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ANALYSIS OF *p*-NITROPHENOL GLUCURONIDATION IN HEPATIC MICROSOMES FROM LACTATING RATS

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Abstract—In the present study, hepatic *p*-nitrophenol glucuronidation was analyzed comparatively in virgin female, lactating mother and nonlactating mother rats (the last two groups 19–21 days post-partum). Enzyme assays were performed in native and activated microsomal suspensions. Activation was achieved either by including UDP-*N*-acetylglucosamine in the incubation mixtures or by preincubating native microsomes with optimal concentrations of Triton X-100 or palmitoyl-lysophosphatidylcholine. When UDP-*N*-acetylglucosamine was used as activator, enzyme activity increased in both lactating (about 80% increment) and nonlactating mothers (about 30% increment) as compared with virgin females. From an analysis of the degree of activation by Triton X-100 and palmitoyl-lysophosphatidylcholine, it can be inferred that the pregnancy–delivery event decreased the latency of UDP-glucuronosyltransferase activity that was detectable even 3 weeks post-partum, irrespective of whether suckling newborns were or were not kept with their mothers (lactating and nonlactating mothers, respectively). The estimation of apparent V_{max} toward UDP-glucuronic acid in palmitoyl-lysophosphatidylcholine-activated microsomes, which allows an estimation of the amount of the enzyme, showed that lactation increased the number of catalytic units (about 40%). Hepatic UDP-glucuronic acid content was 70% higher in lactating rats than in other groups. The lipid composition and membrane fluidity (using 1,6-diphenyl-1,3,5-hexatriene as probe) were also analyzed in microsomes from all groups. A significant decrease in the unsaturation index that correlated with the rigidization of microsomal membranes was consistent with the changes in the degree of enzyme latency observed in lactating and nonlactating mothers. In conclusion, lactating rats exhibited enhanced *p*-nitrophenol UDP-glucuronosyltransferase activity as well as an increase in the hepatic content of UDP-glucuronic acid. These findings and the fact that lactation increased the liver to body weight ratio emphasize the role of the liver in the metabolism of planar phenolic derivatives in these circumstances.

Key words: glucuronidation; *p*-nitrophenol; lactation; microsomal lipid composition; rat liver

UDP-GT† (EC 2.4.1.17) consist of a family of closely related isoenzymes with different ontogeny, substrate specificity and inducibility [1, 2]. Their activity is largely recovered in the microsomal fraction of tissue homogenates, and is at least partially latent [3]. These enzymes catalyze the transfer of glucuronic acid from UDP-GA to a variety of endogenous metabolites and xenobiotics such as planar phenols, enhancing their solubility and facilitating their excretion into bile and urine [3].

Glucuronidation of *p*-nitrophenol, one of these compounds, is catalyzed by more than one UDP-GT isoenzyme [4] and appears to be influenced by sex hormones in several species [5]. In the rat, both pregnancy and treatment with estrogenic agents decrease biliary excretion of many organic anions [6, 7] and specific bilirubin UDP-GT activity [8]. In contrast, bile flow is elevated, and biliary excretion of bile acids and some xenobiotics is increased in lactating rats [9–12]. This phenomenon has been attributed to the role of prolactin in regulating bile secretory function [12]. During lactation, bilirubin conjugating capacity is increased slightly in the rat [8], whereas the influence of this period on *p*-nitrophenol glucuronidation is not known.

In the present study, *p*-nitrophenol UDP-GT activity was determined in hepatic microsomes from lactating-mother, virgin female and nonlactating-mother rats, the last two used as control groups. To estimate the actual *p*-nitrophenol glucuronidating capacity, enzyme activity was measured in the presence of UDP-N-AG, a presumed physiological activator of UDP-GT [13–15]. In addition, hepatic UDP-GA content was assessed since there are some indications that cosubstrate availability may be a determinant of glucuronidation "*in vivo*" [16, 17]. It was also of interest to know how far the lactation period could alter UDP-GT activity by modifications in the amount of enzyme, or by producing microsomal membrane changes. For this purpose, we also analyzed enzyme activity in native microsomes and in preparations pretreated with two membrane perturbants, namely: Triton X-100 and lysophosphatidylcholine. Microsomal lipid content and

