Proc. Natl. Acad. Sci. USA
Vol. 80, pp. 1313–1317, March 1983
Cell Biology

Localization of urinary lactosylceramide in cytoplasmic vesicles of renal tubular cells in homozygous familial hypercholesterolemia

(immunocytochemistry/electron microscopy/hyperlipoproteinemia/plasma exchange/atherosclerosis)

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Communicated by John W. Littlefield, November 17, 1982

ABSTRACT **An average 15-fold increase in lactosylceramide (LacCer) in the sediment of receptor-negative, familial hypercholesterolemic (FH) homozygotes has been reported [Chatterjee, S., Sekerke, C. S. & Kwiterovich, P. O., Jr. (1982) *J. Lipid Res.* 23, 513–522]. We report here the abnormal urinary excretion of significant numbers of renal tubular cells in eight FH homozygotes. The mean activity of γ-glutamyltransferase, a marker for renal tubular cells, was twice as high in urinary sediment of FH homozygotes as in normals. Membrane-enclosed cytoplasmic vesicles that stained strongly positive with a fluorescein-labeled antibody against LacCer were found in the renal tubular cells of all homozygotes except two who had undergone a portacaval shunt. These two had normal urinary levels of LacCer, and the cytoplasmic vesicles were vacuolated. In the other six, most of the fluorescent antibody label was intracellular and perinuclear. The cytoplasmic vesicles stained strongly with polychromatic Papanicolaou stain, periodic acid/Schiff reagent, and oil red O. Electron microscopy revealed perinuclear membrane-enclosed lipid and free lipid droplets. When two FH homozygotes, who excreted increased LacCer, underwent plasma exchange, the cytoplasmic vesicles became empty, and the urinary LacCer level decreased into the normal range. We conclude that the increased urinary excretion of LacCer in FH homozygotes occurs in renal tubular cells and that the intracellular location of LacCer is within cytoplasmic vesicles. The presence of LacCer within these vesicles can be modulated by treatment with plasma exchange.**

Low density (β) lipoproteins (LDL) are the major carriers in plasma of cholesterol and glycosphingolipids (GSL) (1, 2). The pioneering work of Brown, Goldstein, and co-workers elucidated a pathway through which extracellular LDL are taken up and metabolized and exogenous sterol is supplied to the cell (3). Specifically, LDL are bound with high affinity to a cell-surface receptor and internalized, and the apoprotein and cholesteryl ester moieties are hydrolyzed in the lysosomes. These cellular events are associated with a reduction in the activity of hydroxymethylglutaryl-CoA reductase, the rate-limiting enzyme of cholesterol biosynthesis. Excess sterol is reesterified by fatty acyl-CoA cholesterol acyltransferase (3).

Insight into this pathway was facilitated by the use of cultured fibroblasts from patients homozygous for familial hypercholesterolemia (FH), a dominant phenotype that is expressed in double dose by marked increases of plasma total and LDL cholesterol, precocious atherosclerosis, and xanthomas in the first decade of life (3). FH can result from several known mutations at a locus coding for the LDL receptor; the receptor may be functionless (receptor negative), faulty (receptor defective), or incapable of internalizing LDL (internalization defective).

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Our previous work in cultured fibroblasts indicated that there was an alteration of GSL metabolism in certain FH receptor-negative homozygotes (4, 5). Recently, Verdery and Theolis (6) showed that the synthesis of sphingosine, a long-chain base and a precursor of the ceramide component of GSL, was inhibited by LDL in cultured human fibroblasts. This effect of LDL was dependent on the activity of the LDL receptor because LDL did not inhibit the synthesis of sphingosine in fibroblasts from homozygous FH subjects (6). In addition to an *in vitro* abnormality of GSL metabolism associated with FH, we have found that the plasma LDL and the urinary sediment of FH receptor-negative homozygotes contained significantly higher levels of GSL (2, 7). Evidence for an *in vivo* abnormality of GSL metabolism in FH was particularly striking in the urine, where the mean content (nmol of glucose per μg of protein per 24 hr) of lactosylceramide [LacCer; Gal(β-1→4)Glc(β1→1′)Cer] was 15-fold higher in urinary sediments of FH patients than in normals. Cells in the urinary sediments of FH homozygotes were identified and characterized here by histochemical, biochemical, and electron microscopic techniques. Immunocytological procedures were used to determine the intracellular localization of LacCer in the urinary cells of FH homozygotes and to document the presence of LacCer in intracytoplasmic vesicles.

