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Composition and physical properties of lipids from plasma membranes of dog kidney

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Lipid composition, physical state of major phospholipid classes and transbilayer migration of phosphatidylcholine have been determined in plasma membranes of the dog kidney. The lipid composition of brush-border membranes markedly differs from that of antiluminal membranes with respect to: (a) the total phospholipid content; (b) the cholesterol to phospholipid ratio (C/P); (c) the distribution of the major phospholipid classes. Sphingomyelin present in large amounts in both luminal and antiluminal membranes extracts exhibits a transition of phase between 20 and 44°C approximately. In the range of temperature studied (5–55°C) no phase transitions were detected for the other phospholipid species. Our data suggest that: (1) at physiological temperature the higher C/P ratio of brush-border membranes is in large part responsible for their lower fluidity; (2) both the relatively low cholesterol and high sphingomyelin contents contribute to the thermotropic transitions observed in intact membranes. Finally transbilayer migration of phosphatidylcholine in brush-border membranes is a very slow process with a half time of 6.5 h at 37°C which compares with that of other biological membranes.

Introduction

The morphological polarity of kidney epithelial cells is associated with an asymmetrical distribution of enzymes, transport systems and hormones receptors between luminal and antiluminal plasma membranes [1–3]. Recently it has been shown that, like in the intestine [4,5], asymmetry also extends to the physical state of membranes [6–8], brush-border membranes being more rigid than basolateral membranes. This difference is maintained when considering corresponding liposome made from total lipid extracts. This suggests that the lipid composition of these membranes should differ. Such a difference is well established for the intestine [5,9–11] but the available information for the kidney remains limited and to some extent contradictory [8,12–14]. Thus only for the rat kidney was a direct comparison of the lipid composition of basolateral and brush-border membranes made and the recent results of Hise et al. [8] contrast with the conclusions presented by Bode et al. [12].

In addition, our studies on human and dog kidney have also demonstrated the existence of thermotropic transitions in a range of temperature of physiological interest [6,7]. These transitions attributable to lipid constituents which remain to be determined are likely to create defects in the

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Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol; lysoPC, lysophosphatidylcholine.

molecular structure of the membrane. To what extent such phenomenon may affect the transbilayer movement of phospholipids in brush-border membranes is unknown.

Accordingly the present experiments on the dog kidney were designed to answer the following questions: (a) does the lipid composition of basolateral and brush-border membranes differ and can this eventual difference explain the differences in membrane physical state; (b) what are the lipid classes responsible for the thermotropic transitions; (c) is the rate of transbilayer migration of phosphatidylcholine (PC) at 37°C much faster than in other biological membranes. The results indicate that besides marked differences in phospholipid distribution and fatty acid composition between basolateral and brush-border membranes the higher cholesterol to phospholipid molar ratio found in brush-border membranes must be a predominant factor, at 37°C, in the lower fluidity of these membranes. They strongly suggest that both the presence of sphingomyelin and the relatively low amount of cholesterol are responsible for the thermotropic transitions observed in intact membranes. Finally they indicate that the rate of transbilayer migration of PC in brush-border membrane at 37°C is slow and compares to that of other biological membranes.

Materials and Methods

Membrane preparation. Brush-border and basolateral membranes from the kidney of female mongrel dogs were purified as previously described [6,7] using a $CaCl_2$ precipitation method [15] and the method described by Scalera et al. [16], respectively. In brush-border membrane preparations, activities of alkaline phosphatase and γ-glutamyltranspeptidase were enriched 10–12-fold over that of cortical homogenate compared to less than 0.4-fold for $(Na^+ + K^+)$-ATPase, glucose-6-phosphatase and less than 0.2-fold for glucosaminidase. For basolateral membranes, the relative enrichment in $(Na^+ + K^+)$-ATPase or *p*-nitrophenylphosphatase activities was 10–17-fold compared to 2.4-fold for alkaline phosphatase, 1.8-fold for glucose-6-phosphatase and 0.2-fold for glucosaminidase.

