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Articles

Cholesterol-Dependent Modification of Microsomal Dynamics and UDPglucuronyltransferase Kinetics†

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ABSTRACT: The effect of both in vitro incorporation and removal of cholesterol in guinea pig liver microsomes on the lipid composition, dynamic properties of the membrane, and kinetic constants of UDPglucuronyltransferase was studied. No significant changes either in the fatty acid composition or in the distribution of phospholipid classes were observed upon cholesterol incorporation and removal. Lateral and rotational mobility measured by the efficiency of pyrene excimer formation and fluorescence of 1,6-diphenylhexatriene decreased with cholesterol incorporation and increased in parallel to cholesterol removal. These changes were associated with alterations in the kinetic properties of UDPglucuronyltransferase. Whereas V_{max} increased, the K_m of the different steps of the reaction decreased with cholesterol incorporation. The negative homotropic effect and apparent cooperativity of UDP–glucuronic acid decreased when cholesterol was incorporated and increased after cholesterol removal. Moreover, the UDP–*N*-acetylglucosamine-dependent activation of the enzyme decreased in correlation with an increase of cholesterol concentration in microsomes. It has been demonstrated that both the shift of the non-Michaelian kinetics of the enzyme to Michaelian and the decrease of the UDP–*N*-acetylglucosamine-dependent activation of the enzyme are evoked by a change of the physical state of the UDPglucuronyltransferase milieu from a gel phase to a liquid-crystalline phase. Therefore, we must admit that cholesterol incorporation in the microsomes while producing an increased packing of the bulk lipids would also cause the separation of more fluid phospholipids, which increase the proportion of molecules in the liquid-crystalline state within the enzyme environment.

The kinetic properties of several membrane-bound enzymes including liver UDPglucuronyltransferase depend on the composition and physical state of the lipid bilayer phase (Strobel et al., 1970; Zakim & Vessey, 1976). The lipid modulation of this specific enzyme has been interpreted according to the compartmentation theory (Berry & Hallinan, 1976) as an effect on the substrate transport to the deeply located catalytic sites. This theory has been elaborated farther by considering that two enzyme forms, buried and exposed, contribute to the characteristic kinetic properties of the reaction (Onah et al., 1982; Cummings et al., 1984). However, relevant information has been also gathered by Zakim et al. (Zakim & Vessey, 1975, 1978, 1982) to suggest a direct effect on the enzyme molecule.

Since cholesterol is an important modulator of membrane physical properties condensing the lipid bilayer (De Kruijff et al., 1973) above the transition temperature, we investigated the effect of this lipid on the kinetic properties of UDPglucuronyltransferase. This study is important for understanding better not only the mechanism of interaction between lipid bialyers and associated enzymes but also the modulation of the UDPglucuronyltransferase reaction. Besides, it broadens our knowledge on the way cholesterol interacts with membranes and enzymes.

In a previous work (Castuma et al., 1986) we showed that an enhancement of dietary cholesterol in guinea pigs increased the microsomal cholesterol content by decreasing membrane "fluidity" and modifying the kinetic properties of the enzyme. Not only the K_ms and V_m were modified, but also the non-

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Michaelian kinetics of the enzyme was switched toward Michaelian.

Nevertheless, in that experiment the increase of membrane cholesterol was induced in vivo and was joined by an increase of the phosphatidylcholine (PC):phosphatidylethanolamine (PE) ratio. The present work attempts to elucidate if the same effects appear when the cholesterol content of liver microsomes in vitro is modified independently of phospholipid changes.

For these reasons, the cholesterol content of the microsomal membrane of guinea pig liver was modified by direct incubation with liposomes by a mild procedure. The effects produced on the composition, the physical properties of the membrane, and the kinetics of UDPglucuronyl transferase were monitored. The dynamic changes of microsomes were checked by the fluorescence anisotropy of membrane labeled with 1,6-diphenylhexatriene and the efficiency of pyrene excimer formation, which measure rotational and translational mobility, respectively (Shinitzky & Barenholz, 1978; Pownall & Smith, 1973).