SUBJECTS AND METHODS

Patient Population. Eight FH homozygotes (T.B., female, 11 yr; B.A., female, 14 yr; and C.P., male, 17 yr; D.D., female, 44 yr; D.P., male, 7 yr; Ja.P., female, 20 yr; Jo.P., female, 26 yr; and T.H., female, 12 yr) were studied (see Table 1) (1, 7). FH homozygotes T.B., D.D., B.A., C.P., T.H., and D.P. are LDL "receptor-negative" (see Table 2) (ref. 7; E. Schaefer and R. Gregg, personal communication), while Ja.P. and Jo.P. are LDL "receptor-defective," as judged by assays of LDL binding, internalization, and degradation and cholesterol esterification in cultured fibroblasts (3, 7). Two female (S.F. and M.M.) and three male (P.K., P.P., and T.L.) subjects served as similarly aged, normolipidemic controls (see Table 2). Other normolipidemic controls, who donated urinary cells for the measurement of γ-glutamyltransferase (γ-GluTase; EC 2.3.2.2) activity, were: R.S., female, 56 yr; H.K., female, 57 yr; S.M., female, 31 yr; P.B., male, 42 yr; and D.M., male, 31 yr.

The FH homozygotes studied here were undergoing various treatment regimens that affect plasma lipid and lipoprotein levels (Table 1). We previously had studied T.B., B.A., and D.P.

Abbreviations: LacCer, lactosylceramide; FH, familial hypercholesterolemia/hypercholesterolemic; LDL, low density (β) lipoproteins; GSL, glycosphingolipids; Pap stain, Papanicolaou stain; PAS, periodic acid/Schiff reagent; $GbOse_3Cer$, globotriaosylceramide; γ-GluTase, γ-glutamyltransferase.

before the treatment with lipid-diminishing drugs (7). Addition of nicotinic acid, cholestyramine, or neomycin did not alter the urinary excretion of LacCer in these subjects (data not shown). Treatment with plasma exchange transiently decreased the plasma total and LDL cholesterol levels significantly (Table 1) and decreased markedly the urinary excretion of LacCer (7). Surgical treatment by a portacaval shunt also may affect the urinary excretion of LacCer (7).

Preparation of Urinary Sediments for Light Microscopic Studies. Freshly voided urine was collected in the morning. The urine samples (20–40 ml) were centrifuged for 10 min at 3,000 rpm. The supernatants were aspirated, 5 ml of Polysal (Cutter) was added, and the cells were resuspended by mixing. Aliquots of the cell concentrates were collected on glass slides by cytocentrifugation and fixed with alcohol.

Preparation of Fluorescein-Labeled Antibodies Against LacCer and Globotriaosylceramide ($GbOse_3Cer$). LacCer and $GbOse_3Cer$ [Gal(α1→4)Gal(β1→4)Glc(β1-1′)Cer] liposomes were prepared for the isolation of antibodies against LacCer and $GbOse_3Cer$ from rabbit serum (9). The measurements of trapped glucose and immune-specific glucose released by LacCer liposomes and $GbOse_3Cer$ liposomes were carried out as described (9). In the presence of appropriately purified rabbit antibodies, ≈50% of glucose release occurred from LacCer liposomes, and 10–20%, from $GbOse_3Cer$-liposomes. The purified antibodies were monospecific either for LacCer or $GbOse_3Cer$ when tested against both GSL. In addition, the antibody against LacCer did not react with GlcCer [Glc(β1→1′)Cer], GalCer [Gal(β1→1′)Cer], galabiosylceramide [$GaOse_2Cer$; Gal(β1→4)Gal(β1→1′)Cer], and II-SO_3-LacCer. Rabbit immunoglobulin G was obtained from Miles. This immunoglobulin was free of naturally occurring anti-GSL antibodies. The antibody preparations were labeled with fluorescein isothiocyanate and isolated (10).

Light Microscopy, Immunocytochemistry, and Photography. Specimens were stained with polychromatic Papanicolaou (Pap) stain (11), periodic acid/Schiff reagent (PAS), or oil red O. A fourth set was stored at 4°C in acetone and subsequently washed five times with ice-cold phosphate-buffered saline (0.9% NaCl; pH 7.4). Suitable aliquots of the fluorescein-labeled antibodies to LacCer or $GbOse_3Cer$ were layered over the fourth set of cells and then incubated for 1 hr at 37°C. The reaction was terminated by washing the specimens five times with ice-cold phosphate-buffered saline. The specimens were examined under a fluorescent light microscope with epi-illumination and photographed.

