Lipid composition of lysosomal multilamellar bodies of male mouse urine

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Abstract Previous studies from our laboratory have shown that male C57BL/6J mice excrete into the urine multilamellar lysosomal bodies that contain specific neutral glycosphingolipids. These mice excrete approximately 20–30% of their kidney glycolipids each day. The significance and function of this secretion of multilamellar lysosomal organelles is unknown. To characterize these excreted bodies further, we report here their neutral lipid and phospholipid composition. The bodies were collected by differential centrifugation, extracted with chloroform–methanol, and lipids were fractionated into neutral lipids, glycolipids, and phospholipids. The neutral lipids consisted primarily of cholesterol, dolichol, and ubiquinone. The phospholipid fraction consisted primarily of a single molecular species of phosphatidylcholine. This lipid which comprises more than 90% of the total phospholipids was found to contain 16:0 ether and C22:6 n-3 fatty acid as determined by gas–liquid chromatography–mass spectrometry. The glycosphingolipids as reported previously consisted primarily of galabiosylceramides and globotriaosylceramides. This membrane lipid composition is different from any previously reported cellular organelle. —**Gross, S. K., P. F. Daniel, J. E. Evans, and R. H. McCluer.** Lipid composition of lysosomal multilamellar bodies of male mouse urine. *J. Lipid Res.* 1991. **32:** 157–164.

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The mouse kidney increases in weight in response to testosterone and this increase has been shown to involve hypertrophy in cell size, particularly of the proximal tubule cells. In male mice and testosterone-treated females there is a much higher level of lysosomal enzymes, such as β-glucuronidase and β-galactosidase (1), and large percentages of these enzymes are excreted into the urine each day (2, 3). We have previously shown (4) that this increase is accompanied by a concomitant increase in glycosphingolipids in both the kidney and urine of male and testosterone-treated female mice. The complement of urinary glycosphingolipids (GSLs) of male mice is very specific, consisting mainly of dihexosyl and trihexosylceramides, even though the particular pattern varies with the strain (5). When the urine is collected on ice most of the GSLs can be found in multilamellar bodies (MLBs), which are sedimented at 18,000 *g*. Upon examination of the kidneys by electron microscropy, similar MLBs can be seen in the proximal tubule cells and in the tubule lumen. Pigmentation mutants, which have an excretion defect, show an accumulation of lysosomal hydrolases, glycosphingolipids and also an increased number of membranous bodies in their kidneys (2, 6). It thus seems that lysosome-like organelles with a very specific composition are secreted into the urine. Here we report the lipid constituents of these unique membranous bodies of male C57BL/6J mouse urine.

MATERIALS AND METHODS

Animals

Male mice used for these investigations were produced from a breeding colony of C57BL/6J inbred stock maintained at the Shriver Center. All animals used were 6 weeks of age or older.

Abbreviations: Nfa, nonhydroxy fatty acid; Hfa, hydroxy fatty acid; HPLC, high performance liquid chromatography; AUFS, absorbance units full scale; GSLs, glycosphingolipids; SSEA-1, stage specific embryonic antigen 1, Gal(β1-4)[Fuc(α1-3)]GlcNAc-R; Dol, dolichols; FAME, fatty acid methyl esters; PC, phosphatidylcholine; GC-MS, gas chromatography–mass spectrometry; LC-MS, liquid chromatography–mass spectrometry; MLBs, multilamellar bodies; DMA, dimethylacetal; TLC, thin-layer chromatography; CI, chemical ionization; HPTLC, high performance thin-layer chromatography. Nomenclature used for glycosphingolipids is that recommended by IUAC (1976) *Lipids.* **12:** 455–468, GlcCer, glucosylceramide; GalCer, galactosylceramide; $GaOse_2$Cers, galabiosylceramides, Gal(α1-4)Gal(β1-1)Cers; $GbOse_3$-Cers, globotriaosylceramides, Gal(α1-4)Gal(β1-4)Glc(β1-1)Cer; $GbOse_4$-Cer, globotetraosylceramide, GalN Ac(β1-3)Gal(α1-4)Gal(β1-4)Glc(β1-1)Cer.

