, K.W.A. (1984) J. Lipid Res. 25, 27–32
18 Brenner, R.R., Garda, H.A., Leikin, A.I. and Pezzano, H. (1980) Acta Physiol. Latinoam. 30, 225–238
19 Arion, W.J., Lange, A.J., Walls, H.E. and Ballas, L.M. (1980) J. Biol. Chem. 255, 10396–10406
20 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem., 193, 265–175
21 Chen, P.S., Toribara, T. and Huber, W. (1956) Anal. Chem. 28, 1756–1758
22 Allain, C.C., Poon, L.S., Chen, C.S.G., Richmond, W. and Fu, P.C. (1974) Clin. Chem. 20, 470–475
23 Zakim, D. and Edmondson, D.E. (1982) J. Biol. Chem. 257, 1145–1148
24 Arion, W.J., Wallin, B.K., Lange, A.J. and Ballas, L.M. (1975) Mol. Cell. Biochem. 6, 75–83
25 Arion, W.J., Lange, A.J. and Walls, H.E. (1980) J. Biol. Chem. 255, 10387–10395
26 Arion, W.J. and Walls, H.E. (1982) J. Biol. Chem. 257, 11217–11220
27 Nilsson, O.S., Arion, W.J., De Pierre, J.W., Dallner, G. and Emster, L (1978) Eur. J. Biochem. 82, 627–634
28 Zoccoli, M.A., Hoopes, R.R. and Karnovsky, M.L. (1982) J. Biol. Chem. 257, 3919–3924
29 Garda, H.A. and Brenner, R.R. (1982) Acta Physiol. Latinoam. 32, 31–52
30 Strittmatter, P. and Rogers, M.J. (1975) Proc. Natl. Acad. Sci. USA 72, 2658–2661
31 De Pierre, J.W. and Ernster, L. (1977) Annu. Rev. Biochem. 46, 201–262
32 Rogers, M.J. and Strittmatter, P. (1974) J. Biol. Chem. 249, 895–900
33 Rogers, M.J. and Strittmatter, P. (1974) J. Biol. Chem. 249, 5565–5569
34 Strittmatter, P., Rogers, M.J. and Spatz, L. (1972) J. Biol. Chem. 247, 7188–7194
35 Martin, C.E., Hiramitsu, K., Kitajima, Y., Nozawa, Y., Skriver, L. and Thompson, G.A.Jr., (1976) Biochemistry 15, 5218–5227
36 Kasai, R., Kitajima, Y., Martin, C.E., Nozawa, Y., Skriver, L. and Thompson, G.A.Jr. (1976) Biochemistry 15, 5228–5233
37 Skriver, L. and Thompson, G.A.Jr. (1979) Biochim. Biophys. Acta 572, 376–381
38 Brenner, R.R. (1981) Prog. Lipid. Res. 20, 41–47
39 Brenner, R.R., Garda, H.A., de Gómez Dumm, I.N.T. and Pezzano, H. (1981) Prog. Lipid Res. 20, 315–321