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† Abbreviations: UDP-GT, uridine diphosphate-glucuronosyltransferase(s); UDP-GA uridine diphosphoglucuronic acid; UDP-N-AG, uridine diphospho-*N*-acetylglucosamine; DPH, 1,6-diphenyl-1,3,5-hexatriene; PLPC, palmitoyl-lysophosphatidylcholine; UI, unsaturation index; VC, virgin female rats; NLM, nonlactating-mother rats; and LM, lactating-mother rats.

membrane fluidity were also analyzed. The study of the lipid composition included cholesterol–phospholipid ratio, separation and quantification of phospholipid classes, and total lipid fatty acid composition.

MATERIALS AND METHODS

Chemicals

UDP-GA (ammonium salt), *p*-nitrophenol, D-saccharic acid 1,4-lactone, UDP-N-AG (sodium salt), Triton X-100, PLPC, DPH and acid-washed activated charcoal were from the Sigma Chemical Co. (St. Louis, MO). All other reagents were of the highest grade commercially available.

Animals

Adult female Wistar rats weighing 180–250 g were used throughout. They were fed *ad lib.* on a standard laboratory pellet diet and were allowed free access to water.

Three experimental groups were studied: virgin controls (VC), and two groups of mother rats studied 19–21 days after delivery. In one group (to evaluate the possible remaining effect of the pregnancy–delivery event), the animals were separated from their litters immediately after delivery (nonlactating mothers, NLM), while in the other group they were allowed to lactate (lactating mothers, LM). Litters were standardized to 10 pups. All animals were 18 weeks old when they were killed. Animals from the VC group were killed irrespective of the day of estrus, since in preliminary experiments we found no differences in either native UDP-GT activity or percent activation in female rats stratified for stages in the estrus cycle, in concordance with others [18].

Isolation of microsomes

All animals were deprived of food 18 hr before being killed. They were exsanguinated by cardiac puncture under pentobarbital anesthesia (50 mg/kg body weight) between 11:00 and 12:00, to avoid possible effects of diurnal variations. Livers were perfused *in situ* with ice-cold 0.9% NaCl solution through the portal vein and then they were promptly removed and weighed. The liver–body weight ratio was calculated. Microsomal pellets were obtained [19] and resuspended in 0.15 M Tris–HCl buffer (pH 7.40). Protein content of microsomal preparations was determined by the method of Lowry *et al.* [20], with bovine serum albumin as standard. Final microsomal protein concentrations were adjusted to about 16 mg/mL with the same buffer before enzyme assays.

Activation of microsomes

Two methods of activation were used to determine whether UDP-GT activity modifications were due to changes in enzyme latency, to changes in the amount of catalytic unit, or both.

Triton X-100 activation. Triton X-100, a membrane perturbant conventionally employed in *p*-nitrophenol glucuronidation assays [21], serves as a tool to estimate the influence of the membrane environment on glucuronidating activity [22–25]. Optimal Triton X-100 activation was established by mixing variable quantities of the detergent with pools of microsomes obtained from rats of each experimental group (0.025 to 0.5 mg detergent/mg microsomal protein). The mixtures were incubated at room temperature for 20 min, and then UDP-GT activity was assayed as described below. Maximal enzyme activities in all experimental groups were observed at a detergent concentration of about 0.05 mg/mg protein. Therefore, this detergent–protein ratio was systematically used for Triton X-100 activation.

Lysophosphatidylcholine activation. PLPC was incorporated in the incubation medium in excess with respect to normal lipid components of microsomes in order to displace endogenous lipids from the enzyme microenvironment. This methodology serves to abolish possible differences in membrane lipid composition between groups and was used both to estimate the influence of the enzyme environment on the glucuronidating activity and to estimate the amount of catalytic units. For the last purpose, V_{max} toward UDP-GA was calculated in all experimental groups [26].

The optimal concentration of PLPC was determined adding variable quantities of this detergent (0.05 to 1 mg/mg microsomal protein, previously dissolved in 0.15 M Tris–HCl buffer, pH 7.40) to mixtures containing an aliquot of pools of microsomes obtained from rats of each experimental group (about 0.8 mg of microsomal protein). After 2 min of incubation at room temperature, enzyme activities were assayed as described below. The optimal detergent–protein ratio was about 0.1 (w/w) for the three groups and was systematically used in the experiments.