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Urine collection

Twenty four-hour urine samples from male mice were collected on ice. Twenty four mice, six per cage, were maintained in four polycarbonate metabolism cages (Fisher Scientific, Springfield, NJ) on ice for 24 h. Each collecting cup received 1.0 ml of sucrose (0.25 M). The urine samples were immediately centrifuged at 800 *g* for 10 min to remove cellular debris. The supernatants were centrifuged at 18,000 *g* for 20 min to obtain lysosomal MLBs as described by McCluer et al. (4).

Isolation of urinary lipids

One ml of water was added to the urinary lysosomal bodies and an aliquot of the suspension was taken for protein analysis (7). Chloroform (8 ml) and methanol (5 ml) were added and after 1 h at room temperature the suspension was centrifuged for 5 min and the supernatant was removed. The residue was re-extracted with methanol (2 ml) and chloroform (3 ml) and the supernatants were combined to constitute the total lipid extract. Chloroform and water were added to make a solvent ratio of chloroform–methanol–water 8:4:3 (8), the upper phase was discarded, and the lower phase was washed once with theoretical upper phase and then taken to dryness. The dried residue was taken up in 1 ml chloroform and placed on a Unisil column in a Pasteur pipet (125 mg Unisil, Clarksons Chemical Company, Williamsport, PA). The column was eluted with 5 ml of chloroform and the eluate was collected to obtain the neutral lipid fraction. The column was then eluted with 4 ml of acetone–methanol 9:1 (v/v) and the eluate was used for the analysis of the glycosphingolipids. Finally, 5 ml of methanol was used to elute the phospholipids.

Analysis of neutral lipids

The chloroform fraction from the Unisil column was analyzed for neutral lipids by TLC and by HPLC. Silica gel 60 HPTLC plates (E. Merck, Darmstadt, Germany) were used with a solvent system of hexane–diethyl ether–acetic acid 80:20:1 (v/v). Beef heart dolichol and ubiquinone standards were purchased from Sigma Chemical Co., and a neutral lipid standard mixture containing cholesteryl oleate, methyl oleate, triolein, oleic acid, and cholesterol was obtained from Supelco (Supelco Park, Bellefonte, PA). HPLC analysis was performed on a cyanopropyl column (4.6 × 100 mm; Brownlee, Santa Clara, CA) by a slight modification of the method of Palmer, Anderson, and Jolly (9). The sample components were eluted with hexane at a flow rate of 1 ml/min, detected by their UV absorbance at 210 nm, and peaks were integrated by Dynamax (Rainin Instrument Co. Inc., Woburn, MA) chromatography software on a Macintosh SE computer. Standards were made up in hexane and gave the following relative responses: cholesterol 1, dolichol 12.6, and ubiquinone 10.5. The molar abundance of individual dolichol species was determined by isocratic HPLC analysis on a 3-μm octylsilica reversed-phase column (4.6 × 33 mm; Supelco Inc.) in methanol–isopropanol 65:35 (v/v) with UV detection at 210 nm.

Analysis of glycosphingolipids

The acetone–methanol fraction from Unisil was used for the isolation and analysis of lower phase glycosphingolipids. The method of McCluer et al. (4) was used. Briefly, the acetone–methanol fraction obtained from Unisil was evaporated to dryness, and taken up in chloroform (1 ml); methanolic 0.6 M NaOH was added and after 1 h at room temperature the sample was neutralized with 0.4 M aqueous HCl. Chloroform and methanol were added to satisfy Folch conditions (8) and the upper phase was removed. The lower phase was washed twice with theoretical upper phase, taken to dryness, and perbenzoylated with benzoyl chloride in pyridine for HPLC analysis. HPLC was performed as reported previously (10).

Analysis of phospholipids

The methanol eluate from the Unisil column was used for phospholipid analysis. An aliquot was analyzed by HPTLC along with several standard phospholipids for identification purposes. The solvent system used was chloroform–methanol–water 65:25:4 and the lipids were visualized by staining with iodine vapors and by sulfuric acid charring (11). One major component comigrating with phosphatidylcholine (PC) was present along with several minor unidentified lipids. For further characterization, the fraction was analyzed by LC–MS with ammonia (0.6 Torr) chemical ionization on a Finnigan 4500 mass spectrometer as previously described (12, 13).