Materials and Methods

Separation of Microsomal Lipids. Guinea pig liver microsomes obtained from animals weighing 250 g and fed with standard diet (Reid & Briggs, 1953) were used in the experiments. Microsomes were separated by differential ultracentrifugation from livers homogenized in 0.25 M sucrose solution as already described (Castuma & Brenner, 1986), and microsomal lipids were extracted following Folch's procedure (Folch et al., 1957). The total lipid extract (0.6 mg/mL of chloroform) was mixed vigorously with silicic acid (20:1 w/w), filtered, and washed twice with Cl_3CH so as to eliminate neutral lipids. Phospholipids were eluted with methanol 5 times and concentrated in a rotatory evaporator under vacuum. The cholesterol/phosholipid molar ratio decreased from 0.32 in the total lipid fraction to 0.0008 in the phospholipid extract, indicating almost complete elimination of the sterol.

Liposome Preparation. Two types of liposomes were prepared essentially according to the method of Barenholz et al. (1977). One of them was constituted by microsomal phospolipids only (Ph liposomes) and the other by the same liposomes enriched with cholesterol (Ph-chol liposomes). Ph liposomes were prepared in 0.25 M sucrose, 1.5 mM ethylenediaminetetraacetic acid (EDTA), and 0.5 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) buffer, pH 7.0. Appropriate amounts of the corresponding microsomal lipid extract were evaporated, and the same buffer was added to reach a final concentration of 6 mM in phospholipid. They were then stirred in a Vortex for about an hour at laboratory temperature, under N_2, to permit equilibration of the two phases. To prepare cholesterol-enriched liposomes, the appropriate amount of cholesterol was added to the phospholipid to reach a final cholesterol:phospholipid molar ratio of 1:1. Liposomes were prepared by sonicating the lipids in an Ultrasonic sonicator at maximal potency and at 2 °C for 10 min for phospholipid liposomes and 20 min for Ph-chol liposomes. Liposomes were centrifuged at 140000*g* for 2 h so as to discard large multilamellar vesicles (Barenholz et al., 1977) and then stored under N_2 atmosphere at 4 °C for not more than 24 h.

Modification of Cholesterol Content of Microsomes. The transference of cholesterol from cholesterol-rich liposomes to microsomal membranes has been accomplished experimentally by means of lipid transferring proteins (Van Heusden & Wirtz, 1984). In these experiments, the tedious purification of cholesterol transferring protein has been avoided by the direct use of the supernatant of 12000*g* separated by ultracentrifugation of guinea pig liver homogenates for 20 min. This supernatant contains both microsomes and cytosolic transferring proteins. In these mild conditions and temperatures used (5–7 °C), good cholesterol incorporation was obtained. The procedure used was the following: Guinea pig livers were rapidly excised and homogenized in 0.25 M sucrose, pH 7 (1:1.5 w/v). The homogenate was centrifuged at 12000*g* for 20 min, and the supernatant was separated. To incorporate cholesterol, 1 volume of this supernatant was incubated for 2 h at 5–7 °C with 9 volumes of the Ph-chol liposomes and then centrifuged for 1 h at 105000*g*. The pellet constituted by cholesterol-enriched microsomes was resuspended in the same homogenizing solution and recentrifuged in the same conditions and speed.

To extract cholesterol from microsomes, the 12000*g* supernatant was centrifuged at 105000*g* for 1 h and the pellet resuspended in the same solution. This pellet was incubated for 2 h at 5–7 °C with Ph liposomes (9 volumes of liposomes for 1 volume of 12000*g* supernatant) and fresh cytosol (3 and 6 volumes of fresh cytosol for 1 volume of 12000*g* supernatant). The mixture was then centrifuged at 105000*g* for 1 h in the cold, resuspended in the same solution, and washed once as above mentioned. The different types of microsomes were stored at −80 °C until used.

