Urinary cholesterol: its association with a macromolecular protein-lipid complex

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Abstract The cholesterol-containing complexes in the urine of normal subjects and patients with diseases accompanied by hyperexcretion of urinary cholesterol were characterized. In normal subjects, the major portion of the recovered urinary cholesterol was eluted in the void volume fractions after gel chromatography on Bio-Gel A-5m; this suggested an association with a macromolecular complex above 5×10^6 daltons. A comparable elution pattern was seen in most of the urines of the patients with benign or malignant diseases of the kidneys or the urogenital tract. However, in single patients with hyperexcretion of urinary cholesterol, considerable amounts of cholesterol were detected in the included volume of the column. This was caused by additional excretion of high density lipoproteins or both high and low density lipoproteins in the urine which could be identified in these fractions by agarose electrophoresis and immunodiffusion. These results indicate that the macromolecular complex represents the majority of the recovered urinary cholesterol in normal subjects and in disease states with known hyperexcretion. Macroscopically, the isolated cholesterol-containing complex in the void volume fractions was turbid, and electron microscopy showed lipoprotein-like particles with diameters ranging from 300 to 700 Å. The chemical analysis revealed median values of protein (46.0%), triglycerides (16.3%), cholesterol (8.2%), and phospholipids (29.5%) in normal subjects and comparable results in the patients with benign or malignant diseases of the kidney and the urogenital tract. Ethanolamine glycerophospholipids, phosphatidylcholine, sphingomyelin, and phosphatidylserine were the main phospholipid components. After ultracentrifugation in a CsCl gradient, the cholesterol-containing complex was found between densities 1.1 and 1.3 g/ml. By SDS polyacrylamide electrophoresis, up to 17 protein subunits in the molecular weight range of 14,000 to 87,500 were separated. Immunodiffusion studies showed in about 40% precipitin lines against anti-human albumin, but no reactions against anti-human apoHDL and anti-human apoLDL. However, immunodiffusion of the macromolecular complex against anti-liver-specific and anti-kidney-specific lipoproteins revealed single precipitin lines. In conclusion, the isolated cholesterol-containing urinary complex showed many characteristics of membrane-associated protein-lipid particles of the human kidney and even the liver. These proteolipids are the major source of urinary cholesterol in normal and disease states. They could be derived partially from the kidney, but in addition possibly from other parts of the urogenital system, especially the bladder.—**Jüngst, D., H. Weiser, E. Siess, and H. J. Karl.** Urinary cholesterol: its association with a macromolecular protein-lipid complex. *J. Lipid Res.* 1984. **25:** 655–664.

Supplementary key words gel chromatography • ultrafiltration • SDS-PAGE electrophoresis • immunodiffusion • lipoproteins

Small amounts of cholesterol, mainly nonesterified, are present in normal urine (1–7), whereas elevated levels have been reported in patients with benign and malignant diseases of the kidney and the urogenital tract (8–27). On the basis of ultrafiltration studies, excretion as a protein-bound complex has been suggested (28). In normal urine most of the cholesterol seems to be associated with a light particulate fraction, similar to plasma membranes (29). However, in patients suffering from the nephrotic syndrome, a urinary loss of plasma high density (HDL) and in some cases also of low density (LDL) lipoproteins has been reported (30, 31).

It was the aim of this study to characterize the cholesterol-containing complexes in the urine of normal subjects and in disease states with known hyperexcretion. We demonstrate that cholesterol in the urine is associated mainly to a macromolecular protein-lipid complex which might be of cellular origin. In addition, in some cases HDL and LDL could be identified, especially in patients with the nephrotic syndrome.

MATERIALS AND METHODS

Normal subjects and collection of urine

The group of normal subjects included four men and five women, 18 to 46 years old, who had no history or clinical or biochemical signs of a renal or urogenital disease. Microscopic examination of the urine samples was normal, as well as the results of the N-Multistix reagent strips for protein, glucose, bilirubin, blood cells, ketone

Abbreviations: HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein; GLC, gas–liquid chromatography; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; LSP, liver-specific lipoprotein; KSP, kidney-specific lipoprotein; TLC, thin-layer chromatography.

bodies, nitrate, and urobilinogen. The concentrations of creatinine and total protein in serum were normal in all cases and the excretion of urinary protein was below 100 mg/24 hr (Table 1). During the collection period of 24 hr the urine was kept refrigerated (4°C) and sodium azide (0.02%) was added to prevent bacterial growth. Fractionation of urinary cholesterol started within 1 hour after the end of the collection period.

Patients

The group of patients consisted of 12 with benign and 9 with malignant diseases of the kidney or the urogenital tract. Diagnoses were established by routine clinical procedures including urinary protein quantitation, intravenous pyelography, cystoscopy, renal ultrasound, and histological examination of biopsies or resected material. Further clinical data of these patients including protein and creatinine in serum and 24-hr excretion of protein in the urine are given in Tables 2 and 3.