The procedure of Vishnubhatla, Kates, and Adams (14) for determination of diacyl, alkenylacyl, and alkylacyl glycerolphospholipids by GC was adapted to analyze the MLB phospholipid fraction by GC–MS. Samples of the phospholipid fraction equivalent to 0.35 mg of protein, plus 50 μg methyl heneicosanoate (21:0 FAME) internal standard, were methanolyzed by heating at 75°C for 90 min in 0.25 ml of 5% anhydrous methanolic hydrochloric acid in a 1-ml Reacti-Vial (Pierce Chemical Co., Rockford, IL). A biphasic system was then formed by the addition of 0.25 ml of distilled water and 0.25 ml of chloroform. After mixing, the phases were allowed to separate and the lower phase containing fatty acid methyl esters, fatty aldehyde dimethyl acetals, and lysoalkyl phosphatidic acids was removed to a clean Reacti-Vial. The upper phase was re-extracted twice with 0.25 ml of chloroform and the combined chloroform extracts were dried with a stream of nitrogen. The residue was dissolved in 100 μl n-hexane; 50 μl was removed to be used for monoalkyl gly-

cerol diacetate determination (see below) and the remainder was used for analysis by GC–MS of fatty acid methyl esters (FAME) and dimethylacetals (DMA) from any plasmalogen present.

GC–MS was performed with a Finnigan 4500 mass spectrometer equipped with a Hewlett Packard 5890 Gas Chromatograph and a Teknivent Vector/One GC–MS data system. Ammonia (0.6 Torr) chemical ionization was used under full scan (*m/z* 240–*m/z* 380) conditions to determine the FAME and DMA composition and then repeated under selected ion monitoring conditions for the $[M + NH_4]^+$ ion of each fatty acid for its measurement. A 30 m × 0.25 mm i.d. DB-225 (0.15 μm phase thickness) fused silica capillary column (J & W Scientific Inc., Folsom, CA) was directly connected to the mass spectrometer ion source and used with helium (20 psi) as the carrier gas. The column temperature was programmed from 180°C to 220°C at 4°C/min and held at the final temperature for 2 min to elute FAMEs from 14:0 through 22:6. Fatty acids were measured by comparison of the peak area response ratio of the $[M + NH_4]^+$ ions for the fatty acids of interest to the internal standard (FAME 21:0), with reference to those ratios obtained from standard FAME mixtures.

Monoalkyl glycerol diacetate in the methanolysate was measured by GC–MS after acetolysis. The hexane was evaporated and the samples were acetolyzed in 0.25 ml of acetic acid–acetic anhydride 3:2 (v/v) at 150°C for 48 h. The reagent was removed by evaporation via a stream of nitrogen and the residue was mixed vigorously with 0.4 ml of 90% methanol and 0.4 ml of n-hexane. The upper hexane phase was removed and the lower phase was extracted twice more with 0.4 ml of n-hexane. After removal of the solvent from the combined hexane extracts, the residue containing the monoalkyl glycerol diacetate was re-dissolved in n-hexane for GC–MS analysis. A 30 m × 0.25 mm DB-1 (0.25 μm phase thickness) fused silica capillary column (J & W Scientific, Inc. Folsom, CA) was used for these analyses. The column temperature was programmed from 200°C to 275°C at 7.5°C/min. Electron impact (70 eV) spectra were collected at 1.7-sec intervals over the range from *m/z* 40 to 460. Peak areas for extracted ion plots *m/z* 159 and *m/z* 340 (M^+) were measured for monoalkyl glycerol diacetate and the internal standard (21:0 FAME), respectively. The amount of monoalkyl glycerol diacetate present in the samples was calculated by comparison of the peak area ratio of monoalkyl glycerol diacetate to 21:0 FAME internal standard in the sample to that ratio obtained from separately processed known amounts of 1-O-hexadecylglycerol (Sigma, St. Louis, MO) also containing 21:0 FAME internal standard. Platelet activating factor (L-α-phosphatidylcholine, β-acetyl-, γ-O-hexadecyl, Sigma Chemical Co.) was also analyzed to validate the method.