Enzymatic Measurements. UDPglucuronyltransferase activity was determined at 37 °C as previously described (Castuma & Brenner, 1986) with *p*-nitrophenol as glucuronyl acceptor. Spectrophotometric measurements were carried out at 400 nm to follow the disappearance of *p*-nitrophenol during the conjugation reaction. Mannose-6-Pase activity was estimated according to Arion et al. (1980) to check the integrity of the membrane. Whenever UDP–*N*-acetylglucosamine was used, concentrations were indicated in figures. Initial rates of reaction for control and UDP–*N*-acetylglucosamine-stimulated preparations were directly proportional to the concentration of microsomal protein over a wide range of protein concentrations.

Fluorescence Anisotropy and Pyrene Excimer Formation Measurements. The labeling of the microsomes with 1,6-diphenyl-1,3,5-hexatriene (DPH) (Aldrich Chemical Co.) and pyrene (Fluka) as well as steady-state fluorescence anisotropy (r_s) measurements was described elsewhere (Castuma & Brenner, 1986). For fluorescence anisotropy measurements with DPH, the final lipid concentration was 25 μM and final label concentration 0.25 μM. Measurements were made at 37 °C (excitation light at 350 nm). 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 elsewhere (Castuma & Brenner, 1986). Pyrene concentrations fell between 2 and 7 μM, and the emission spectra were obtained at 25 °C in an Aminco-Bowman spectrofluorometer. The fluorescence of pyrene was excited at 320 nm and registered at 390 (monomers) and 470 nm (excimers).

Lipid Analysis. Lipids were separated by thin-layer chromatography and analyzed as described previously in detail (Castuma & Brenner, 1986). Fatty acid composition was studied by gas–liquid chromatography (Castuma & Brenner, 1986). Phospholipid phosphorus was determined according to Toritara et al. (1956) and cholesterol with the method described by Huang et al. (1961).

Results

Lipid Composition of Modified Guinea Pig Liver Microsomes. The modification of cholesterol content in guinea pig liver microsomes was evoked by their incubation with liposomes prepared with those lipids of the same microsomes with and

Table I: Cholesterol and Phospholipid Content of Modified Microsomes[a]

type of microsomes	phospholipid/protein (μmol/mg of protein)	cholesterol/protein (μmol/mg of protein)	cholesterol/phospholipid (molar ratio)
N	0.33 ± 0.01	0.12 ± 0.01	0.364
N + Ph-chol	0.37 ± 0.02	0.15 ± 0.01	0.405
N^s + Ph	0.38 ± 0.02	0.14 ± 0.01	0.368
N^s + Ph-chol	0.39 ± 0.01	0.20 ± 0.02	0.513
N + Ph^3	0.37 ± 0.02	0.11 ± 0.01	0.297
N + Ph^6	0.37 ± 0.02	0.10 ± 0.02	0.270

[a] N = normal microsomes; N + Ph-chol = normal microsomes incubated with Ph-chol liposomes; N^s + Ph = 12000*g* supernatant incubated with Ph liposomes; N^s + Ph-chol = 12000*g* supernatant incubated with Ph-chol liposomes; N + Ph^3 = normal microsomes incubated with Ph liposomes and cytosol (3 volumes); N + Ph^6 = normal microsomes incubated with Ph liposomes and cytosol (6 volumes). Results are the mean of five experiments ± SE of the mean.