Enzyme assay

Glucuronidation of *p*-nitrophenol was measured by the disappearance of substrate absorbance using a spectrophotometric method [27]. D-Saccharic acid 1,4-lactone (2 mM) was systematically included in the incubation medium to inhibit enzymatic hydrolysis of *p*-nitrophenol glucuronides. The optimal concentrations of the reagents in the mixtures were: 0.8 mM *p*-nitrophenol, 5 mM UDP-GA and 9 mM $MgCl_2$. The incubation medium also included native or activated microsomes (about 0.8 mg of microsomal protein). For activation, the microsomes were preincubated with Triton X-100, PLPC or added directly to incubation medium containing UDP-N-AG (final concentration 2 mM). The nucleotide was included in the mixtures at a concentration similar to that found in hepatocytes [28]. For kinetic studies performed using PLPC-activated preparations, enzyme activity was measured at different UDP-GA concentrations (0.5, 0.8, 1.5, 3.0, 5.0 and 10.0 mM) to estimate the apparent V_{max} value toward UDP-GA. The final volume was 0.4 mL (0.15 M Tris–HCl buffer, pH 7.40) for all experimental conditions. After incubation at 37° in a shaking water bath, the reaction was stopped with 2.5 mL of glycine buffer (0.2 M, pH 10.40). Time of incubation varied from 10 to 30 min, depending on each reaction rate, to maintain linearity since substrate concentration became rate-limiting when more than 60% of the substrate had reacted.

Kinetic analysis. Apparent V_{max} toward UDP-

GA was determined with the aid of a non-linear square-fitting program [29].

Determination of hepatic UDP-GA concentration

Hepatic UDP-GA concentration was measured by a one-dimensional paper-chromatography method [28]. About 2 g of tissue was weighed, and a 25% (w/v) homogenate was prepared in ice-cold 0.3 M $HClO_4$. After centrifugation at 5000 *g* for 10 min, nucleotides were separated from the supernatant by using activated charcoal. Elution of the nucleotides, evaporation of the eluent, and chromatography were performed as described by Zhivkov [28]. The spots corresponding to UDP-GA were eluted with water, and the nucleotide concentrations were determined spectrophotometrically at 262 nm.

Steady-state fluorescence anisotropy measurements

DPH (2 mM; 0.5 μL) in tetrahydrofuran was added to 4 mL of microsomal suspension (0.05 mg protein/mL) with rapid agitation. Another aliquot of the same suspension mixed with an equivalent volume of tetrahydrofuran was used as a reference blank to correct fluorescence intensities from non-specific light-scattering excitation. Samples were gently swirled for at least 1 hr at 37° in the dark to allow equilibration of the probe with membrane lipids. Measurements were made at 37° in a Perkin–Elmer spectrofluorometer equipped with two polarizers. DPH was excited at 352 nm, and its fluorescence was detected at 435 nm. The rotational motion of the probe molecule is characterized by the steady-state fluorescence anisotropy defined by

$$r = \frac{I_{vv} - G \cdot I_{vh}}{I_{vv} + 2 \cdot G \cdot I_{vh}}$$

where I_{vv} and I_{vh} are the fluorescence intensities parallel and perpendicular, respectively, to the plane of polarization of the excitation beam, and G, the grating correction factor, takes account of parallel diffraction anomalies introduced by the monochromator [30].

Lipid analysis

Aliquots of the suspended microsomes were extracted by the procedure of Folch *et al.* [31]. Phospholipids were separated by TLC with a double development [32] using the solvents $Cl_3CH/CH_3OH/NH_3/H_2O$ (70:25:25:1.5) in the first run and Cl_3CH/CH_3OH/acetic acid/H_2O (80:10:2:0.75) in the second run. Total cholesterol was determined by the cholesterol oxidase method [33]. Lipid phosphorus was measured by the method of Chen *et al.* [34].