Fractionation of urinary cholesterol

For fractionation of urinary cholesterol usually the whole 24-hr urine volumes were used, and were passed through a Selecta filter no. 1117 1/2 (Schleicher Schüll). This filtration removes larger particles (5–10 μm in diameter) such as intact cells and larger cellular debris. After filtration NaCl was added to the urinary samples (final concentration 0.56 M) to precipitate the Tamm-Horsefall mucoprotein (32). This was removed by centrifugation (8000 rpm for 10 min) and the supernatant, considered as the upper 90% of the volume, was collected. Recovery studies of urinary cholesterol in single specimens revealed a loss of ~10% during these procedures. Ultrafiltration was done in a stirred cell (Amicon model 402 and Amicon model 52) using PM 10 membranes at 4°C to a final volume of 3–5 ml. This concentration step was the most critical in regard to the loss of urinary cholesterol since some of the lipid perhaps adhered to the PM 10 membranes or might even pass the membrane. Aliquots of 2–3 ml of the concentrated urine were used for gel chromatography on a 1.2 m × 2.0 cm Bio-Gel A-5 m column (BioRad), mesh 200–400, according to Sata, Havel, and Jones (33). Elution was performed with 0.15 M NaCl in water containing 0.01 M EDTA and 0.02% NaN_3 at pH 7.0. Usually 48 to 50 fractions (8.5 ml) were collected. Absorbance at 280 nm was determined in a spectrophotometer (Beckman, model 24).

Total cholesterol was measured by gas–liquid chromatography. The cholesterol-containing fractions were eluted usually at the void volume. These fractions were pooled and concentrated with PM 10 membranes (Amicon) to a final volume of 1–2 ml. Further characterization was done by chemical assay, electron microscopy, density gradient ultracentrifugation, immunodiffusion, agarose electrophoresis, and SDS-polyacrylamide gel electrophoresis. Cholesterol-containing fractions in the included volume of the column were studied separately by agarose electrophoresis and immunodiffusion. A considerable amount of urinary cholesterol was lost as a consequence of the different fractionation procedures, especially the two ultrafiltration steps. Recovery studies in single urinary specimens for cholesterol showed a total loss of urinary cholesterol between 30 and 50% at the end of the second ultrafiltration.

Chemical assays

Total cholesterol was analyzed in 1.0-ml aliquots of the 24-hr urine collection and the 8.5-ml fractions after gel chromatography. After extraction with 8 ml of chloroform–methanol 3:1 (v/v) and centrifugation, 5 ml of chloroform was removed and dried under a stream of nitrogen. The residue was hydrolyzed with 0.5 ml of ethanolic KOH at 60°C for 60 min and reextracted with 6 ml of n-hexane and evaporated. The residue from 5 ml of the extract was dissolved in 0.1 ml of the internal standard solution (10 mg of androstendione/dl of isooctane). Gas–liquid chromatographic determination of cholesterol was performed using a 1.8 × 2 mm, 1% XE 60 column, column temperature 220°C, with flame ionization detectors.

Triglycerides were measured enzymatically with commercial test kits (Boehringer Mannheim) (34).

Phospholipids in the lipid extracts (chloroform–methanol 3:1, v/v) were determined by phosphorus analysis (35). The phospholipids were fractionated by TLC on 20 × 20 cm glass plates coated with silica gel (Merck). After two-dimensional TLC in the solvent systems chloroform–methanol–25% aq. ammonia 60:20:5 and chloroform–acetone–methanol–acetic acid–water 3:4:1:1:0.5, the separated phospholipids were made visible with iodine vapor; they were individually recovered and assayed for phosphorus content (36).

Protein was measured by the method of Kashyap, Hynd, and Robinson (37).

Electron microscopy

Electron microscopy was performed with a Siemens Typ 101 instrument and a negative staining technique (38).

Density gradient centrifugation

Ultracentrifugation was performed on a CsCl-gradient (density 1.05–1.35 g/ml) at 50,000 rpm in a Beckman SW-60 rotor for 68 hr. Eleven or 12 fractions were obtained by pipetting from the top. Absorbance at 280 nm

and total cholesterol content were measured in each fraction (39).

Electrophoresis and immunodiffusion

Electrophoretic separation was done on 1% agarose gels according to Noble (40). For SDS-polyacrylamide gel electrophoresis, aliquots of the fractions were lyophilized and then mixed with Tris-HCl (37.5 mM, pH 8.8) containing 2% SDS, 4% 2-mercaptoethanol, and 10% sucrose. After incubation for 2 hr at 50°C, protein subunits were separated in 1.5-mm vertical slab gels using 4–22.5% acrylamide concentrations (41). After fixing the gel for 30 min in 20% (w/v) trichloracetic acid, the proteins were stained with Coomassie brilliant blue (G 250). Molecular weights were estimated comparing with the low molecular weight calibration kit (Pharmacia Fine Chemicals) (42).