Electron Microscopy. Urinary sediment cells were collected from two FH homozygotes (T.B., B.A.), fixed in 3% glutaraldehyde/0.1 M phosphate buffer (pH 7.3), and post-fixed in 1% osmium tetroxide in 0.1 M phosphate buffer. After dehydration in ethanol, toluene, and Epon, the cells were embedded in Epon Araldite-6005 and cut with a Sorvall MT-2-B ultramicrotome. Thin sections were stained with uranyl acetate and lead citrate. An AEI series transmission electron microscope was used.

Cell Culture. Normal human kidney (HK) proximal tubular cells were obtained from A. Trifillis (12). Normal neonatal human urinary cell cultures were obtained from J. Felix and J. W. Littlefield (13). Human skin fibroblasts (L.W.) were cultured as described (14). Cells were grown in Eagle's minimal essential medium containing 10% fetal calf serum, penicillin, streptomycin, and insulin (1 μg/ml). Approximately 1×10^6 cells were seeded in a 60-mm plastic Petri dish in 5 ml of culture medium. Medium was changed on the third day. On the seventh day, confluent monolayers of cells (passage numbers 2–5) were harvested, and the viable cell count was measured by Trypan blue exclusion (15).

Measurement of γ-GluTase Activity in Cell Homogenate. γ-GluTase activity in normal fibroblasts, urinary cells, and human kidney proximal tubular cells grown in tissue culture and in urinary cells from seven of the eight FH homozygotes was measured exactly as described (16). Boiled homogenates served as controls. Specific activity of γ-GluTase was expressed as nmol of *p*-nitrophenylanaline released per min/mg of protein.

Measurement of Urinary GSL Levels. Twenty-four-hour urine from a FH homozygote, C.P., was centrifuged, and the GSL in the urinary cell pellet were isolated and quantified (7, 17).

RESULTS

Histochemical Studies. Light microscopy of Pap-stained urinary cells from all FH homozygotes revealed primarily renal tubular cells. These cells from T.B., D.D., B.A., T.H., Ja.P., and

Table 1. Plasma lipid and lipoprotein cholesterol levels in homozygous FH subjects on the day of their urine collection

Subject	Treatment status	Total triglycerides, mg/100 ml	Cholesterol, mg/100 ml				Lipoprotein pattern
			Total	VLDL	LDL	HDL	
T.B.†	Nicotinic acid, 2 g/day						
	Before PE*	67	456	45	356	55	IIa
	After PE	42	247	5	209	33	
D.D.‡	Before PE*	116	486	65	388	33	IIa
	After PE	38	129	ND	ND	ND	
B.A.†	Cholestyramine, 12 g/day	224	876	ND	797	34	IIb
C.P.‡	Nicotinic acid, 2 g/day						
	Before PE						
	After PS	110	355	ND	ND	ND	IIa
D.P.‡	No drugs						
	After PS	122	536	72	434	30	IIa
T.H.‡	Nicotinic acid, 2 g/day	155	794	79	682	33	IIb
Ja.P.‡	No drugs	272	557	54	455	48	IIb
Jo.P.‡	No drugs	146	505	29	442	34	IIb

PE, plasma exchange; PS, portacaval shunt; ND, not determined.

*Samples were obtained the morning before plasmapheresis and 48 hr after the procedure.

†Lipid and lipoprotein levels were determined in the Johns Hopkins laboratory (8).

‡Lipid and lipoprotein levels were determined in the laboratory of the Molecular Disease Branch, National Institutes of Health, Bethesda, MD (1).

Jo.P. contained Pap stain-positive material in the form of spherical, intracytoplasmic membrane vesicles of different sizes and non-membrane-bound secretory substances having a greenish-blue color (Fig. 1*A*). Few renal tubular or transitional epithelial cells were identified in the five control specimens, and no Pap stain-positive cytoplasmic vesicles were found. Forty-eight hours after plasma exchange (T.B. and D.D.) (Table 1), the Pap-stained tubular cells contained predominantly empty cytoplasmic vesicles (Fig. 1*B*). C.P. and D.P., FH homozygotes who had undergone portacaval shunts, had mostly empty cytoplasmic membrane vesicles in the renal tubular cells (Fig. 1*C*).