RESULTS

Total neutral lipids

TLC analysis of the MLB neutral lipids in the chloroform eluate from Unisil (**Fig. 1**) indicated that ubiquinone, dolichol, and cholesterol were the major components, with much smaller amounts of triglycerides and other less polar constituents. Kidney neutral lipids gave a similar TLC profile (data not shown), with the exception of a greater abundance of triglycerides. Quantification of the major neutral lipids was achieved by normal phase HPLC on a cyanopropyl column, and the results are included in **Table 1**. The HPLC elution profile is shown in **Fig. 2**. Eight minor peaks (many of them fused) were eluted prior to ubiquinone (peak 1). Ubiquinone was the major component observed on HPLC because the UV response of cholesterol is one-tenth that of ubiquinone. The minor peaks probably correspond to dolichyl and cholesteryl esters, squalene, triglyceride, etc. There was at least one unknown component eluting between ubiquinone and dolichol that was not readily apparent by TLC analysis. Cholesterol was the most abundant neutral lipid (9.9 μg/mg protein), with smaller amounts of ubiquinone and dolichol (4.4 and 1.2 μg/mg protein, respectively). Comparable values for cholesterol, ubiquinone, and dolichol in male C57BL/6J kidney were 36.6, 9.1, and 2.1 μg/mg protein, respectively. Levels of triglyceride in

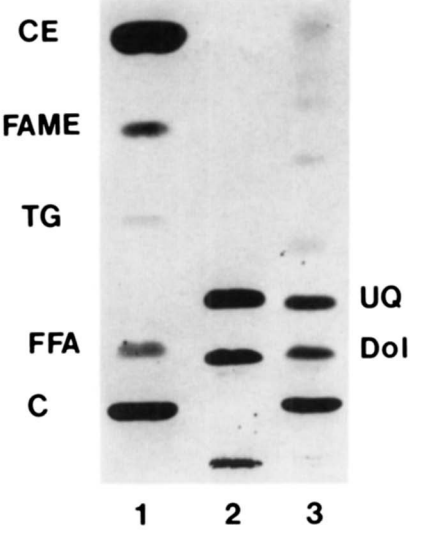

Fig. 1. HPTLC analysis of neutral lipids from multilamellar bodies isolated from male C57BL/6J urine. The neutral lipid fraction, prepared as described in Materials and Methods, was analyzed on a silica gel 60 HPTLC plate developed with hexane–diethyl ether–acetic acid 80:20:1 and components were visualized by charring with 50% sulfuric acid. Lanes 1 and 2 consist of the indicated standards: CE, cholesteryl ester; FAME, fatty acid methyl ester; TG, triglyceride; UQ, ubiquinone; Dol, dolichol; FFA, free fatty acids; and C, cholesterol. Lane 3, an aliquot of the MLB neutral lipid fraction.

TABLE 1. Lipid composition of urinary multilamellar bodies

Lipid	Protein	Moles/Mole Cholesterol
	nmol/mg	
Ubiquinone	5.1	0.2
Dolichols	0.9	0.04
Cholesterol	25.6	1.0
Total glycosphingolipids[a]	29.5	1.2
Total phospholipids[b]	160.0	6.3

[a]Galabiosylceramides ($GaOse_2Cer$); globotriaosylceramides ($GbOse_3Cer$).
[b]The phospholipids are estimated to be 92.5 mole % 1-O-hexadecyl-2-*cis*-4,7,10,13,16,19-docosahexaenoyl-3-phosphocholine.

kidney were 9.3 μg/mg protein (based on the UV response of standard triolein).

The relative abundance of each individual isoprenologue in the dolichol fraction isolated from mouse urine sediment was determined by reversed-phase HPLC. The following results were obtained (expressed as a percentage of total dolichol): Dol-17, 4.4%; Dol-18, 34.5%; Dol-19, 42.7%; Dol-20, 13.6%; Dol-21, 3.6%; and Dol-22, 1.1%. In contrast, the relative abundance of Dol-18 and Dol-19 was reversed in C57BL/6J kidney; there was also much less Dol-21 and only a trace of Dol-22. Interestingly, the dolichol profile in urine sediment from male C57BL/6J mice closely resembled the kidney dolichol profile in male light ear (le/le) and pale ear (ep/ep) mice; these are pigmentation mutants that express a defect in the excretion of MLBs in their urine (P. F. Daniel, M. H. Meisler, and R. H. McCluer, unpublished results).