Ouchterlony immunodiffusion was performed in 1.5% agar (Difco) at 20°C on glass plates (5 × 2 cm) for 48 hr. Antisera against human serum, human albumin, α- and β-lipoproteins were obtained from Behring (Marburg). Special antisera against cellular lipoproteins of liver (anti-LSP) and kidney (anti-KSP) were kindly provided by U. Behrens, Immunopathology Laboratory, VAM Center, Bronx, NY. The antibodies against α- and β-lipoproteins were identical with antibodies to apoHDL and apoLDL, respectively. The manufacturer used LDL and HDL isolated by preparative ultracentrifugation from normal human plasma for immunization. Density intervals of 1.020–1.050 and 1.063–1.21 g/ml were used for LDL and HDL antigen isolation, respectively. The anti-HDL and anti-LDL antibodies did not react with lipoprotein-free plasma obtained by preparative ultracentrifugation of plasma at d > 1.25 g/ml. There was no cross-reactivity between anti-HDL with LDL and anti-LDL with HDL.

For the preparation of antibodies against LSP and KSP, the antigens were isolated by Behrens from normal human liver and kidney that showed no significant histologic changes nor evidence of autolysis (43). Essentially the same method was used as described by McFarlane et al. (44). Tissue slices were washed free of blood in 0.25 M sucrose, adjusted to pH 8.0 with 1 M Tris, and then homogenized. The homogenate was centrifuged at 100,000 *g* in a SW-27 rotor and the supernatant was placed on a column of Sephadex G-100 (gel bed 90 × 2.5 cm), previously equilibrated with 0.1 M Tris-HCL–0.2 M NaCl–1 mM EDTA, pH 8.0 (Tris/EDTA buffer). The first peak fractions were concentrated and then chromatographed on a column of Sepharose 6 B using the Tris/EDTA buffer for equilibration and elution. The first peak material contained LSP and KSP and was further subjected to ultracentrifugal flotation in a CsCl gradient.

LSP and KSP were recovered between the densities 1.107 and 1.202 g/ml. Analysis by SDS-PAGE revealed that the relative mobilities of LSP and KSP components were similar and the molecular weights of the monomers ranged from 40,000 to 96,000. At least seven out of eight recognizable LSP and KSP bands had the same R_f value, with one of them corresponding to that of human albumin. Antisera against the purified antigens were produced in rabbits and absorbed with lyophilized human plasma. Anti-LSP sera were tested by indirect immunofluorescence and immunoenzyme light microscopy on cryostat sections. They reacted with cell membranes not only of liver cells but also of smooth muscle fibers, thyroid follicles, intestine, spleen, kidney tubules, and glomeruli (43). Identical staining patterns were obtained with anti-KSP sera by Behrens and Paronetto (43). These findings of Behrens and Paronetto demonstrate the non-organ specificity of LSP and KSP. By immunodiffusion anti-LSP serum formed precipitin lines with LSP and KSP, as well as with SDS- or papain-solubilized isolated liver cell membranes (43). No reaction of anti-LSP and anti-KSP sera was seen by immunodiffusion against human albumin or total human plasma. These observations were made with the anti-LSP and anti-KSP sera used in this study.

RESULTS

Normal subjects

After gel filtration on the Bio-Gel A-5 m column, urines of normal subjects showed similar elution patterns. The characteristic profile illustrated in **Fig. 1** shows that the major portion of the cholesterol recovered from urine was eluted in the void volume. The total amount of urinary cholesterol in the 24-hr samples and the percentage

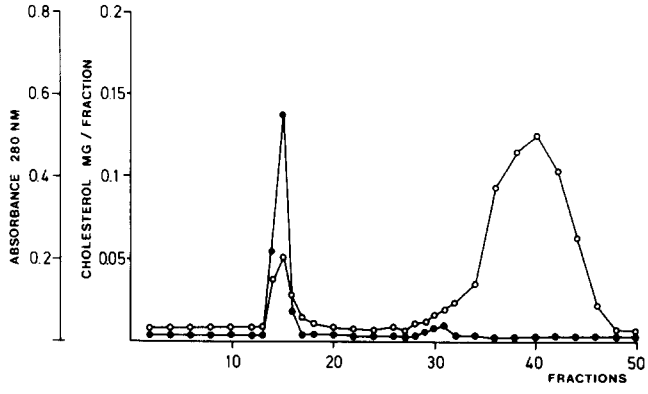

Fig. 1. Gel chromatographic elution pattern of a concentrated urinary sample from a normal subject on Bio-Gel A-5m. Column size 120 × 2.0 cm. Individual points represent values of cholesterol (● —— ●, mg/10-ml fraction) and the absorbance at 280 nm (○ —— ○).