Fatty acid composition was determined by gas–liquid chromatography of the methyl esters [35] in a GOW-MAC chromatograph model 580. A Krompex column filled with 15% DEGS on 100–120 chromosorb W-HP was used.

Statistical analysis

Results are expressed as means ± SD. Statistical analyses were performed using the Newman–Keuls multiple-range test [36], which includes ANOVA. Values of $P < 0.05$ were considered to be statistically significant.

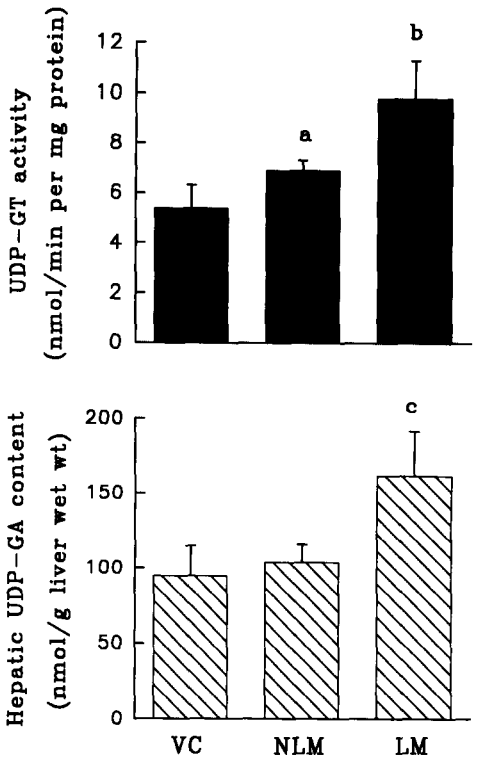

Fig. 1. *p*-Nitrophenol glucuronidation rate (closed bars) and hepatic content of UDP-GA (hatched bars) in virgin controls (VC), nonlactating mothers (NLM) and lactating mothers (LM;). The UDP-GT assay was carried out in the presence of UDP-N-AG (2 mM) as activator and fixed concentrations of *p*-nitrophenol (0.8 mM) and UDP-GA (5 mM) (N = 4–8). Hepatic content of UDP-GA was measured by a chromatographic method (N = 3). Error bars represent the SD. Key: (a) significantly different from VC ($P < 0.05$), (b) significantly different from VC and NLM ($P < 0.01$), and (c) significantly different from VC and NLM ($P < 0.05$).

RESULTS

Liver–body weight ratio

The liver–body weight ratio (expressed as percentage) was significantly higher in LM (4.77 ± 0.12) compared with VC (3.77 ± 0.14, $P < 0.01$) and NLM (3.70 ± 0.43, $P < 0.05$) (N = 4–8).

p-Nitrophenol UDP-GT activity

The glucuronidation rate of *p*-nitrophenol was measured in the presence of UDP-N-AG as enzyme activator (Fig. 1). Hepatic UDP-GT activity increased significantly in LM as compared with both VC and NLM. Enzyme activity was slightly higher in NLM than in VC.

The effects of Triton X-100 and PLPC on *p*-nitrophenol glucuronidation are depicted in Table 1. As was observed for UDP-N-AG-activated microsomes, enzyme activity assessed in the native preparation was somewhat increased in NLM as compared with VC. Native UDP-GT activity from

Table 1. Effect of Triton X-100 and PLPC on *p*-nitrophenol glucuronidation

	Native	Triton X-100	% Activation	PLPC	% Activation
VC	3.8 ± 0.5	20.0 ± 4.8	420 ± 48	44.6 ± 4.8	1077 ± 81
NLM	5.1 ± 0.7*	22.4 ± 2.8	329 ± 16‡	47.7 ± 3.9	830 ± 76‡
LM	7.5 ± 1.5†‡	30.0 ± 3.4†‡	306 ± 44‡	72.0 ± 6.8†‡	856 ± 108*

Hepatic *p*-nitrophenol glucuronidation in native and activated microsomes is expressed as nanomoles per minute per milligram of microsomal protein. For activation, microsomes were incubated with either Triton X-100, at a detergent–protein ratio of 0.05 (w/v), or PLPC, at a detergent–protein ratio of 0.1 (w/w). Enzyme assay was performed using fixed concentrations of *p*-nitrophenol (0.8 mM) and UDP-GA (5 mM). Values are means ± SD of 4–8 animals. Statistical analysis (see Materials and Methods) was used for comparison among all groups of enzyme activities and percent of detergent activation. Abbreviations: VC, virgin control; NLM, nonlactating mothers; and LM, lactating mothers.