The renal cells of FH homozygotes (T.B., B.A., D.D., T.H., Ja.P., and Jo.P.) reacted strongly positive (++++) to an oil red O stain (Fig. 1*D*). The intracytoplasmic vesicles contained several deeply red-stained structures, most likely due to the nonpolar components of LacCer (18). The urinary specimens from normal subjects and two FH homozygous subjects (C.P. and D.P.) who had undergone a portacaval shunt were oil red O negative. Cells used for the oil red O staining procedure were fixed in formaldehyde, which may have contributed toward some loss of preservation of morphology (Fig. 1*D*) when compared with cells in Fig. 1*A* (which were wet-fixed in ethanol and showed excellent preservation of morphology).

The cytoplasmic vesicles in cells from all FH homozygotes except C.P. and D.P. were PAS positive, indicating the presence of carbohydrate material (cells not shown). Cells from normals did not react with the PAS reagent.

Immunocytochemical Localization Studies. The cytoplasmic vesicles in the FH renal cells of T.B., D.D., T.H., and Ja.P. stained strongly positive (++++) with the fluorescein-labeled antibody against LacCer (Table 2); the renal cells of two other FH homozygotes, B.A. and Jo.P., were less strongly positive (++) with the same labeled antibody. The label was localized intracellularly in discrete perinuclear vesicles, confirming the accumulation of LacCer (Fig. 1*E*). Urinary specimens from five normal and two FH homozygous subjects (C.P. and D.P.) who had portacaval shunts did not show fluorescence with the fluorescein-labeled antibody against LacCer. The positive reaction in T.B. and D.D. was not observed when the cells were obtained 48 hr after plasma exchange (Table 2). However, 10 days after plasma exchange, the urinary cells in T.B. again reacted positively with the fluorescein-labeled antibody against LacCer. The reaction was correlated with the levels of LacCer in the urinary sediment (Table 2).

A replicate set of specimens (T.B.) stained with fluorescein-labeled antibody against $GbOse_3Cer$ showed only weak fluorescence, and that was associated with the plasma membrane. No fluorescence was observed when the specimens were incubated with fluorescein-labeled rabbit immunoglobulins. These findings indicate the specific nature of the fluorescent antibody-labeling technique.

Electron Microscopic Studies. Electron micrographs showed well-preserved, renal tubular cells that contained microtubules emerging from the plasma membrane, a large nucleus, and several perinuclear membrane-enclosed cytoplasmic vesicles (Fig. 2). Most of the cytoplasmic vesicles were empty (Fig. 2) because the use of alcohol, toluene, and Epon during the dehydration steps most likely removed the lipids. An occasional vesicle was filled with electron-dense material that presumably represented lipid (Fig. 2). Several small cytoplasmic vesicles (large arrowhead in Fig. 2) were observed that may be precursors of the large cytoplasmic vesicles. Univesicular bodies, multivesic-