TABLE 1. Clinical data and relative percentage of cholesterol in the void volume fractions after gel chromatography on the Bio-Gel A-5m column in normal subjects

Subject	Serum Protein	Serum Creatinine	Urinary Protein	Urinary Cholesterol	% Cholesterol in Void Volume
	g/dl	*mg/dl*	*mg/24 hr*	*mg/24 hr*	
A	7.8	0.8	65	0.6	84.0
B	8.3	0.7	50	0.9	96.0
C	7.4	1.0	42	0.4	93.4
D	7.9	0.6	83	1.8	95.3
E	7.5	0.8	92	2.1	86.2
F	8.2	0.9	34	0.8	91.7
G	7.2	1.0	76	0.7	94.6
H	7.3	0.6	52	0.8	87.5
I	7.8	1.0	67	1.3	98.8

of the recovered cholesterol in the void volume fractions is given in **Table 1.** Between 84 and 99% of the cholesterol appeared in the void volume, indicating that it was associated with a macromolecular complex of more than 5×10^6 daltons. Only a minor portion of the cholesterol was found in the included volume of the column. Although it is possible that small amounts of HDL were present in these fractions, immunodiffusion against anti-α- and β-lipoproteins and agarose-electrophoresis failed to demonstrate these lipoproteins.

Patients

In 8 out of 12 urines of patients with benign diseases of the kidneys and the urogenital tract a comparable elution pattern as in normal subjects (Fig. 1) was seen after gel filtration on the Bio-Gel A-5 m column. In these cases 84 to 98% of the cholesterol was found in the void volume fractions after gel chromatography (**Table 2**).

However, in one patient with a renal cyst and in three patients with the nephrotic syndrome, only 44 to 75% of the fractionated urinary cholesterol was eluted in the void volume (Table 2). Measurements of cholesterol in the single fractions revealed two (**Fig. 2**) or three (**Fig. 3**) cholesterol peaks. In the second peak immunodiffusion showed precipitin lines against anti-apoLDL and in the third peak precipitin lines against anti-apoHDL were seen (**Fig. 4** and **Fig. 5**).

In patients with malignant disease of the kidneys or the urogenital tract, 44 to 100% of the urinary cholesterol was found in the void volume fractions (**Table 3**). In single cases, HDL and LDL were detected in the lower molecular weight fractions as judged by agarose electrophoresis and immunodiffusion against anti-apoLDL and anti-apoHDL. These results demonstrate that urinary cholesterol is mainly excreted as a macromolecular complex in normal subjects, as well as in patients with benign or malignant diseases of the kidneys or the urogenital tract.

TABLE 2. Clinical data and relative percentage of urinary cholesterol in the void volume fractions after gel chromatography on the Bio-Gel A-5m column in patients with benign diseases of the kidneys and the urogenital tract

Diagnosis	Subject	Serum Protein	Serum Creatinine	Urinary Protein	Urinary Cholesterol	Cholesterol % in Void Volume
		g/dl	*mg/dl*	*g/24 hr*	*mg/24 hr*	
Urolithiasis	J	7.5	0.9	0.18	3.5	85.2
	K	8.1	1.1	0.14	0.7	98.4
	L	7.7	0.8	0.32	5.4	87.3
Renal cyst	M	8.3	2.2	0.41	1.8	88.6
	N	7.2	1.1	0.35	7.3	68.1
Nephrotic syndrome						
Glomerular nephritis	O	5.5	1.4	8.2	59.0	58.3
Lupus erythematosus	P	5.2	2.5	12.1	81.0	44.2
Diabetic glomerular sclerosis	Q	5.8	2.0	4.3	18.1	75.2
Glomerular nephritis	R	5.9	1.2	5.5	5.9	98.6
Glomerular nephritis	S	5.5	1.1	3.7	14.7	92.3
Prostatic adenoma	T	8.5	0.9	0.22	3.4	84.3
	U	7.9	0.8	0.34	11.5	95.8

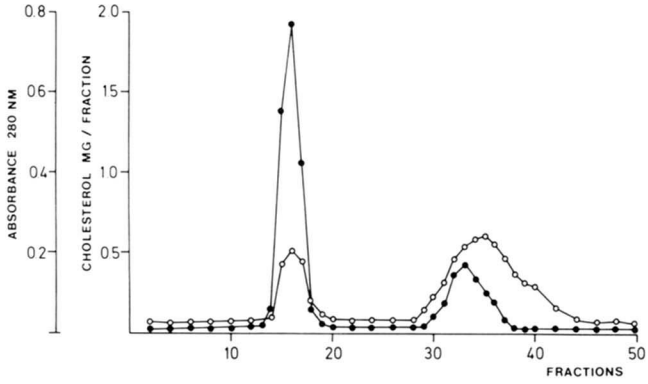

Fig. 2. Gel chromatographic elution pattern of a concentrated urinary sample from a patient with a nephrotic syndrome on Bio-Gel A-5m. Column size 120 × 2.0 cm. Individual points represent values of cholesterol (● —— ●, mg/10-ml fraction) and the absorbance at 280 nm (○ —— ○).