Table II: Dynamic Parameters of Modified Microsomes[a]

microsomes	fluorescence anisotropy	slope of I_e/I_m vs. pyrene concentration
N^s + Ph	0.112 ± 0.002	0.025
N^s + Ph-chol	0.154 ± 0.001	0.017
N + Ph^6	0.102 ± 0.003	0.028

[a] Symbols correspond to those explained in Table I.

without cholesterol addition so as to incorporate or extract cholesterol, respectively. This cholesterol exchange was enhanced by the presence of cytosolic transport proteins. As is shown in Table I, the incubation of liver microsomes for 2 h at 5–7 °C with Ph liposomes or Ph-chol liposomes only produced a small increase of the cholesterol:phospholipid molar ratio (N + Ph-chol). On the contrary, when cytosol was added, the cholesterol exchange was markedly increased and the cholesterol:phospholipid molar ratio was enhanced from 0.364 to 0.513 (Table I). When increasing volumes of cytosol were added in the presence of Ph liposomes (N + Ph^3 and N + Ph^6) (Table I), cholesterol was delivered from microsomes according to the decrease in the cholesterol:phospholipid molar ratio from 0.364 to 0.270. Table I also evidences a small increase in the phospholipid/protein ratio in all types of microsomes compared to the normal ones, probably due to the incubation procedure. Liposomes and microsomes prepared as stated under Materials and Methods differ only in the cholesterol content, and consequently, no change in the other lipid components was expected during the incubation.

In order to check this, fatty acid and total lipid composition were analyzed and compared to control microsomes evidencing no differences between the fatty acid composition of control and modified microsomes. The phospholipid distribution was also maintained in normal and modified microsomes (data not shown).

Efficiency of Pyrene Excimer Formation and Fluorescence Anisotropy of Modified Microsomes. The lateral diffusion in control and treated microsomes was evaluated by measuring the efficiency of pyrene excimer formation. The effect of cholesterol incorporation and extraction upon the lateral mobility is shown in Figure 1, where the I_e/I_m ratio was plotted vs. pyrene concentration. The efficiency of pyrene excimer formation increased linearly with the probe concentration while the increase or decrease of the I_e/I_m ratio corresponded to opposite changes in the cholesterol content of the modified microsomes (Figure 1 and Table II).

Table II also indicates the fluorescence anisotropy of labeled membranes of the different types of microsomes. The changes in the DPH fluorescence anisotropy went together with the

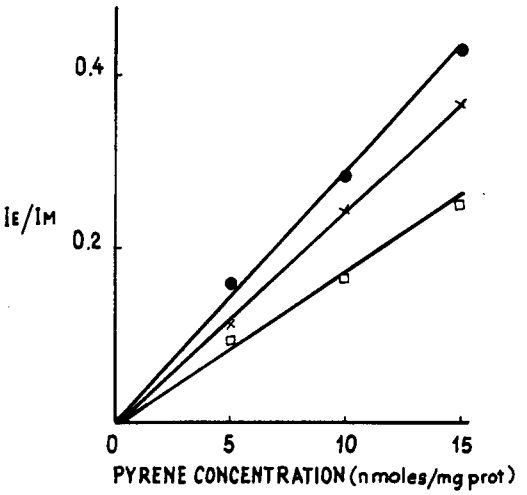

FIGURE 1: Efficiency of pyrene excimer formation at different pyrene concentrations in control, N^s + Ph (×), and modified microsomes, N + Ph^6 (●) and N^s + Ph-chol (□). N + Ph^6, normal microsomes incubated with Ph liposomes and 6 volumes of cytosol; N^s + Ph, control microsomes; N^s + Ph-chol, 12000*g* supernatant incubated with Ph-chol liposomes.

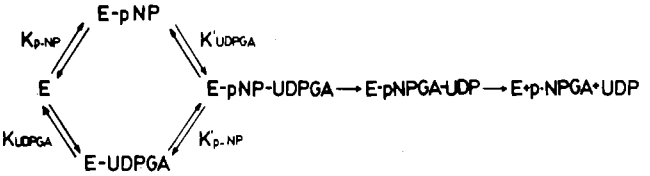

FIGURE 2: Postulated reaction mechanism for UDPglucuronyltransferase. K_{pNP} and K_{UDPGA} correspond to the dissociation constants for the binary complex. K'_{pNP} and K'_{UDPGA} correspond to the dissociation constants for the ternary complex.

cholesterol levels of modified microsomes: increasing in cholesterol-enriched microsomes (N^s + Ph-chol) and decreasing in cholesterol-depleted microsomes (N + Ph^6). Thus, the enhancement or decrease of cholesterol content of microsomes produced a corresponding decrease or increase of both the wobbling dynamics of DPH and translational mobility of pyrene molecules in the membrane lipid matrix.