Lipid analysis. Lipids were extracted using the acidic Bligh and Dyer method [17]. Extraction was repeated three times to obtain a 100% recovery using [^{3}H]cholesterol and [^{14}C]phosphatidylglycerol as markers. Extracts were pooled, evaporated to dryness under nitrogen and solubilized in 2 ml chloroform/methanol mixture (2 : 1, v/v). Thin-layer chromatography was done on precoated slica gel thin-layer plates (Whatman K_5, Baker Si 250) using benzene/diethyl ether/ethanol/acetic acid (50 : 40 : 2 : 0.2, v/v) as developing solvent for neutral lipids and either chloroform/methanol/water/acetic acid (65 : 25 : 4 : 1, v/v, phospholipid I) or chloroform/acetone/methanol/acetic acid/water (10 : 4 : 2 : 2 : 1), v/v, phospholipid II) for phospholipids. Individual components were detected by exposure to iodine vapors and identified by comparison with authentic standards. The spots were scraped off and transfered into acid-washed test tubes. Known amounts of cholesterol and defined phospholipids were treated in the same way to be used as internal standards. Cholesterol and cholesterol esters were determined chemically as previously described [18,19]. The phosphorus content of phospholipid classes was determined according to Broekhuyse [20].

For fatty acid analysis, lipids were extracted as described above excepted that 50 mg/l of butylated hydroxytoluene was added to prevent oxydation of polyunsaturated fatty acids. Lipid extracts were separated using the developing solvent 'phospholipid I'. After visualization by iodine vapors, phospholipid classes were eluted from the gel with 3 × 6 ml of chloroform/methanol (2 : 1, v/v). For PC, PE, PS + PI methyl esters were prepared as previously described [19]. Methyl esters of sphingomyelin were prepared using methanol/water/HCl (83 : 9.4 : 8.6, v/v, 70°C for 18 h). Methyl esters were subjected to gas-liquid chromatography in a Hewlett-Packard model 5720 A apparatus (columns contained the following: 15% DEGS WHE on 80/100 mesh; 10% Silar-10C on 100/120 Gas-chrom Q mesh). Fatty acids were identified by their retention times relative to the retention time of standard methyl ester mixtures (lipid standards 189-1, Sigma Chemical Co., St. Louis, MO, and GLC Ref Standards D-104, Serdary, Res. Lab., London, Canada).

Fluorescence polarization studies. Fluorescence

polarization measurements using 1,6-diphenyl-1,3,5-hexatriene as a probe were performed on a SLM 4000 apparatus (SLM Inc, Urbana, IL) as previously described [6,7]. Diphenylhexatriene was added to chloroform/methanol (2:1, v/v) solutions of phospholipid classes to maintain a molar ratio of approx. 1 molecule of probe for 300 phospholipid molecules. After drying of the solutions under nitrogen, dispersions of labeled phospholipids were prepared by adding to the test tube 2 ml of phosphate buffer (0.1 M phosphate/35 mM NaCl/1 mM KCl/2 mM $MgCl_2$, pH 7.2) preheated at 65°C. Several cycles of vortexing in the presence of glass beads provided homogeneous dispersions. Results of steady-state depolarization experiments are expressed in terms of fluorescence anisotropy, r, with $r=[I_{\parallel}-I_{\perp}]/[I_{\parallel}+2I_{\perp}]$. $I_{\parallel}$ and $I_{\perp}$ are the fluorescence intensities observed with the analysing polarizer parallel and perpendicular, respectively, to the polarized excitation beam.