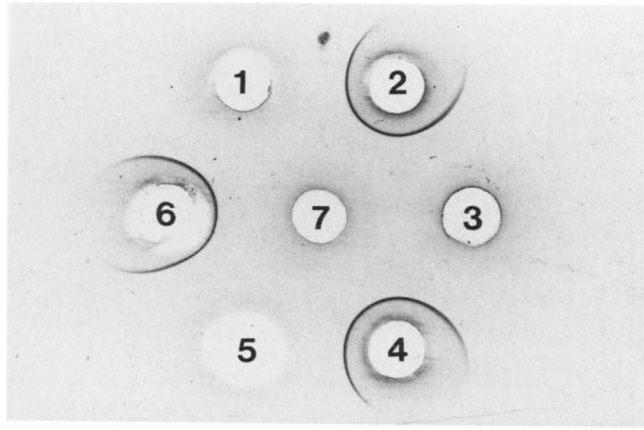

Fig. 4. Immunodiffusion of the isolated protein-lipid complex which was eluted in the second peak fraction (Fig. 3) (1, 3, 5, 7) against anti-apoLDL (2, 4, 6).

Characterization of the macromolecular urinary complex

Macroscopically, the isolated cholesterol-containing complex in the void volume fractions was turbid, appeared to be labile, and showed a tendency to aggregate after storage at 4°C for several days. In regard to the exclusion limit of the Bio-Gel A-5 m column, the estimated molecular weight of this complex was above 5×10^6 daltons.

Electron microscopy showed lipoprotein-like particles with diameters ranging from 300 to 700 Å (**Fig. 6**). The chemical analysis revealed the presence of protein, triglycerides, and phospholipids in addition to cholesterol. The compositions (%) of these constituents (ranges and median values) are illustrated in **Table 4.** Fractionation of the phospholipids by two-dimensional thin-layer chromatography (TLC) showed ethanolamine phosphoglycerides, phosphatidylcholine, sphingomyelin, and phosphatidylserine as the main components (**Table 5**).

Electrophoretic separation of the macromolecular complex on 1% agarose gels was not possible, since all the material remained at the origin. Density ultracentrifugation in a CsCl gradient showed the cholesterol-containing complex between the densities 1.1 and 1.3 g/ml (**Fig. 7**). The protein subunit constituents of the complex were studied by SDS-polyacrylamide gel electrophoresis. In a molecular weight range of 14,400 to 87,500, up to 17 protein subunits were separated (**Fig. 8**). Immunodiffusion studies showed no precipitin lines against anti-human-apoHDL and anti-human-apoLDL but in about 40% against anti-human albumin. However, immunodiffusion of the macromolecular complex against

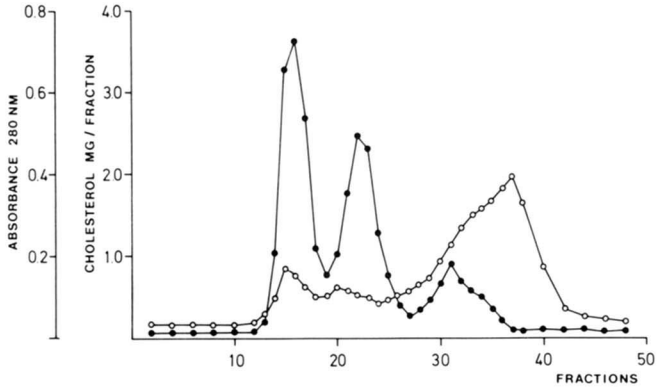

Fig. 3. Gel chromatographic elution pattern of a concentrated urinary sample from a patient with a nephrotic syndrome on Bio-Gel A-5m. Column size 120 × 2.0 cm. Individual points represent values of cholesterol (● —— ●, mg/10-ml fraction) and the absorbance at 280 nm (○ —— ○).

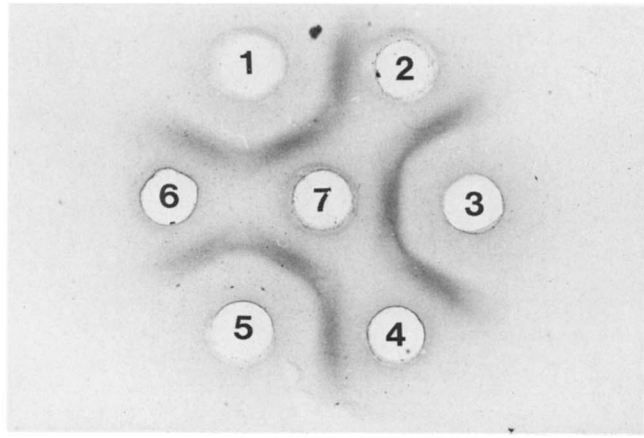

Fig. 5. Immunodiffusion of the isolated protein-lipid complex which was eluted in the third peak fraction (Fig. 3), (2, 4, 6, 7) against anti-apoHDL (1, 3, 5).