Glycolipids

The lower phase glycolipids were isolated from the acetone–methanol eluate from Unisil and analyzed by HPLC. As previously reported (4), $GaOse_2Cers$ and $GbOse_3Cers$ were the major glycolipids present. Monohexosylceramides, such as GlcCer and GalCer and the tetrahexosylceramides ($GbOse_4Cers$), which are constituents of the male kidney glycolipids, were present at a very low level or completely absent in the urinary MLBs (**Fig. 3, Table 2**). Gangliosides and longer chain neutral glycolipids such as SSEA-1, present in the normal and mutant kidney, were also not detectable in the urinary multilamellar bodies.

Phospholipids

The methanol eluate from the Unisil column was analyzed for phospholipids. HPTLC showed that only a single phospholipid, migrating with the PC standard, was present in an appreciable amount; traces of other unidentified phospholipids migrating faster than phosphatidylethanolamine were observed when a large amount of the fraction was spotted (**Fig. 4**). LC-MS analysis of the phospholipid fraction showed a single molecular species of PC, 1-O-hexadecyl-2-docosahexaenoyl-3-phosphocholine, as the only identifiable PC. Interpretation of this spectrum is illustrated in the fragmentation diagram present in **Fig. 5**. The major ions present are MH^+(*m/z* 792), $[M + NH_4]^+$-$N(CH_3)_3$ (*m/z* 750), diglyceride ions (*m/z* 644 and 609), 22:6 fatty acid + NH_4^+ (*m/z* 346), and phosphocholine specific ion (*m/z* 142), which are all consistent with the proposed structure (12). GC-MS was used to confirm the identification of the PC molecular species and to measure the fatty acids and glycerol ethers in the PL fraction. The total amount of fatty acid was 131 nmol/mg protein with a mole percent distribution of: 22:6, 84.8%;

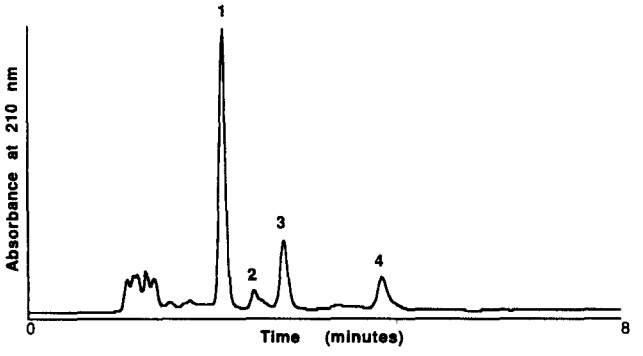

Fig. 2. HPLC analysis of neutral lipids from MLBs. The neutral lipid fraction was analyzed on a cyanopropyl Brownlee column (4.6 × 100 mm) eluted with hexane at a flow rate of 1 ml/min and detection at 210 nm. Peak 1, ubiquinone; peak 2, unknown; peak 3, dolichol; peak 4, cholesterol. Peaks were identified by comparison with standards as described in Materials and Methods.