Integrity of Membrane Structure. Despite the mild procedures used either to incorporate or remove microsomal cholesterol in vitro, it was meaningful to check if this manipulation of membranes did not disrupt microsomal integrity and alter enzyme properties. For this reason, microsomal integrity was assayed by measuring mannose-6-Pase latency (Arion et al., 1980). Mannose-6-Pase latencies were 85, 81, and 78% for N^s + Ph, N^s + Ph-chol, and N + Ph^6 microsomes, respectively, indicating that the experimental treatment did not evoke a significant disruption either in control or in modified microsomes.

Kinetic Measurements of UDPglucuronyltransferase. The UDPglucuronyltransferase has been defined as a random ordered sequential reaction by Vessey and Zakim (1971) (Figure 2). In this work, glucuronyltransferase activity was measured by using different fixed concentrations of UDP–glucuronic acid or *p*-nitrophenol and by varying the other corresponding substrate as explained in detail elsewhere (Castuma & Brenner, 1986). When data are plotted in double-reciprocal form, $K_{m,UDPGA}$ and $K_{m,pNP}$ are obtained from the interception on the $1/s$ axis. These K_ms are the dissociation constants of the binary enzyme–substrate complex. Secondary plots of the interception on the $1/v$ axis vs. the inverse of the concentration of the fixed substrate yield the $1/V_{max}$ value and a second K_m corresponding to the dissociation constants for

Table III: Kinetic Constants for Forward Reaction of UDPglucuronyltransferase[a]

microsomes	K_{UDPGA} (mM)	K'_{UDPGA} (mM)	K_{pNP} (mM)	K'_{pNP} (mM)	V_{max} [nmol min^{-1} (mg of protein)$^{-1}$]	Hill coefficient
N^s + Ph	12.0 ± 0.20	10.3 ± 0.30	0.17 ± 0.01	0.12 ± 0.01	10.1 ± 1.3	0.46 ± 0.03
N^s + Ph-chol	8.9 ± 0.12	6.2 ± 0.11	0.10 ± 0.02	0.08 ± 0.02	14.3 ± 1.2	0.74 ± 0.01
N + Ph^6	13.1 ± 0.20	10.9 ± 0.1	0.20 ± 0.01	0.15 ± 0.01	8.7 ± 0.8	0.38 ± 0.01

[a] Symbols correspond to those explained in Table I. Results are the mean of five experiments with standard errors.

the ternary enzyme–substrate complex (Vessey & Zakim, 1971).

By this procedure, the four K_ms and V_{max} of the forward reaction were calculated and tabulated (Table III). Table III summarizes the kinetic properties estimated for normal and modified microsomes. UDP-glucuronic acid shows lower affinity than *p*-nitrophenol for the enzyme, and the incorporation of cholesterol in vitro evokes an increase of the affinity for all the reaction steps, also enhancing the specific activity of the UDPglucuronyltransferase. A shift of the non-Michaelian kinetics of the enzyme to Michaelian was also evidenced by the increase in the Hill coefficient approaching to unity. Similar results were found when cholesterol was incorporated in vivo. On the contrary, the release of cholesterol from the membrane demonstrated by the decrease in the cholesterol:phospholipid molar ratio slightly decreased the Hill coefficient.