Flip-flop experiments. Transbilayer movements of PC in brush-border membranes were studied using a phosphatidylcholine exchange protein, a gift of Dr. J.A.F. op den Kamp (University of Utrecht, The Netherlands) as described by Kamp and Wirz [21] and Van Meer et al. [22]. Liver microsomal membranes were prepared from fasting Sprague-Dawley rats injected intraperitoneally with 30 μCi of [*methyl*-^{14}C]choline (Amersham, Canada) 45 min before excision of livers and stored at −70°C until used [21]. The day of experiment an aliquot of the microsomal suspension was thawed, sonicated for 5 min in an ice bath and centrifuged twice at 20000 × *g* for 20 min. The supernatant containing the microsomes was used as a donor of ^{14}C-PC for exchange experiments. 0.35 ml microsomal membranes (1.2 μmol PC) was mixed with 0.25 ml concentrated brush-border membranes (0.7 μmol PC) in incubation buffer (0.15 M NaCl/0.001 M EDTA/0.01 M Tris (pH 7.4), 37°C), final volume 1.1 ml, containing or not 15 μg of the exchange protein (specific activity: 7000 nmol PC transferred/min per mg protein), under constant stirring. After various times of incubation ranging from 15 min to 6 h, 0.1 ml of the incubation mixture was withdrawn, diluted by 1 ml cold buffer and the brush-border membranes were pelleted by centrifugation (13000 × *g*, 15 min). After washing in the same buffer to remove the microsomes, the sample of brush-border membranes was dissolved in sodium lauryl sulfate (20 mM) for the subsequent determination of its ratioactivity and protein content. At the end of the experiments, an aliquot of the brush-border pellet was extracted using the Bligh and Dyer method [17] and the specific activity of PC determined. Calculation of the percentage of the phosphatidylcholine present in brush-border membranes which had equilibrated with microsomal PC was done as described by Van Meer et al. [22]. Calculations of the readily available pool of phosphatidylcholine as well as the half time for transbilayer migration of PC were done using a semilogarithmic plot of the percentage of the total phosphatidylcholine in brush border which had not equilibrated with microsomal PC as function of time [22].

Enzyme and protein determination. Activities of the marker enzymes ($Na^+ + K^+$)-ATPase, alkaline phosphatase and glucose-6-phosphatase were determined as previously described [6]. Ouabain-sensitive *p*-nitrophenylphosphatase activity, γ-glutamyltranspeptidase activity and glucosaminidase activity of membrane preparations were determined as described by Colas and Maroux [23], Glossmann and Neville [24] and Scalera et al. [16], respectively. Protein determinations were made by the method of Lowry et al. [25] after precipitation with 10% trichloroacetic acid using bovine serum albumin as a standard.

Results

Cholesterol and phospholipid contents of brush-border and basolateral membranes

Phospholipid and cholesterol content of dog kidney brush-border and basolateral membranes is given in Table I. The amount of free cholesterol present in both membrane types is similar (146 μg/mg membrane protein for brush-border vs. 130 μg/mg membrane protein for basolateral membranes; difference is not significant, n.s.). Cholesterol esters, however, are more abundant in basolateral membranes where they represent 25% of total cholesterol than in brush-border membranes where they account for only 11% of total cholesterol. The amount of total phospholipids

TABLE I

CHOLESTEROL AND PHOSPHOLIPID CONTENTS OF BRUSH-BORDER AND BASOLATERAL MEMBRANES

Values are means ± S.E. Value in parenthesis are numbers of dogs. * $P < 0.05$; ** $P < 0.01$.

	Brush-border		Basolateral
Cholesterol (μmol · mg protein^{-1})	0.38 ± 0.04 (8)		0.34 ± 0.02 (7)
Cholesterol esters (μmol · mg protein^{-1})	0.05 ± 0.01 (4)	*	0.13 ± 0.03 (4)
Lipid phosphorus (μmol · mg protein^{-1})	0.59 ± 0.03 (10)	*	0.73 ± 0.05 (8)
Cholesterol/phospholipid (mol · mol^{-1})	0.64 ± 0.04 (8)	**	0.47 ± 0.03 (7)

present in membranes is also significantly higher in basolateral than in brush-border membranes. Accordingly the ratio of free cholesterol to total phospholipids (C/P), largely below unity in brush border (0.64), is still significantly decreased in basolateral membranes.

Sphingomyelin is the most abundant phospholipid of brush-border membranes (Table II) whereas phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylserine (PS) and phosphatidylinositol (PI) account for 22, 17, 14 and 4%, respectively, of total phospholipids. Lysophosphatidylcholine (lysoPC) is present only in very small quantities. The phospholipid composition of basolateral membranes markedly differs: the sphingomyelin content drops to 24% while PE and PC increase to 29 and 27% of total phospholipids, respectively. Although the percentage of their negatively charged phospholipids is not significantly modified, basolateral membranes contain more PI than PS a situation which contrasts with that of brush-border membranes. The amount of lysoPC found in basolateral membranes is low and does not differ from that brush border membranes.