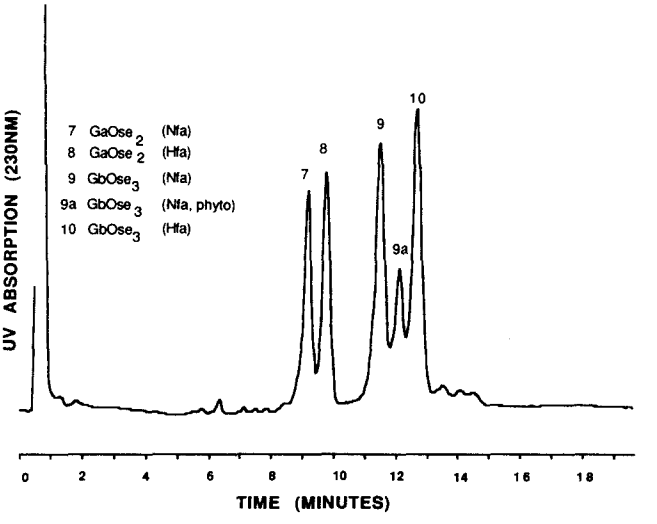

Fig. 3. HPLC analysis of perbenzoylated neutral glycolipids from urinary MLBs. The glycolipid fraction was isolated and perbenzoylated as described in Materials and Methods. The perbenzoylated lipids were analyzed on a Zipax column eluted with a gradient of dioxane in hexane at 2 ml/min and 230 nm detection as previously described (10).

TABLE 2. HPLC of glycosphingolipids in the urinary multilamellar bodies of male C57BL/6J mice

Peak No.	Peak Name	Protein	% of Total
		nmol/mg	
7	$GaOse_2$ (Nfa)	7.39	25.1
8	$GaOse_2$ (Hfa)	7.39	25.1
9	$GbOse_3$ (Nfa)	5.97	20.2
9[a]	$GbOse_3$ (Nfa, Phyto)	2.68	9.1
10	$GbOse_3$ (Hfa)	6.06	20.5
Total		29.49	99.0

Abbreviations: Nfa, nonhydroxy fatty acid; Hfa, hydroxy fatty acid; Phyto, phytosphingosine.

20:4, 5.6%; 16:0, 5.4%; 18:2, 1.8%; 18:0, 1.2%; and 18:1, 1.1%. The 22:6 FAME peak had retention time and mass spectrum identical to authentic methyl 4,7,10,13,16,19-docosahexaenoic acid, indicating that it is the C22:6 (n-3) fatty acid found in marine animals. No fatty aldehyde dimethyl acetals were observed by GC-MS in the phospholipid fraction indicating that plasmalogens are minor sample components, if present at all. GC-MS analysis of the monoalkyl glycerol diacetates in this fraction showed a peak for the 16:0 glycerol ether (107 nmol/mg protein) and a peak for 18:0 glycerol ether with less than 1% of the area of 16:0. This amount of 16:0 glycerol ether indicates that more than 90% of the fatty acids measured in the phospholipid fraction are bound to glycerol ether as the single molecular species, 1-O-hexadecyl-2-docosahexaenoyl-3-phosphocholine.

DISCUSSION

The results indicate that the urinary multilamellar organelles excreted by male mice have a unique lipid composition. The major neutral lipid components were identified by HPTLC, HPLC, and mass spectrometry to be cholesterol, dolichol, and ubiquinone as indicated in Fig. 1 and Table 1. The minor unidentified components migrating above dolichol are probably dolichyl and cholesteryl esters, squalene, and triglycerides. The major pool of dolichol in liver is known to be in lysosomes (15) and dolichyl esters and ubiquinone are also reported to be normal lysosomal lipid components (16). The reversed-phase HPLC analysis of the individual dolichol molecular species revealed a profile that resembled that obtained from dolichol isolated from the kidneys of male light ear and pale ear pigmentation mutants more closely than that obtained from normal male C57BL/6J kidneys. This result is consistent with the concept that normal male mice excrete a population of multilamellar lysosome-like bodies that accumulate in the kidneys of the pigmentation mutants because these organelles cannot be excreted.

Previous studies from this laboratory indicated that the glycolipids found in the 18,000 *g* sediment, as prepared in the present study, are associated with MLBs purified by Percoll gradient centrifugation (4). Further evidence that $GaOse_2Cer$ and $GbOse_3Cer$ are specific components of the lysosomal-like MLBs has been obtained from studies of the pigmentation mutants with excretion defects (2). The glycolipids most elevated in the kidneys of the pigmentation mutants with severe excretion abnormalities i.e., beige, light ear, and pale ear (6), are the same glycolipids that are normally excreted into the urine, namely $GaOse_2Cers$ and $GbOse_3Cers$. The urinary glycolipids of these mutants is less than 10% of that of normal mice. The data obtained in the present study are entirely consistent with the urinary glycolipid results obtained earlier (6). Although glycolipids are usually thought to be associated with plasma membranes, their association with lysosomes, or rather tritosomes, was reported as early as 1973 (17, 18). Chatterjee et al. (19) reported that lactosylceramide is localized in cytoplasmic bodies of human proximal tubule cells and Symington et al. (20) also reported that lactosylceramide is primarily located in cytoplasmic organelles.