Effect of Cholesterol on UDP–N-Acetylglucosamine Stimulation of UDPglucuronyltransferase. In unaltered microsome membranes, UDP–*N*-acetylglucosamine stimulates UDP-glucuronyltransferase (Pogell & Leloir, 1961; Vessey et al., 1973). As is shown in Figure 3, fixed concentrations of UDP–*N*-acetylglucosamine increased the rate of glucuronidation of *p*-nitrophenol. The extent of the enhancement was dependent on the concentration of UDP–glucuronic acid, there being no enhancement at infinite concentrations of UDP–glucuronic acid. Figure 3 also shows the effect of cholesterol concentration in microsomes on the UDP–*N*-acetylglucosamine-dependent activation of UDPglucuronyltransferase monitored by the measurement of the initial rates of glucuronidation. The inset shows that the increase of cholesterol level from depleted microsomes to control microsomes and enriched microsomes decreases this percent activation of UDPglucuronyltransferase.

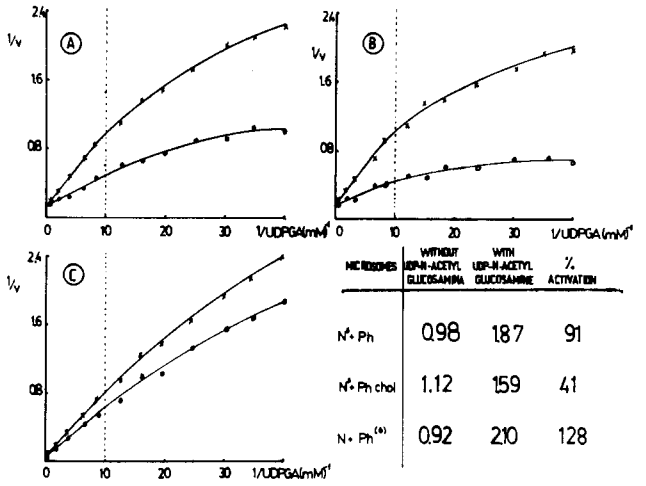

FIGURE 3: Double-reciprocal plots of the rate of synthesis of (*p*-nitrophenyl)glucuronide in the presence (○) and absence (×) of UDP–*N*-acetylglucosamine as a function of the concentration of UDP–glucuronic acid (UDPGA) in (A) control (N^s + Ph), (B) cholesterol-deficient (N + Ph^6), and (C) cholesterol-enriched (N^s + Ph-chol) microsomes. The concentrations of *p*-nitrophenol and *N*-acetylglucosamine (UDPNAG) when present were 0.2 and 2.0 mM, respectively. Inset shows the initial rate [nmol min^{-1} (mg of protein)$^{-1}$] and percent activation of UDP–*N*-acetylglucosamine for a fixed concentration of UDP–glucuronic acid (0.1 mM).

Discussion

Effect of Cholesterol Content on Structural and Dynamic Properties of Microsomes. The presence of cholesterol in membranes poses relevant questions about the molecular interaction and organization of the cholesterol–phospholipid system (Martin & Yeagle, 1978; Jacobs & Oldfield, 1979; Cullis et al., 1978). Besides, cholesterol may alter the kinetic properties of membrane-bound enzymes (Garda et al., 1982; Garda & Brenner, 1984; Castuma & Brenner, 1986), evoking changes of biochemical and physiological relevance.

In this experiment, we incorporated and removed cholesterol into microsomal membranes by incubation with cholesterol–phospholipid liposomes or phospholipid liposomes prepared with the phospholipids of the same microsomes (Garda & Brenner, 1985). Under these conditions, either cholesterol-enriched or cholesterol-depleted microsomes maintained practically the same fatty acid and lipid distribution of original microsomes, except for the change of cholesterol content. Moreover, the integrity of the microsomal membrane was maintained.

Cholesterol incorporation produced an increase in the fluorescence anisotropy of membranes labeled with DPH (Table II), evidencing a restriction in the rate and range of the wobbling diffusion of the acyl chain due to the packing effect of cholesterol (Godici & Landsberger, 1975; Kinosita & Ikegami, 1985). On the contrary, the removal of cholesterol decreased the steady-state anisotropy, enhancing the molecular motions. Similar results were found by measuring pyrene excimerization (Figure 1) where the translational diffusion of pyrene in the plane of the membrane and, moreover, the frequency of collisions were dependent on the cholesterol level of the microsomes.