TABLE 3. Clinical data and relative percentage of urinary cholesterol in the void volume fractions after gel chromatography on the Bio-Gel A-5m column in patients with malignant diseases of the kidneys and the urogenital tract

Diagnosis	Subject	Serum Protein	Serum Creatinine	Urinary Protein	Urinary Cholesterol	Cholesterol % in Void Volume
		g/dl	*mg/dl*	*g/24 hr*	*mg/24 hr*	
Carcinoma of the kidney	AA	6.9	1.0	0.35	2.5	86.2
	BB	7.1	0.8	0.21	0.9	91.5
	CC	7.8	1.1	0.42	15.5	97.3
Carcinoma of the bladder	DD	6.8	0.7	0.31	0.8	100.0
	EE	7.4	1.1	0.27	12.7	76.1
Carcinoma of the prostate	FF	8.1	0.7	0.16	3.8	100.0
	GG	8.5	1.4	0.15	1.1	94.6
	HH	7.1	1.0	0.29	2.7	75.3
	II	7.8	0.6	0.30	2.2	44.3

anti-LSP and anti-KSP revealed single precipitin lines, suggesting that this macromolecular protein-lipid complex is of cellular origin (**Fig. 9**).

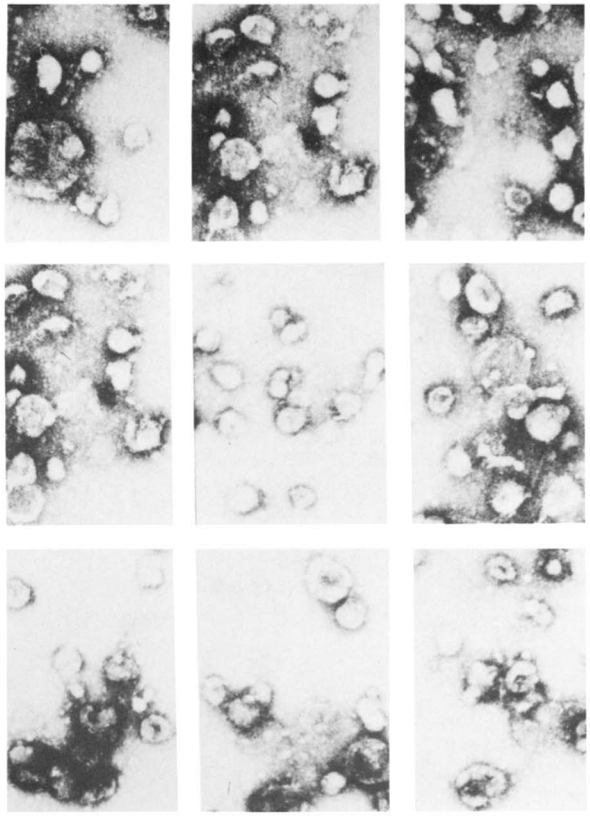

Fig. 6. Electron micrograph of the isolated macromolecular urinary protein-lipid complex negative stained with uranium acetate and observed in a Siemens 101 electron microscope (×120,000).

DISCUSSION

Cholesterol in the urine of normal male and female individuals is mainly associated with a macromolecular protein-lipid complex which appears to be of cellular origin. Only small amounts of cholesterol could be removed by filtration using Selecta filter 1117 1/2, presumably reflecting the presence of small numbers of intact cells, such as leucocytes and erythrocytes, and of larger cellular debris. During the whole fractionation procedure a considerable loss of lipids occurred, especially at the two ultrafiltration steps. This might be due to some adherence of the protein-lipid complex to the PM 10 membrane. In addition, some of the lipid was lost directly through the membrane, most likely as a consequence of delipidation. Recovery studies for cholesterol in single urinary specimens showed a total loss of urinary cholesterol between 30 and 50% at the end of the fractionation procedures. Most of the cholesterol was lost during ultrafiltration; the recovery after gel chromatography was nearly 95–100%. The form of the cholesterol lost during fractionation has not been investigated. However, it seems impossible that cholesterol could pass the PM 10 membrane in association with an intact lipoprotein. It might be present in the form of free molecules which originate from the macromolecular protein-lipid complex.

Plasma lipoproteins were not detected in the normal urinary samples investigated. However, a minor portion of the recovered urinary cholesterol was eluted in the lower molecular weight range after gel filtration on the Bio-Gel A-5 m column. This might be caused by small amounts of HDL undetectable by Ouchterlony immunodiffusion. These suggestions were supported by the findings of Segal and co-workers (45) who detected apoproteins of HDL in highly concentrated urine of normal subjects. However, their study showed neither apoprotein

TABLE 4. Relative composition of lipid and protein in the macromolecular complex isolated from urinary samples of normal subjects and patients with benign or malignant diseases of the kidneys or the urogenital tract (range and median values)

	Normal Subjects (5)[a]	Benign Diseases (7)	Malignant Disease (5)
		%	
Triglycerides	11.4–24.8 (16.3)	9.8–21.7 (14.3)	7.4–18.0 (11.8)
Cholesterol	3.1–14.5 (8.2)	4.2–15.3 (9.1)	3.7–12.4 (7.5)
Phospholipids	19.5–38.9 (29.5)	12.7–31.1 (19.8)	13.6–29.7 (20.9)
Protein	39.6–53.1 (46.0)	45.8–68.2 (56.8)	44.3–72.5 (59.8)

[a] Number of subjects in each category in parentheses.