* Significantly different from VC ($P < 0.05$).
† Significantly different from NLM ($P < 0.01$).
‡ Significantly different from VC ($P < 0.01$).

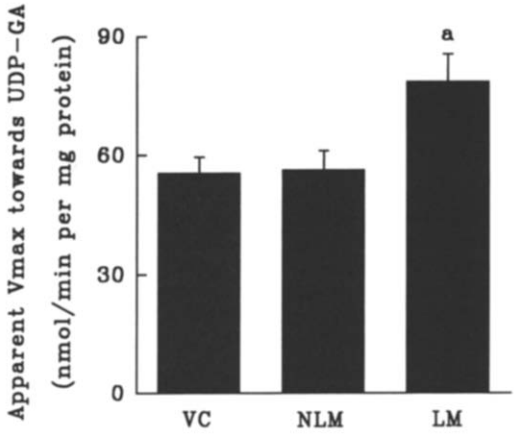

Fig. 2. UDP-GT activity estimated at an infinite UDP-GA concentration in virgin control (VC), nonlactating mothers (NLM) and lactating mothers (LM). Kinetic assays were performed using a fixed concentration of *p*-nitrophenol (0.8 mM),variable concentrations of UDP-GA (0.5 to 10.0 mM), and microsomes pretreated with 0.1 mg of PLPC per mg of microsomal protein. Apparent V_{max} toward UDP-GA was estimated using a non-linear square-fitting program. K_m values did not differ statistically between groups (data not shown). Values are means ± SD of 4–8 animals. Key: (a) significantly different from VC and NLM ($P < 0.05$).

LM was clearly higher than that from either VC or NLM. Percent increments in enzyme activity due to PLPC were invariably higher than those produced by Triton X-100, probably due to differences in their mechanism of action [22, 23, 26]. This parameter was significantly lower in the postpartum (NLM) and lactating period (LM) than in virgin females (VC), irrespective of the perturbant used. These results are indicative of a minor degree of UDP-GT latency in both lactating and nonlactating mothers compared with virgin controls. Figure 2 shows UDP-GT activities at apparent V_{max} toward UDP-GA for the three experimental groups. V_{max} was about 40% higher in LM than in VC and NLM, indicating an increase in the amount of isoenzymes glucuronidating *p*-nitrophenol in response to lactation.

Hepatic UDP-GA concentration

The hepatic content of UDP-GA is depicted in Fig. 1. This parameter was about 70% higher in LM than in NLM and VC, whereas no statistical difference was observed between the last two groups.

Membrane lipid composition and DPH fluorescence anisotropy

Cholesterol and phospholipid composition of hepatic microsomes is shown in Table 2. Phospholipid composition remained virtually unchanged in all the groups studied, but the cholesterol–phospholipid molar ratio was reduced in NLM and LM when compared with VC. This ratio also differed significantly between the NLM and LM groups.

Microsomal fatty acid composition is summarized in Table 3. It was found that in LM, microsomal fatty acid composition presented a significant decrement in linolenic (18:3), eicosatrienoic (20:3) and docosahexaenoic (22:6) acids and an increment in stearic acid (18:0) compared with VC. Furthermore, LM showed a higher content in stearic acid and a lower content in docosahexaenoic acid than NLM. It was also found that the pregnancy–delivery event affected membrane fatty acid composition, since contents in linolenic and docosahexaenoic acids were decreased significantly in NLM with respect to VC. As was expected, a progressive reduction in the unsaturation index (UI) was observed in NLM and LM compared with VC.

The fluorescence anisotropy (r) of the membrane was determined by a physicochemical method, as described in Materials and Methods. The results were: LM = 0.129 ± 0.005 significantly higher than VC = 0.117 ± 0.003 ($P < 0.001$) and NLM = 0.122 ± 0.002 ($P < 0.05$) ($N = 6$–8). The last two fluorescence anisotropy values also differed statistically ($P < 0.05$). These results indicate a rigidization of microsomal membranes in response to the pregnancy–delivery event; this rigidization was extended during lactation.