TABLE II

PHOSPHOLIPID COMPOSITION OF BRUSH-BORDER AND BASOLATERAL MEMBRANES

Data are means ± S.E. Number of animals: brush-border membranes nine dogs, basolateral membranes seven dogs. The sum of all fractions account for 98% of total lipid phosphorus in brush-border and basolateral membranes. Sph, sphingomyelin. n.s., not significant.

Phospholipid	mol% of the total		
	Brush-border	Basolateral	*P*
Sph	38.7 ± 0.9	23.5 ± 0.9	0.001
PE	22.1 ± 0.7	28.8 ± 1.0	0.01
PC	17.0 ± 0.7	27.8 ± 0.4	0.001
PS	14.4 ± 0.6	6.1 ± 0.4	0.001
PI	3.9 ± 0.4	9.1 ± 0.4	0.001
LysoPC	2.1 ± 0.4	2.6 ± 0.4	N.S.

Fatty acid composition of phospholipids

Fatty acid composition of the main phospholipid classes is compilated in Table III. In accordance with the determinations on other membrane types, the composition of sphingomyelin is characterized by (1) a high proportion of saturated fatty acids. (2) The presence of long chain fatty acids. (3) The presence of nervonic acid (C 24 : 1) as the principal monoenoic acid. Essentially because of the lower content in palmitic (C 16 : 0) and of the higher percentage in nervonic acids the sphingomyelin appears less saturated in basolateral than in brush-border membranes. Arachidonic acid (C 20 : 4), the major fatty acid of the PE species, represents 32 and 46% of the total fatty acids in basolateral and brush-border membranes, respectively. The other unsaturated fatty acids present in significant amounts are essentially oleic (C 18 : 1) and linoleic (C 18 : 2) acids. In contrast, palmitic acid accounts for about 50% of the total fatty acids found in PC. For this phospholipid the differences in fatty acid composition between the two plasma membranes are much less marked than for sphingomyelin and PE. Stearic (C 18 : 0), arachidonic and palmitic acids constitute 80% of the total fatty acids of the negatively charged phospholipids PS + PI, pooled for practical rea-

TABLE III

FATTY ACID COMPOSITION OF PHOSPHOLIPIDS EXTRACTS OF DOG BRUSH-BORDER AND BASOLATERAL MEMBRANES

Total phospholipid (sphingomyelin + PE + PC + PS + PI) calculated by multiplying the molar fraction of the fatty acid considered by the relative proportion of the phospholipid species in the membrane. BB, brush-border membranes; BL basolateral membranes. Fatty acids are presented by the number of carbon atoms: number of double bonds. Satd./Unsatd., ratio saturated/unsaturated fatty acids.

Fatty acid	mol/100 ml								Total phospholipids	
	Sphingomyelin		PE		PC		PS + PI			
	BB	BL	BB	BL	BB	BL	BB	BL	BB	BL
C 14:0	1.6	6.9		2.2	0.9	1.5	0.9	2.8	1.5	3.3
C 16:0	33.9	23.4	19.4	23.0	52.1	46.3	19.4	25.4	31.1	30.0
C 18:0	23.7	19.6	9.9	13.7	4.4	4.4	31.3	34.0	18.5	15.7
C 18:1		1.5	14.8	18.4	15.8	14.8	7.1	10.3	7.5	11.8
C 18:2			6.6	7.4	15.3	17.7	5.4	4.0	5.2	7.9
C 18:3							1.0	1.2	0.2	0.2
C 20:0	10.9	13.6							4.4	3.4
C 20:4			46.2	31.7	10.0	13.8	32.9	20.7	18.5	16.9
C 22:0	7.8	11.8							3.2	3.0
C 24:0	8.6	8.0							3.5	2.0
C 24:1	9.5	14.6							3.9	3.7
Satd./ Unsatd.	9.1	5.2	0.43	0.68	1.38	1.13	1.11	1.72	1.76	1.42

sons. Principally due to a decrease in the arachidonic content, the fatty acids of the PS + PI species are more saturated in basolateral than in brush-border membranes. The large change in the proportion of these two phospholipid species (Table II) does not allow for further analysis of the origin of this difference.