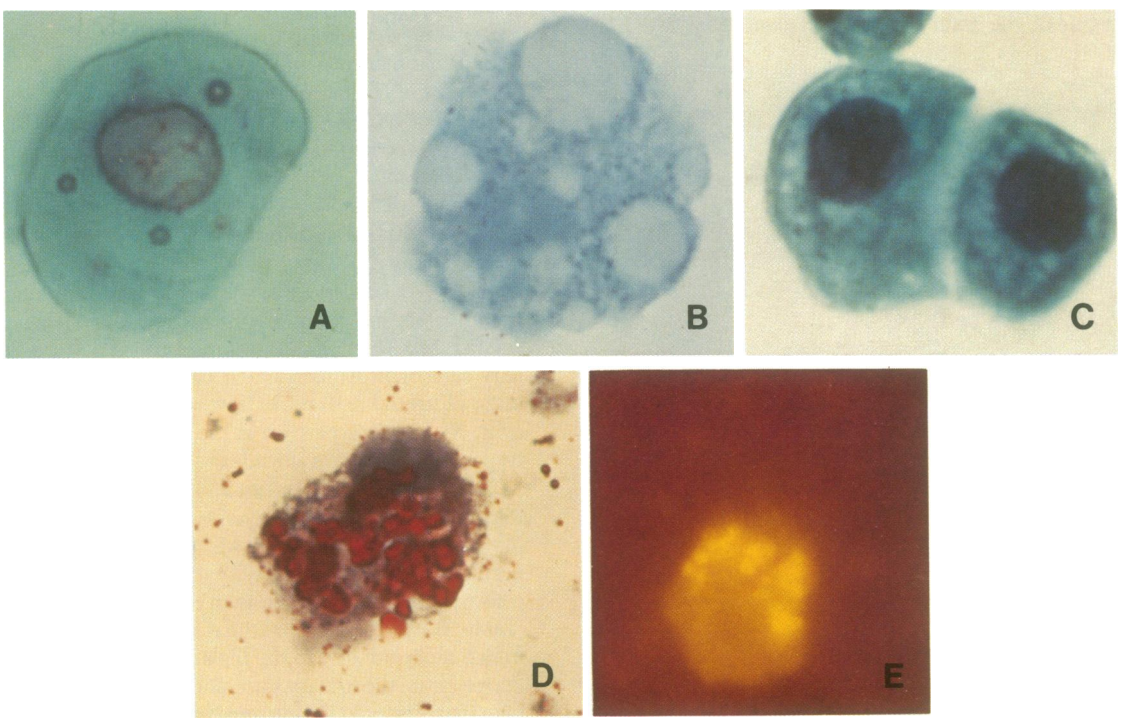

FIG. 1. Histochemical and immunocytochemical demonstration of lipid accumulation in renal tubular cells from the urinary sediment of FH receptor-negative homozygotes. (×240.) (*A*) Renal tubular cell from urine of T.B. obtained before plasma exchange and stained with polychromatic Pap stain. Note the presence of several cytoplasmic vesicles filled with sudanophilic material. (*B*) Renal tubular cell from urine of T.B. obtained 48 hr after plasma exchange and stained with polychromatic Pap stain. Note the presence of several empty cytoplasmic vesicles. (*C*) Renal tubular cells from urine of C.P., who had undergone a portacaval shunt operation, stained with polychromatic Pap stain. Note the presence of several vacuolated cytoplasmic vesicles. (*D*) Renal tubular cells from urine of B.A. stained with oil red O. Note the deeply red-stained stuctures (presumably due to nonpolar components of LacCer) in the perinuclear cytoplasmic vesicles and also the presence of non-membrane-bound lipid. (*E*) Renal tubular cells from urine of T.B. stained with fluorescein isothiocyanate-labeled antibody against LacCer. Note the presence of fluorescence in discrete perinuclear cytoplasmic vesicles. Compare with *C*.

Table 2. Biochemical and morphological* analysis of the urinary sediment from normal human subjects and FH homozygotes

FH homozygotes	LacCer levels,† nmol/mg of protein	Cytoplasmic vesicle–LacCer Ab reaction‡
LDL receptor negative		
T.B. before PE	27.7	++++
2 days after PE	0.7	-
10 days after PE	4.2	++
D.D. before PE	7.5	++++
2 days after PE	ND	-
B.A.	2.6	++
C.P. after PS	0.8	-
D.P. after PS	ND	-
T.H.	ND	++++
LDL receptor defective		
Ja.P.	ND	++++
Jo.P.	ND	++
Normal range	0.1–0.7	-

ND, not determined; PE, plasma exchange; PS, portacaval shunt; Ab, antibody.

* Renal tubular cells were present in the urinary sediment from FH homozygotes at all treatment stages indicated but were absent in that from normal subjects.

† In urinary sediment. Data taken from ref. 7 except for C.P. (see *Subjects and Methods*). The urinary sediment of D.P. contained 1.8 nmol of LacCer per mg of protein before the PS.

‡ Reaction of cytoplasmic vesicles in the FH renal cells with fluorescein-labeled antibody against LacCer.

ular bodies, several lysosomes, peroxisomes, and mitochondria were also visible (Fig. 2).

Number of Renal Tubular Cells in Urine from Normals and FH Homozygotes. The total urinary cell counts in two FH homozygotes (T.B. and B.A.) were 2.2×10^4 and 3.0×10^4 cells per ml of urine, respectively. The renal proximal tubular cells accounted for 68% (1.5×10^4 cells per ml of urine) of the cells in T.B. and 80% (2.4×10^4 cells per ml of urine) in B.A. The total urinary cell count in a normal man (S.C.) and a normal woman (S.E.) were 6.5×10^3 and 8.5×10^4 cells per ml of urine, respectively. No renal tubular cells were found in the urine from the normal man and only five renal cells per ml of urine were found in the normal woman. Squamous cells of the vagina accounted for 99.9% of the urinary cells in the latter case. Thus, renal cells accounted for less than 0.1% of the total normal urinary sediments.