Table 2. Cholesterol and phospholipid composition of hepatic microsomal membranes

	VC	NLM	LM
Phospholipid composition (mol %)			
Phosphatidylcholine	53.5 ± 3.9	50.4 ± 0.9	54.4 ± 5.5
Sphingomyelin	3.8 ± 0.5	3.2 ± 0.3	3.9 ± 0.7
Phosphatidylethanolamine	27.1 ± 2.5	30.8 ± 1.1	30.0 ± 3.1
Phosphatidylserine + phosphatidylinositol	15.6 ± 1.1	15.6 ± 1.3	11.7 ± 2.6
Interlipid relationships (mol/mol)			
Cholesterol/phospholipid	0.16 ± 0.02	0.13 ± 0.01*	0.10 ± 0.01†‡
Phosphatidylcholine/sphingomyelin	14.4 ± 1.1	15.8 ± 1.7	13.9 + 2.0

Values are means ± SD of 4–8 animals. Abbreviations: VC, virgin controls; NLM, nonlactating mothers; and LM, lactating mothers.

* Significantly different from VC ($P < 0.05$).

† Significantly different from NLM ($P < 0.01$).

‡ Significantly different from VC ($P < 0.01$).

Table 3. Fatty acid composition of liver microsomes

Fatty acid	VC	NLM	LM
16:0	19.7 ± 1.0	23.4 ± 2.2	21.2 ± 2.9
16:1	2.5 ± 0.4	3.4 ± 2.8	2.1 ± 0.7
16:2	1.3 ± 0.5	0.9 ± 0.4	1.3 ± 0.4
18:0	20.9 ± 3.9	22.2 ± 2.0	28.9 ± 3.0*†
18:1	15.1 ± 1.6	12.3 ± 1.7	14.3 + 2.6
18:2	12.7 ± 2.3	16.6 ± 1.4	14.2 ± 2.3
18:3	6.9 ± 2.4	1.8 ± 0.3*	1.8 ± 0.7*
20:3	2.0 ± 0.4	1.0 ± 0.4	1.0 ± 0.2‡
20:4	13.0 ± 3.9	16.4 ± 3.2	14.2 ± 1.9
22:6	5.9 ± 2.1	2.0 ± 0.5*	1.0 ± 0.2*†
UI§	1.50 ± 0.10	1.39 ± 0.06*	1.12 ± 0.09*†

The methyl esters of the fatty acids were analyzed by GLC as described in Materials and Methods. Values indicate the relative content (% weight) of each individual fatty acid and are expressed as means ± SD of 3–6 animals. Abbreviations: VC, virgin controls; NLM, nonlactating mothers; and LM, lactating mothers.

* Significantly different from VC ($P < 0.01$).

† Significantly different from NLM ($P < 0.05$).

‡ Significantly different from VC ($P < 0.05$).

§ Unsaturation index (UI) = $\Sigma n_i \cdot x_i / FA$, where n_i = number of double bonds in each fatty acid, x_i = mol of each fatty acid, and FA = total mol of fatty acid.

DISCUSSION

The present study was undertaken to determine whether hepatic p-nitrophenol glucuronidating capacity in the rat may be modified during the lactating period and to give an insight into the possible mechanism involved in such a modification.

Three groups of animals were used in the experiments: virgin-female (VC), lactating-mother (LM) and nonlactating-mother (NLM) rats. The last group was included to evaluate possible remaining effects of pregnancy and delivery but without the influence of the lactation process. Thus, LM differs from NLM only in the physiological events related to lactation.

Enzyme activity was determined in native microsomes and in microsomal preparations activated with UDP-N-AG, Triton X-100 or PLPC. It was suggested that UDP-N-AG may be a key physiological activator of the enzyme [13–15], being effective in enhancing the conjugation of both endogenous and exogenous substrates. When the nucleotide was included in the incubation mixtures in a concentration similar to that found in the hepatocytes [28], lactating rats showed a higher UDP-GT activity not only with respect to VC but also with respect to NLM. This was emphasized by the fact that the liver to body weight ratio was also enhanced in LM (microsomal protein per gram of liver did not differ statistically between groups, data not shown). These findings and the fact that UDP-GA content was also increased in LM clearly indicate an improvement in hepatic capacity to detoxify planar phenolic derivatives.