Physical state of phospholipids

Diphenylhexatriene-labeled dispersions of phospholipids in phosphate buffer were used to investigate the physical state of sphingomyelin, PE, PC and PS + PI isolated from three different preparations of basolateral and brush-border membranes (Fig. 1). Broad phase transitions extending from 18–22°C to 43–45°C are observed in the sphingomyelin species. The transition is, however, more pronounced for brush-border sphingomyelin because of a higher degree of order of the acyl chains towards the low temperature end. This difference in ordering vanishes for temperatures higher than 37°C. For the other phospholipid species tested there was no detectable phase transition of the range of temperature studied (5–55°C).

'Flip-flop' of PC in brush border

To further characterize the physical properties of kidney plasma membranes, the rate of transbi-

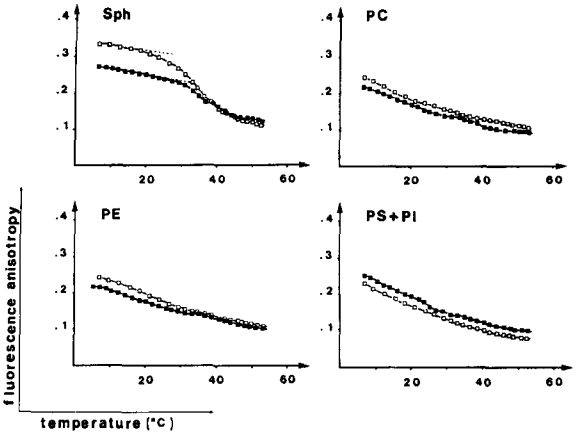

Fig. 1. Physical state of major phospholipid classes of brush-border and basolateral membranes. Representative experiment on phospholipid dispersions labelled by diphenylhexatriene as described in Materials and Methods. □, Lipids from brush-border membranes; ■, lipids from basolateral membranes. Sph, sphingomyelin.

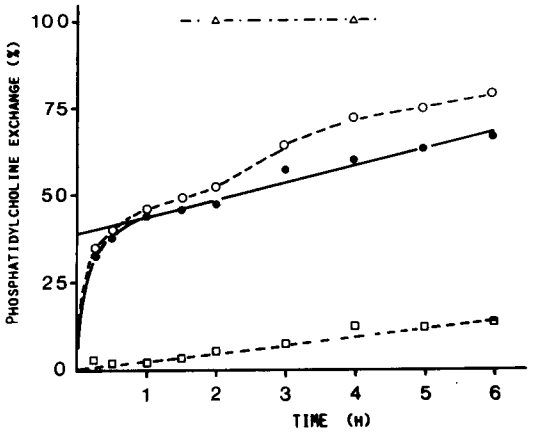

Fig. 2. Exchange of phosphatidylcholine from dog kidney brush border. Dog kidney brush-border membranes were incubated, at 37°C, with [^{14}C]phosphatidylcholine containing rat liver microsome (see Materials and Methods). The rapid exchange phase corresponds to the labelling of the external membrane leaflet. □, Brush-border membranes + microsomes; ○, brush-border membranes + microsomes + phosphatidylcholine-specific exchange protein; ●, net protein-dependent exchange of PC; △, brush-border membranes + microsomes + PC-exchange protein + Triton X-100 (0.05%).

layer migration of PC in brush-border membranes has been assessed by using the PC exchange protein technique [21,22]. Microsomes from rat liver were used as donors of [^{14}C]phosphatidylcholine, the labeling being achieved biologically by injecting intraperitoneally [*methyl*-^{14}C]choline to the rats. As shown in Fig. 2a rapid exchange which corresponds to the labeling of the external membrane leaflet is followed by a gradual additional incorporation of ^{14}C-PC in the membranes. This second pool of slowly exchangeable PC likely corresponds to the migration from the inner leaflet of unlabeled PC which becomes readily accessible for exchange. This hypothesis is supported by the observation that low concentrations of Triton X-100 (0.05%) suffice to allow a complete equilibration of the PC present in brush-border membranes in less than two hours. Using a semilogarithmic plot of the residual phosphatidylcholine [22] a half time of 6.5 ± 0.7 h for the 'flip-flop' rate of this phospholipid at 37°C was obtained from three experiments on three different membrane preparations. These experiments also indicate that 39 ± 5% of the PC present in the brush-border membranes are in the rapidly exchangeable pool, i.e. on the external leaflet of the membrane preparation.