Effect of Microsome Cholesterol Content on Kinetic Properties of UDPglucuronyltransferase. The modification of cholesterol content of microsomes also modified the kinetics of the membrane-bound UDPglucuronyltransferase (Table III). In cholesterol-enriched microsomes, all the K_ms of the forward steps of the reaction were decreased, the V_{max} was increased, and the Hill coefficient was enhanced toward 1.

These results are in accordance with previous experiments in which the cholesterol incorporation was produced by in vivo administration (Castuma & Brenner, 1986). However, in that experiment the high cholesterol diet also increased the PC/PE ratio in microsomes. In this experiment, the in vitro incorporation of cholesterol in the microsomes did not modify significantly the pattern of membrane phospholipids, demonstrating in consequence fairly well that cholesterol concentration in the bilayer is responsible for the structural dynamic and kinetic changes observed in both types of experiments. The Hill coefficient was one of the cholesterol-dependent kinetic data changed. A Hill coefficient value of 1 is considered to correspond to Michaelian kinetics. Microsomes of control animals showed a Hill coefficient inferior to 1, indicating a non-Michaelian kinetics for the enzyme. An increase of membrnae cholesterol in vivo or in vitro evoked a decrease of

bulk lipids fluidity and switched the reaction from non-Michaelian to Michaelian. This switch is mathematically equivalent to a loss of the negative cooperativity of UDP–glucuronic acid on the enzyme. But equivalent mathematical curves could be obtained if we have two populations of transferase molecules (Cummings et al., 1984) or one enzyme with two binding sites of different affinity for the substrate (Hochman et al., 1983).

On the other hand, the present experiment also shows that the cholesterol release was correlated to an increase of membrane "fluidity" together with a decrease of the Hill coefficient.

Magdalou et al. (1982) have found that choline phospholipids are required specifically by UDPglucuronyltransferase, and Hochman et al. (1983) have shown that the isolated and delipidated UDPglucuronyltransferase of pig liver presents a non-Michaelian kinetics when the enzyme is reconstituted into unilamellar vesicles of phosphatidylcholine in the gel phase. Binding studies indicated that the basis for this kinetic pattern was the presence of two subunits in a single molecule of UDPglucuronyltransferase, one with high affinity and another with low affinity for UDP–glucuronic acid. Moreover, Hochman and Zakim (1983) presented evidence that UDP-glucuronyltransferase of normal pig liver microsomes at 37 °C is in a gel-phase lipid environment. Furthermore, Hochman et al. (1983) also found that a phase transition from gel to liquid crystalline in the phospholipids of reconstituted pig liver UDPglucuronyltransferase vesicles was associated with a switch from non-Michaelian to Michaelian kinetics and the disappearance of the functionality of one of the binding sites.

Therefore, Hochman and Zakim's (1983) results indicating that the change of physical properties of the phospholipid milieu surrounding UDPglucuronyltransferase influence the Hill coefficient of the enzyme must be considered in order to understand the effect of cholesterol. The basic facts are as follows: (1) the bulk lipids of liver microsomes of guinea pigs fed on a standard diet are in a liquid-crystalline state (Eletr & Zakim, 1973); (2) non-Michaelian kinetics of UDPglucuronyltransferase, as is found in microsomes of guinea pigs fed a low cholesterol diet and in isolated microsomes with relatively low cholesterol content, requires a phospholipid milieu in the gel phase (Hochman et al., 1983). This implies that the physical state of enzyme boundary lipids in the control microsomes must be in a gel phase and that they are different from the bulk lipids of the bilayer. Cholesterol incorporation rigidizes the membrane, turning at the same time the UDP-glucuronyltransferase from a non-Michaelian toward a Michaelian kinetics. Therefore, we consider that cholesterol incorporation while increasing the packing of the bulk lipids must produce an opposite effect on the lipid milieu of the enzyme.