B nor apoprotein E in the urine, suggesting that VLDL and LDL are not excreted in the urine of normal subjects. In accordance with these observations we were not able to detect LDL or VLDL in normal urine. In patients with benign or malignant diseases of the kidneys or the urogenital tract, usually most of the excreted urinary cholesterol could be found in the void volume fractions after gel filtration. These findings show that hyperexcretion of urinary cholesterol in these diseases is caused mainly by increasing amounts of the macromolecular protein-lipid complex in urine.

Only in single samples with elevated urinary cholesterol, especially in patients with the nephrotic syndrome, were major portions of urinary cholesterol eluted in the lower molecular weight fractions after gel chromatography on the Bio-Gel A-5 m column. In these fractions HDL and LDL could be detected by agarose electrophoresis and immunodiffusion against specific antisera. The results, for the nephrotic syndrome urine, were in accordance with studies performed by Kashyap and coworkers (31) who found HDL as well as LDL in concentrated urinary samples of several patients with the nephrotic syndrome. Using double immunodiffusion and lipoprotein electrophoresis, they detected HDL in the urine of nine nephrotic patients investigated. No plasma lipoproteins were found in urine of normal subjects or patients with primary hyperlipoproteinemia and normal renal function. LDL was detected in the urine of only two patients with the nephrotic syndrome, most likely due to its higher molecular weight in comparison to HDL. However, our findings show that in the nephrotic syndrome also, a considerable portion of the total urinary cholesterol is associated with a macromolecular proteolipid which was eluted in the void volume fractions after gel filtration on the Bio-Gel A-5 m column, in contrast to HDL and LDL. This macromolecular protein-lipid complex had an opalescent appearance similar to the light particulate fraction isolated by ultracentrifugation from normal urine by Cenedella and Belis (29).

However, the electrophoretic profile of proteins recovered from their 100,000 *g* (60 min) urine pellet was slightly different from the electrophoretic separations in this study. This might be due to the different methods of isolation, ultrafiltration, and gel chromatography versus ultracentrifugation. The isolated urinary protein-lipid complex had a diameter of 300 to 700 Å as judged by electron microscopy, and the particles showed a globular shape. The chemical assays were in accordance with a proteolipid which seemed to be partially delipidated in regard to the broad density range of 1.1 to 1.3 g/ml.

Albumin could be identified in 40% of the samples as the only plasma protein of the isolated urinary protein-lipid complex and might be considered as a nonspecific contaminant. The nature of the other protein components is unknown although there is some evidence that they could be of cellular origin. Cellular protein-lipid com-

TABLE 5. Relative composition of phospholipids in the macromolecular complex isolated from urinary samples of normal subjects and patients with benign or malignant diseases of the kidneys or the urogenital tract (range and median values)

	Normal Subjects (5)[a]	Benign Diseases (7)	Malignant Diseases (5)
Ethanolamine glycerophospholipids	22.4–42.1 (31.5)	19.8–38.1 (27.4)	24.1–41.5 (33.4)
Phosphatidylcholine	21.3–36.4 (28.1)	28.3–38.9 (34.6)	19.8–33.7 (26.2)
Sphingomyelin	16.6–26.2 (19.6)	12.1–23.7 (15.3)	13.3–24.1 (17.6)
Phosphatidylserine	15.9–26.1 (20.8)	17.8–27.2 (22.7)	16.3–26.1 (22.8)

[a] Number of subjects in each category in parentheses.

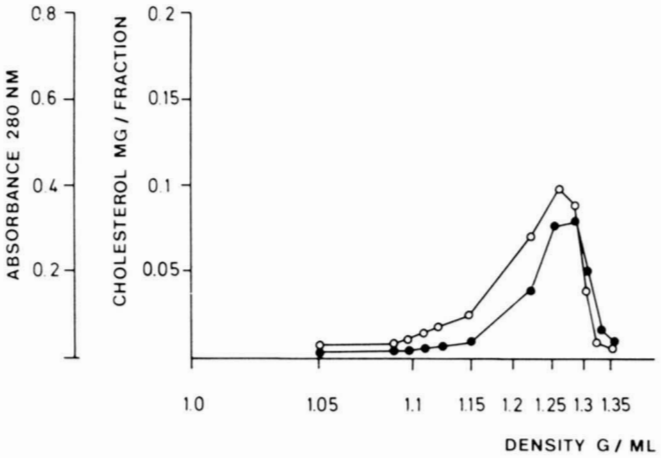

Fig. 7. Density ultracentrifugation in a CsCl gradient of the isolated macromolecular urinary protein-lipid complex in a Beckman SW-60 rotor (68 hr). Individual points represent values of cholesterol (● —— ●, mg/fraction) and the absorbance at 280 nm (○ —— ○).