Discussion

As predictable from fluorescence polarization experiments on liposomes made from total lipid extracts, the lipid composition of dog kidney brush-border membranes significantly differs from that of corresponding basolateral membranes. This observation, in accordance with the well established lipid asymmetry of intestinal epithelial cells also supports the conclusions of the recent study of Hise et al. [8] on the rat kidney plasma membranes. These data strongly suggest that the polarity of the plasma membrane of epithelial cells, besides enzymes and transport systems also extend to their lipids components.

In differences in the lipid composition reported here several points need to be examined more in detail.

(A) Purity of membrane preparations. Basolateral membrane preparations enriched 10–17-fold in specific marker enzymes were also slightly enriched in alkaline phosphatase (2.4-fold). Consequently the differences in lipid composition between the two plasma membranes reported here have to the considered as minimal differences.

(B) The total phospholipid content and the C/P ratio. Our results indicate that the C/P ratio is significantly lower in basolateral than in brush-border membranes. This observation compares well with what has been described for the intestine [5,9–11] but is at variance with data on the rat kidney [8,12]. This discrepancy seems attributable to the fact that, in contrast to the dog kidney, the amount of total phospholipids present in basolateral membrane from the rat kidney does not exceed that of brush-border membranes. The C/P ratio of dog kidney brush-border membranes, largely below unity (0.64) is identical to those determined by Quirk and Robinson [26] in rabbit and by Hise et al. [8] in rat.

(C) The distribution of phospholipid classes. Comparing the phospholipid distribution in brush border to that found in basolateral membranes, the most striking changes are the drop in sphingomyelin associated with the increase in PC and the reversed proportions of negatively charged

phospholipids. Similar modifications in the sphingomyelin-PC distributions were reported for the rat kidney plasma membrane by Hise et al. [8] and, although less marked, by Bode et al. (Ref. 12, Table IV). In that species, however, the PS-PI distribution in both plasma membranes was comparable. Possible explanation for the different distribution of phospholipids in brush border and basolateral membranes could reside in the activity of biosynthetic pathways at the level of the plasma membrane. Plasma membrane is a site of active synthesis of sphingomyelin from PC [27]. A lower activity of the enzyme(s) involved in this biosynthesis at the level of basolateral membranes would lead to the result observed, i.e. a decrease in percentage of sphingomyelin associated with an increase in PC, the total amount of PC + sphingomyelin being unchanged. Differences in activity of this biosynthetic pathway could also explain why for the rat brush-border membranes, Schwertz et al. [28] have described a phospholipid composition very similar to the one we obtained, whereas in the work of Hise et al. [8] and Bode et al. [12] the percentages of sphingomyelin were lower and those of PC higher. Finally it has to be pointed out that the percentage of lysoPC found in brush-border preparations is low and comparable to that of basolateral membranes. This implies that for the dog kidney brush-border membranes the activity of phospholipases A2 is not greatly, if at all, enhanced during the $CaCl_2$ precipitation step. This contrasts with the experiments on intestinal cells where the percentage of lysophospholipids is drastically increased by the use of the $CaCl_2$ precipitation method [29].