The phospholipid composition of the urinary MLBs is unusual because PC is practically the only phospholipid present as revealed by HPTLC and LC-MS. It is estimated that PC constitutes more than 90% of the total phospholipids; LC-MS CI mass spectrometry of this

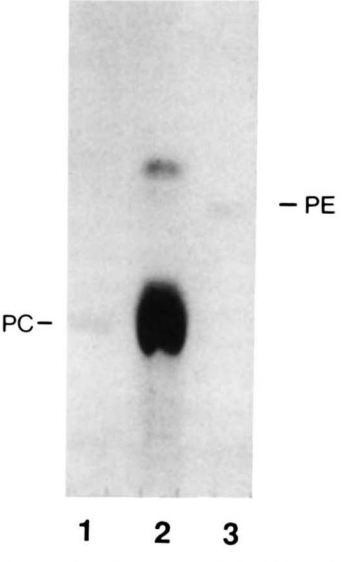

Fig. 4. HPTLC analysis of the phospholipid fraction from urinary MLBs. Approximately 100 μg of the methanol fraction from the Unisil column was spotted in lane 2. Lanes 1 and 3 contain approximately 2 μg of PC and phosphatidylethanolamine, respectively. The plate was developed with chloroform-methanol-water 65:25:4 and the lipids were visualized with iodine vapors. The plate was also examined with a phosphate spray and by charring as described in Materials and Methods. The minor component migrating above phosphatidylethanolamine has not been identified.

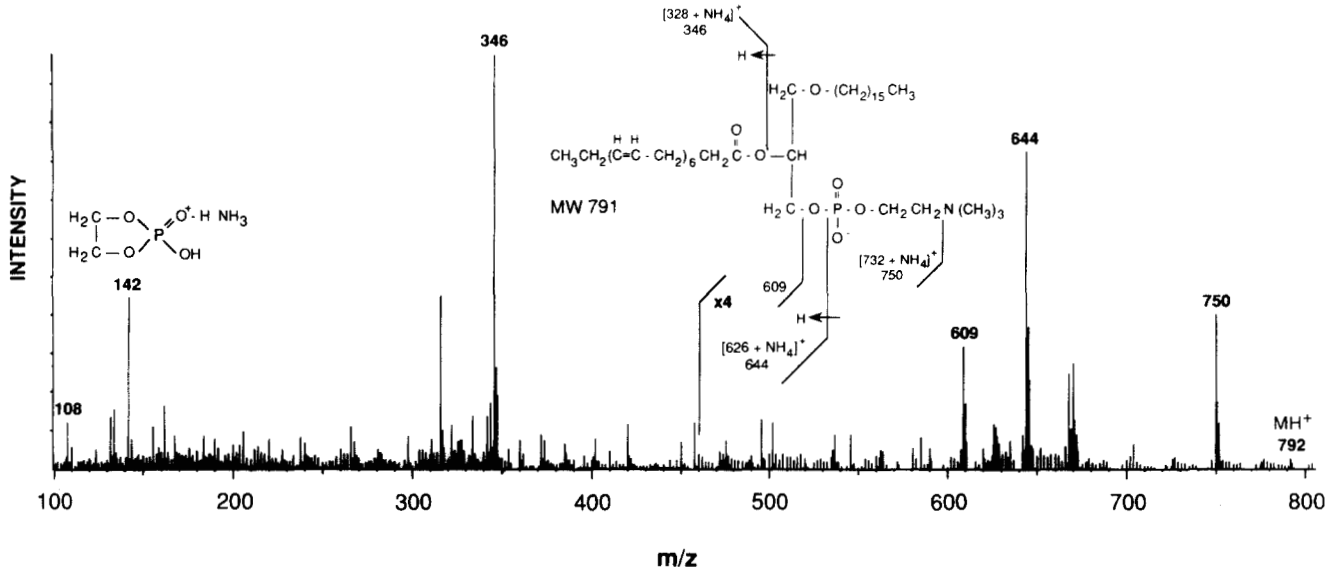

Fig. 5. Ammonia chemical ionization mass spectrum averaged over the PC peak from the LC-MS analysis of the phospholipid fraction of urinary MLBs.