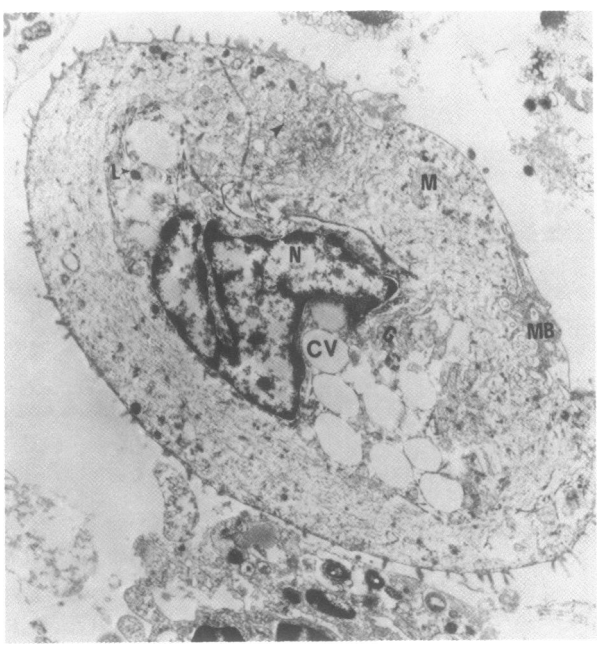

FIG. 2. Transmission electron micrograph of a renal tubular cell from the urinary sediment of a FH receptor-negative homozygote (T.B.). (×17,100.) Several filamentous microvilli are present that are typical of renal tubular cells. Note the presence of large, perinuclear, vacuolated, cytoplasmic vesicles and some cytoplasmic vesicles filled with electron-dense material, presumably representing lipid. Several small cytoplasmic vesicles (see large arrowhead), which may be the precursors of the large cytoplasmic vesicles, are also present. Compare this figure with Fig. 1*E*. CV, cytoplasmic vesicle; G, Golgi; L, lysosome; M, mitochondria; MB, multivesicular body.

Table 3. γ-GluTase activity of normal human cells and urinary cells from FH subjects

Cells	γ-GluTase, nmol/min per mg of protein
Normal humans	
Urinary cells*	33.6 (20.5–43)
Cultured urinary cells	34.1
Cultured proximal tubular cells	166.6
Cultured fibroblasts	16.6
FH homozygotes†	
Urinary cells	67.5 (53.7–115)

Normal human skin fibroblasts (L.W.), human kidney proximal tubular cells, and neonatal human urinary cells were grown in tissue culture medium and homogenized as described in the text. Freshly obtained human urinary cells from five normolipidemic subjects (R.S., H.K., S.M., P.B., D.M.) and seven FH homozygotes (T.B., D.D., B.A., D.P., T.H., Ja.P., Jo.P.) were washed with phosphate-buffered saline and homogenized. Suitable aliquots of cell homogenates were withdrawn for the measurements of protein content (21) and γ-GluTase activity (16).

* Average value (range in parentheses) of five normolipidemic controls.

† Average value (range in parentheses) of seven FH homozygotes.

Cell Viability and γ-GluTase Activity in Normal Cells and Cells from FH Homozygotes. γ-GluTase activity is enriched in human kidney proximal tubular cells (19, 20). Therefore, the activity of γ-GluTase was measured in urinary cells obtained from five normolipidemic controls and seven FH homozygotes. γ-GluTase activity was determined also in cultures of either urinary cells or proximal tubular cells from normals. Normal human cultured skin fibroblasts were used as a control for nonrenal cells. Ninety-nine percent of the normal and FH cells excluded trypan blue and were considered viable (15). Urinary cells from FH homozygotes had a mean 2-fold higher activity of γ-GluTase than did urinary cells from normals, and the enzymatic activity in each of the homozygotes was outside the range found in the normals (Table 3). The activity of γ-GluTase in normal urinary cells was similar to that found in cultures of normal urinary cells (Table 3). Cultures of proximal tubular cells obtained directly from human kidney had the highest activity of γ-GluTase of cells derived from the urinary tract, and this activity was 10-fold higher than that observed in cultured cells of nonrenal origin (Table 3).