Effect of Microsome Cholesterol Content on UDP-N-Acetylglucosamine-Dependent Activity of UDPglucuronyltransferase. Preceding conclusions also agree with results collected in Figure 3, where it is shown that cholesterol incorporation or extraction in the microsomes changes the activation of UDP-*N*-acetylglucosamine on the enzyme. In intact guinea pig microsomes, UDPglucuronyltransferase is stimulated by UDP-*N*-acetylglucosamine (Vessey et al., 1973), but the activation effect is lost in a delipidated enzyme (Hochman & Zakim, 1983) or in microsomes treated with detergents (Winsnes, 1971). When added to the delipidated enzyme, only phosphatidylcholine in the gel phase can restore the UDP-*N*-acetylglucosamine-dependent activation of UDPglucuronyltransferase (Hochman & Zakim, 1983).

In our experiment, as is shown in Figure 3, the extraction of cholesterol from the microsomal membrane (N + Ph^6 of Figure 3) leads to an increase in the percent activation of UDP-*N*-acetylglucosamine. This result indicates that the enzyme is in a more packed microenvironment, opposing the fluid state of the bulk lipids produced by cholesterol release.

On the contrary, when cholesterol is incorporated (N^s + Ph-chol of Figure 3), rendering the bulk lipids of the bilayer less fluid, the UDPglucuronyltransferase is less sensitive to the activation by UDP-*N*-acetylglucosamine, evidencing a more fluid lipid milieu of the enzyme. Therefore, these results are in accordance with the previous postulation that cholesterol increase in the membrane while enhancing bulk lipids packing fluidizes the enzyme boundary phospholipids.

Further Aspects of the Model. This postulation finds support on experimental results showing that cholesterol evokes phase separations in membranes (Melchior et al., 1980) and separation of cholesterol–phospholipid domains from free phospholipid domains (Jain, 1983). Moreover, it has been shown (Van Dyck et al., 1976; Demel et al., 1977; Housley & Stanley, 1982) that in mixtures with phospholipids cholesterol preferentially interacts with sphingomyelin ≫ phosphatidylserine = phosphatidylglycerol > phosphatidylcholine ≫ phosphatidylethanolamine. Besides, Schroeder (1984) considers that possibly cholesterol interacts specifically with gel-phase lipids in phase-separated systems, excluding the more fluid parts. Models for cholesterol–phospholipid interaction in membranes propose the formation of complexes at 20, 33.3, or 50 mol % of the sterol (Presti et al., 1982).

In this experiment, the molar concentration of cholesterol calculated for the phospholipid bilayer varied from about 21 to 34%. According to Presti et al. (1982), the formation of different cholesterol–phospholipids complexes depends on the relative concentration of both lipids. They would produce domains of 1:2 and 1:1 cholesterol–phospholipid stoichiometry. Below 20%, cholesterol molar concentration would be constituted by rows of 1:2 cholesterol–phospholipid plus boundary lipid regions. The amount of interphasic boundary phospholipids decreases above 20 mol % cholesterol and disappears at 33.3 mol % cholesterol. At this composition, only the cholesterol-rich domain would mainly remain. Therefore, phospholipids principally available for the UDPglucuronyltransferase would be constituted by interphasic lipids between cholesterol–phospholipid domains. This would emphasize the importance of even mild specific cholesterol–phospholipid affinities to deviate fluid molecules of phospholipids to the UDPglucuronyltransferase lipid milieu.

In this respect, Gruyer and Bloch (1984) have shown that cholesterol rigidizes phospholipid vesicles of different fatty acid composition in the following order: bisaturated lecithins > monounsaturated lecithins > diunsaturated lecithins. This selectivity would possibly lead to a specific segregation of the lecithin molecules containing unsaturated acids and therefore lower transition temperature from cholesterol–phospholipid domains, fluidizing the boundary lipids of UDPglucuronyltransferase and decreasing the Hill coefficient of the enzyme.

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