phospholipid fraction showed only a single identifiable molecular species: 1-O-hexadecyl-2-docosahexaenoyl-3-phosphocholine. This identification was confirmed by GC-MS analysis of the fatty acid methyl esters obtained after methanolysis with methanol-HCl. The 22:6(n-3) fatty acid 4,7,10,13,16,19-docosahexaenoic acid was found to constitute 85% of all the fatty acids detected. The presence of essentially only 16:0 ether was confirmed by the GC-MS analysis of monoalkyl glycerol diacetates obtained after methanolysis and acetylation. Thus, HPTLC, LC-MS, and analysis of the methanolysis products are all consistent with the conclusion that about 85% of the phospholipids from the urinary MLBs consist of a single molecular species of PC. As far as we are aware, this degree of uniformity in the phospholipid composition of a biological membrane has not been previously reported and can be postulated to have some relevant significance in terms of membrane properties and behavior.

Morphological studies by Koenig et al., (21, 22) showed a high content of vesicular organelles in kidneys and urine of testosterone-treated female mice. These studies indicated that testosterone induces an increased production of lysosomes that are discharged, apparently by exocytosis, into the tubule lumen and subsequently appear in the urine as the MLBs. The parallel excretion of these bodies and lysosomal enzymes supports the concept that these bodies are secondary lysosomes or teleolysosomes. The neutral lipid composition of the urinary MLBs is consistent with previous reports on the composition of lysosomal membranes (17, 23). Thus, several lines of evidence indicate that these bodies are lysosomes: *1*) morphologically similar organelles stain for acid phosphatase in the kidney proximal tubule cells (24, 25); *2*) there is a parallel induction and excretion of lysosomal enzymes and MLB glycolipids in developing male and testosterone-treated female mice; and *3*) there is an accumulation of lysosomal enzymes and morphologically abnormal MLBs, which apparently cannot be excreted (6) in pigmentation mutants with blocks in the secretion of lysosomal enzymes.

However, the unusual phosphatidylcholine in these bodies and the absence or presence of only a small amount of phosphatidylethanolamine, sphingomyelin, phosphatidylserine (usual lysosomal components), and bis(monoacylglycero)phosphate, a generally accepted lysosomal lipid marker (26-30) suggest that it may not be useful to refer to these organelles as lysosomes. It should be noted that the urinary MLBs studied here bear a striking morphological similarity to the lamellar bodies of type II pneumocytes (31). Lung surfactant, a lipid-protein complex that lines the alveoli, is stored in the type II pneumocytes in characteristic multilaminar organelles known as lamellar bodies, which are secreted into the liquid layer covering the alveolar surface. Lamellar bodies and surfactant contain a characteristically high concentration of dipalmitoylphosphatidylcholine and the phospholipids of human lung lamellar bodies contain 70% PC. The bodies contain lysosomal enzymes, but it has been shown that lamellar body fractions purified from human lung contain a distinct α-glucosidase distinguishable from lysosomal acid α-glucosidase (31). Thus, it has been concluded that even though lamellar bodies contain a spectrum of lysosomal-type enzymes, they are distinct organelles that can be isolated free of lysosomes. It will be of interest to analyze the proteins of these urinary MLBs

and determine the presence or absence of the characteristic lysosomal membrane-associated glycoproteins, (LAMP-1 and LAMP-2). The protein composition may help establish their relationship to proximal tubule cell lysosomes. It may be that the MLBs formed in the mouse kidney proximal tubule cells and excreted into the tubule lumen play a protective role analogous to the lung lamellar bodies. Because the glycolipids in these bodies are almost entirely $GaOse_2Cer$ and $GbOse_3Cer$, which are known receptors for Shiga and Shiga-like toxin (32), and $GbOse_3Cer$ is also a receptor for uropathogenic *E. coli* (33), we propose that the excretion of MLBs that contain receptors for toxins and pathogenic bacteria may provide protection from these agents.

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