DISCUSSION

The major findings here are: first, the urinary sediment of FH homozygotes contains an increased number of renal tubular cells; second, the intracytoplasmic, membrane-enclosed bodies in these cells are enriched in LacCer; and, third, the presence of LacCer in these structures was modulated by treatment with plasma exchange.

Renal tubular cells were seen frequently by light microscopy in urinary sediment of FH subjects but were seen only occasionally in the urine of controls. In electron micrographs (Fig. 2), cells from FH urine had a brush border typical of renal tubular cells. Biochemical (20), histochemical (12), and immunochemical (21) studies have demonstrated that the activity of γ-GluTase is enriched in proximal tubular cells from normal human kidney. Increased activity of γ-GluTase was present in the urinary cells from each of seven FH homozygotes. Considered together, our morphological and biochemical data indicate that the urinary sediment of FH homozygotes contains an increased number of kidney tubular cells, including those from the proximal tubules. Because both the untreated FH homozygotes and those who had undergone plasma exchange or portacaval shunt shed renal tubular cells, this process appears to be continuous, even when the urinary levels of LacCer are normal. The etiology of the increased urinary excretion of tubular cells in FH is unknown.

The strong reaction of LacCer in the renal tubular cells of FH homozygotes with a fluorescent-labeled antibody was consistent with our previous biochemical findings of significantly increased urinary LacCer in untreated FH homozygotes (7). The immunocytochemical localization of LacCer to intracytoplasmic vesicles in cells from FH homozygotes was substantiated further by three different procedures. The oil red O-positive cytoplasmic vesicles most likely represented a reaction with the fatty acid component of LacCer because the amounts of cholesterol, triglycerides, and total phospholipids in the urinary sediment of these patients were normal (7). When the cells were counterstained with Pap stain after reaction with the fluorescent antibody against LacCer, strongly positive Pap-stained cytoplasmic vesicles were found, indicating that the two techniques were in fact detecting the same cytoplasmic vesicles. Finally, electron microscopy revealed the presence of several discrete cytoplasmic vesicles in the perinuclear area of these cells.

The effect of treatment by plasma exchange on the presence of LacCer in the intracytoplasmic bodies was striking. Plasma exchange therapy decreases LDL cholesterol levels significantly, and the simplest explanation for our data is that the decreased plasma LDL and GSL levels were accompanied by a lower renal GSL level. The return of increased GSL excretion and LacCer-positive bodies 10 days after exchange, when the LDL levels had returned to preexchange levels, is compatible with this interpretation. However, other lipoproteins (such as HDL) and other plasma proteins are decreased as the result of plasma exchange, and our data do not exclude the possible effect of these processes on altering LacCer excretion. A simple relation between the plasma LDL (and GSL) levels and the appearance of LacCer in the urine is questioned further by the data in the FH homozygotes who had a portacaval shunt. These patients had "empty" cytoplasmic vesicles despite the persistence of relatively high LDL levels. In contrast, FH homozygotes who have not undergone a portacaval shunt but who are treated by plasma exchange develop "full" cytoplasmic vesicles 2 wk after exchange, at a time when their LDL levels have increased to approximately the same levels as those in the FH homozygotes who had a portacaval shunt. The simplest explanation for the apparent discrepancy is that a portacaval shunt, known to have pleotrophic metabolic and biochemical effects (22, 23), affects lipid metabolism in the kidney. Alternatively, the "threshold" for storage of LacCer in FH renal cells may differ, and this might be related to the homeostasis between endogenous synthesis of LacCer (24) and exogenous supply through LDL.

The function and nature of the intracytoplasmic bodies are not known. The availability of viable renal tubular cells from normal and FH homozygotes make feasible studies of the role of lipoproteins and lipoprotein receptors on the regulation of GSL metabolism in the kidney.

We thank Ms. Hazel Smith and Ms. Lynda Reynolds for expert technical assistance, Ms. Lucille Jeffries for electron microscopy, Dr. Jean Olson for interpretation of the electron micrographs, Dr. Robert Heptinstall for useful discussions, and Drs. Ernie Schaefer and Richard Gregg for urine and plasma samples from C.P., D.P., D.D., T.H., Ja.P., and Jo.P. This research was supported by the William Penn Foundation of Philadelphia, National Heart and Lung Institute Contract HV-2158L, and Grant GRC-RR-52 from the General Research Center Program of the Division of Research Resources, National Institutes of Health.

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