One important feature of the transferase is its latency in microsomes: its full activity can be detected only after disruption of microsomal membranes by certain treatments [3] such as detergents. In fact, the percent increase in UDP-GT activity produced by a detergent reflects the average degree of restriction of all native p-nitrophenol glucuronidating isoenzymes in expressing their activities (latency).

In the present report, enzyme latency was studied using two different membrane perturbants: Triton X-100 and PLPC [22–26]. Even though the degree of enzyme activation differed between Triton X-100 and PLPC (see Table 1), both led to the same conclusion. The degree of latency clearly decreased both in rats that underwent the pregnancy–delivery process and in rats that underwent the same events followed by lactation. These findings could be a consequence of changes in the enzyme environment. When microsomal lipid composition was analyzed, changes in the relation of cholesterol to phospholipid as well as in the fatty acid pattern were observed, whereas phospholipid classes remained unchanged (see Tables 2 and 3). The cholesterol–phospholipid ratio decreases in rats during postpartum, and this decrement was magnified when suckling litters were kept with their mothers. Regarding fatty acid

composition, the decrement in linolenic (18:3) and docosahexaenoic (22:6) acids observed in NLM was extended in LM by a decrease in eicosatrienoic acid (20:3). It is known that an increase in the unsaturation index (UI) and a decrease in the cholesterol–phospholipid ratio affect membrane fluidity in an opposite way [37]; a higher UI increases fluidity, whereas a lower cholesterol–phospholipid ratio decreases it. Since NLM and LM showed a progressive increase in r values, the effect of fatty acid changes appears to exceed those of the cholesterol–phospholipid ratio and, in consequence, regulate membrane fluidity. In fact, the association between membrane fluidity (estimated by DPH) and the degree of unsaturation of fatty acids, irrespective of modifications in other parameters (e.g. cholesterol–phospholipid ratio) was observed previously in other circumstances when membrane preparations from humans, rats and guinea pigs were assayed [38–40]. Recently, Dannenberg *et al.* [26] reported a good correlation between a decrement in docosahexaenoic acid and an increment in the *p*-nitrophenol glucuronidating activity per enzyme molecule during development in rats. Therefore, although measurements of membrane fluidity and lipid composition may not reflect the characteristics of the microenvironment of UDP-GT, the changes in fatty acid composition observed in LM and NLM are consistent with the modifications in enzyme latency.

PLPC was also used to estimate the number of catalytic units involved in *p*-nitrophenol glucuronidation. This was achieved by calculating UDP-GT activity at infinite UDP-GA concentration (apparent V_{max} toward UDP-GA [26]). Using this approach, the enzyme activity in the lactating period was greater than in VC and NLM (see Fig. 2). In consequence, while the pregnancy–delivery event did not modify the amount of UDP-GT, lactation produced an increase of about 40% in the number of catalytic units.

Lactating rats differed from the others mainly in the fact that the suckling newborns were kept with their mothers. It is well established that the newborn suckling stimulus leads to an increase in plasma prolactin levels [41]. On the other hand, Zabala and García-Ruíz [42] described a role of prolactin in regulating phosphoenolpyruvate kinase activity (cytosolic form) by stimulating the expression of its specific mRNA in lives of lactating rats. Then, a modification in prolactin level or another mechanism related to the newborn suckling stimulus [43, 44] could be responsible for the enhanced amount of UDP-GT in response to lactation.

In conclusion, lactating rats exhibited an enhanced *p*-nitrophenol UDP-GT activity that can be explained both by a decrease in enzyme latency and by an increase in the number of catalytic units. This improvement in the enzyme activity, reinforced by the increment in the hepatic UDP-GA content, would be important to prevent the eventual transfer of active planar phenolic derivatives to the suckling newborns since, as it is well known, several drugs administered to lactating mothers are detectable in maternal milk [45].

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