(D) The fatty acid composition. Besides the distribution of major phopholipid classes, the fatty acid composition of each class is different according to its basolateral or brush-order origin. The differences are, however, more pronounced when each class is taken separately than when the total fatty acid composition is considered. This suggests that deacylation-acylation processes at the level of the plasma membrane itself may take part to the observed differences between corresponding classes. The high proportion of arachidonic acid in PE and in PS + PI is in accordance with data obtained on a large number of systems (see Tables 23 and 24, Ref. 30) and, taking into account the relative proportions of the phospholipids species, with the data of Schwertz et al. [28] on LLC-PK_1 cells. Comparison with the global fatty acid composition of plasma membranes from rat kidney reported by Hise et al. [8] was done after transformation of the data of their Table 6 in terms of mol%. This form of expression of data allows to standardize the fatty acid composition of membranes having different total lipid contents. The results thus obtained show that in the rat as in the dog kidney: (1) The three major fatty acid species are C 16:0, C 18:0 and C 20:4. (2) There are only minor variations between basolateral and brush-border membranes in the relative amounts of the different fatty acid species.

From previous fluorescence polarization and ESR experiments it has been concluded that: (a) the order of membrane lipids is higher in brush-border than in basolateral membranes from human, dog, and rat kidneys [6–8]; (b) broad phase separations take place between approximately 20 and 42°C in both brush-border and basolateral membranes [6,7]. When pooled, sphingomyelin, PC, PE, PS and PI represent more than 95% of total membrane phospholipids. The anisotropy corresponding to the mixture of these phospholipids has been calculated using $\Sigma x_i \cdot r_i$ where x_i is the molar fraction and r_i the anisotropy of the phospholipid class under consideration. At 37°C values of 0.146 and 0.133 are obtained for brush-border and basolateral membranes, respectively, i.e., phospholipids of brush-border membranes are slightly more ordered than phospholipids of basolateral membranes. The difference in ordering is however much less marked than that observed for total lipids extracts which in the present experiment gave corresponding values at 37°C of 0.206 and 0.168. This observation can be explained by the higher C/P ratio found in brush-border preparations: it is well known that for a given phospholipid composition, an increase in the C/P ratio results in an increase in the order of membrane lipids [31]. Thus it is likely that, at physiological temperature, the difference in C/P ratios plays a predominant role in the difference in fluidity between brush-border and basolateral membranes.

In what concerns the existence of phase separation phenomena, our data strongly suggest that the thermotropic transitions observed in kidney

plasma membranes and their total lipid extracts are attributable both to the low cholesterol content (C/P ratios significantly below unity) and to the transitions of the sphingomyelin species. Thus (a) in basolateral as in brush-border membranes extracts the sphingomyelin species are the only phospholipid species showing a marked phase transition. (b) Temperatures of transitions correspond well to those observed in membrane preparations. Such high transition temperatures are not surprising: sphingomyelin of biological origin, because of their long chains and the high percentage of saturated fatty acids, often exhibit transitions in a domain of temperature physiologically relevant [32]. (c) Sphingomyelin constitutes a major part of the membrane phospholipids. Because of the localization of sphingomyelin in membranes [33] our data suggest that the thermotropic transitions detected primarily concern the external leaflet of the kidney plasma membranes.

Finally experiments using the phosphatidylcholine exchange protein indicate that, in dog kidney brush border membranes, about 40% of the PC is readily accessible for the exchange and suggest that in these membranes like in other biological membranes [34] the rate of PC 'flip-flop' is a very slow process. The value obtained (6.5 h) also suggests that the existence of a phase separation ending around 42°C [6] does not drastically alter the transbilayer migration of PC. In fact the real flip-flop rate is probably even lower if one considers that: (a) during the time of the incubation, part of the vesicles may have opened spontaneously; (b) part of the slowly exchangeable PC may correspond to 'masked' species present in the outer leaflet. However, due to the highly probable presence of all the sphingomyelin (39% of total phospholipids) plus 40% of PC (7% of total phospholipids) and certainly of a few percent of negatively charged phospholipids in the outer leaflet, the contribution to the process of 'masked' PC species must be minimal.

In conclusion our data demonstrate that the lipid composition of brush border is markedly different from that of basolateral membranes. They suggest, that the lower fluidity of brush-border membranes is, at physiological temperature, mainly attributable to their higher C/P ratio. They also suggest that both the sphingomyelin species and the low cholesterol content of membranes are responsible for the thermotropic transition observed in the corresponding membrane preparations. Finally, in spite of the thermotropic transition, the transbilayer migration of phosphatidylcholine in brush-border membranes remains, like in other biological membranes, a very slow process.

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