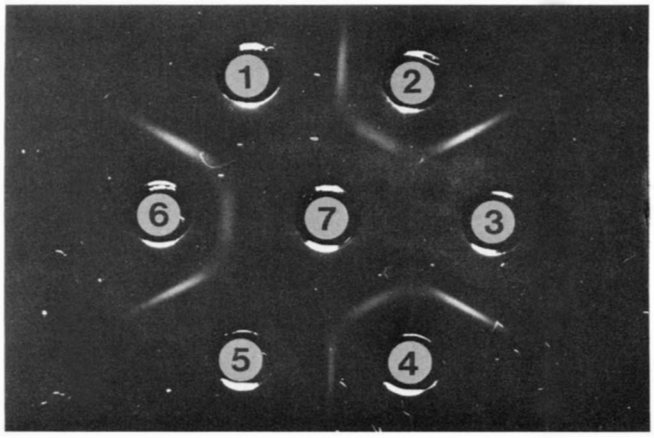

Fig. 9. Immunodiffusion of the isolated macromolecular urinary protein-lipid complex (1, 3, 5, 7) in 1.5% agar (Difco) at 20°C for 48 hr against anti-KSP (2, 4) and anti-LSP (6).

plexes have been isolated from the liver and recently from the kidney (43, 46). They were found in the 150,000 *g* supernatant of liver or kidney homogenates, but immunofluorescent studies indicate that they were derived from cell membranes (43, 44). After gel filtration on Sepharose 6B columns, these protein-lipid complexes were eluted in the void volume fractions, suggesting a high molecular weight above 5×10^6 daltons (43, 44). SDS-PAGE of the associated proteins separated up to 13 subunits in a molecular weight range between 30,000 and 90,000 daltons. Since there were similarities between these cellular proteolipids and the isolated urinary protein-lipid complex, further identification by immunodiffusion studies was undertaken. Anti-LSP and anti-KSP sera were provided by Behrens and Paronetto (43). These antisera did not react with plasma lipoproteins or other plasma proteins but showed precipitin lines against cytosolic proteins in liver or kidney cells which are not associated with lipids (43). However, they reacted with cell membranes not only of liver or kidney cells but also of smooth muscle fibers, thyroid follicles, intestine, and spleen. No studies were performed against tissue of the bladder or other parts of the urinary tract. In our study immunodiffusion of these antisera against the isolated macromolecular urinary proteolipid revealed single precipitin lines which support the view that cells were the source of this complex. In kidney disease we suppose that most of this urinary complex is of renal origin. In diseases of the bladder it is much more likely that this organ is the major source of urinary cholesterol. In regard to the immunofluorescent studies, the urinary protein-lipid complex might be a fragment of a plasma membrane. This is supported by the findings of Cenedella and Belis (29). These authors described a high activity of 5′-nucleotidase, a marker enzyme for plasma membranes, in their light particulate cholesterol-containing fraction.

In conclusion, the isolated macromolecular cholesterol-containing urinary complex showed many characteristics of membrane-associated protein-lipid particles of the hu-

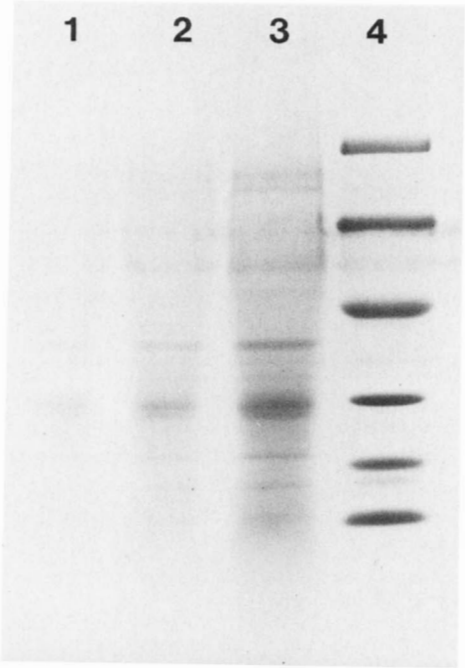

Fig. 8. SDS-PAGE of the isolated macromolecular urinary protein-lipid complex in 1.5-mm vertical slab gels using 4–22.5% acrylamide concentrations (lanes 1, 2, 3). Standard proteins phosphorylase b (94,000), albumin (67,000), ovalbumin (43,000), carbonic anhydrase (30,000), trypsin inhibitor (20,100), and α-lactalbumin (14,000) were used (lane 4).

man kidney and even the liver and is most likely a fragment of the plasma membrane.

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