

Neutral Lipid Storage with Acid Lipase Deficiency: a New Variant of Wolman's Disease with Features of the Senior Syndrome

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Summary

A girl presented with small stature, obesity, tapetoretinal degeneration, deafness, psychomotor regression, seizures, acanthosis nigricans, hepatomegaly, and chronic tubulointerstitial nephropathy. She died at age ten with renal insufficiency and uncontrolled seizures. Histochemistry showed lipid storage in hepatocytes, histiocytes, smooth muscles and, to a much lesser extent, kidney tubules and cortical neurons. The liver had increased cholesterol esters (5-fold) and triacylglycerols (8-fold), and decreased phospholipids (50%). Methylumbelliferyl-oleate, oleylcholesterol, trioleylglycerol, and tripalmitylglycerol lipase activities were markedly reduced in the liver, in the range found in Wolman's disease. In cirrhotic fatty livers these activities ranged from 7-87% of the normal mean. The patient's brain had limited neutral lipid storage and normal methylumbelliferyl-oleate lipase. Trioleylglycerol lipase activity was 14-60% of controls; tripalmitylglycerol lipase activity 14-25% of controls; and oleylcholesterol lipase activity 12-33% of controls.

Speculation

Neutral lipid storage in the kidney was minimal suggesting that unidentified factors were responsible for the tubulointerstitial nephropathy. Acid lipase activity should be investigated in the Senior syndrome and other related disorders.

A number of distinct phenotypes have been associated with a neutral lipid storage disorder. Wolman's disorder is characterized by hepatosplenomegaly, malabsorption with steatorrhea, bilateral adrenal calcification, and failure to thrive. The disease occurs in infancy and is nearly always fatal before age one (7). Cholesterol ester storage disease (CESD) is a comparatively benign disease characterized by hepatomegaly, which may not be recognized until adulthood. Patients may present bilateral adrenal calcification. Cholesterol esters account for most of the stored lipid whereas there is little accumulation of triglycerides (7). A severe congenital triglyceride storage disorder (TGSD) leading to death in the neonatal period has been reported in a unique family under the name of Fatty Metamorphosis of the Viscera (21). Other patients have been described with phenotypes similar to those of Wolman's disease but with a longer life span (36). A severe deficiency of the lysosomal acid lipase, acting on both cholesterol esters and triglycerides has been demonstrated in these different conditions (2, 7, 20). In this paper we give a follow up on a unique patient with a disease characterized by generalized accumulation of neutral lipids also due to a profound deficiency of acid lipase (22).

MATERIALS AND METHODS

[carboxy-¹⁴C]-Oleate (54 mCi/mmole), glyceryl-[carboxy-¹⁴C]-trioleate (40 mCi/mmole), glyceryl-[carboxy-¹⁴C]-tripalmitate

(37), triolein, tripalmitin, cholesteryl oleate, phosphatidylserine and phosphatidylethanolamine (38) were used to prepare lipase substrates.

Thin layer chromatography of neutral lipids was conducted by sequential runs in diethylether:benzene:ethanol:acetic acid (80:100:4:0.4) and hexane:diethyl ether (94:6) on 250 μ Silica Gel G (8). Lipids were visualized by exposure to iodine vapor or two-dimensional scanning (39). Lipid areas were scraped and counted in Aquasol (37). Lipid extracts (250 μ g) from normal human serum were used as standards for lipid identification. Enzyme extracts were prepared by extracting tissues with 9 volumes of water in a Potter-Elvehjem fitted with a Teflon pestle.

Control livers and brains and cirrhotic livers from alcoholics were obtained from the autopsy room and kept frozen at -20°C from 3 wk to 14 years. Tissues from patients with Wolman's disease included four autopsy specimens and three open liver biopsies. Tissues from the present case were obtained at autopsy. Parental consent was given for all tests and biopsies.

Triacylglycerol lipase was determined by a modification of Kaplan's procedure (15). The labeled substrate was diluted with unlabeled lipid to provide a specific activity ranging from 200-30,000 cpm/nmole. The lipids (300-500 nmole) were mixed with 20-160 mg Triton X-100 and 800 nmole phosphatidylserine in 1 ml hexane, dried under nitrogen at 40°C and emulsified by adding water dropwise in a Potter-Elvehjem held at 50°C up to a volume of 1 ml. Fresh substrate was prepared for each set of determinations. The reaction mixture included 15-130 nmole substrate, 12.5 nmole citrate-phosphate buffer (pH 4.0) or 5 nmole acetate buffer (pH 4.25), and 15-400 μ g of extract protein in a final volume of 75-100 μ l. The reaction was stopped and the liberated fatty acids extracted according to Kaplan (15). Incubations were carried in triplicate for 0, 30, and 60 min. Reactions were generally linear for 2 h. Free fatty acid recovery varied from 34-50% when labeled oleate was substituted for labeled triacylglycerol in the reaction mixture. Pathologic specimens were always analyzed simultaneously with controls, and fatty acid recovery was monitored for each series. Alternatively, incubations were stopped by adding 3 ml chloroform:methanol (2:1) and analyzing neutral lipids by thin layer chromatography. Under these conditions label recovery was generally about 80% although it ranged from 75-105%. When labeled oleate was mixed with serum lipids, 60% was recovered with the free fatty acids, 15% each with monoacylglycerol and diacylglycerol, and 5% each with triacylglycerol and origin, suggesting that adsorption onto other lipids was responsible for the low and often erratic recoveries. Thin layer chromatographic analysis also allowed to follow the breakdown of the labeled triglycerides. After 10 min incubation, labeled diacyl and monoacylglycerols and free fatty acids were close to the theoretical 2/1. Monoacylglycerol was a minor fraction, generally less than 2% of labeled substrate, and did not increase linearly with time after 30 min incubation. The sum of the three labeled products equalled the decrement in labeled substrate. No evidence

of transesterification with phospholipids was found. Compared to 600 g supernates, tissue homogenates generally had about one-third less acid lipase activity, but gave more reproducible results. Extracts were stable at -18°C for several wk, although in one instance a cirrhotic liver extract lost 50% activity in 4 days. Patient's extracts were stable for at least 10 wk. Cholesterol esterase was determined by the procedure of Sloan and Fredrickson (31) and acid lipase by that of Aubert-Tulkens and Van Hoof (1) using 4-methylumbelliferyl (MU)-oleate. Total lipids (5), triglycerides (4), free and esterified cholesterol (18), phospholipids (28), and protein (19) were determined according to published procedures.

Extracts from acetone powders had the same MU-oleate lipase activity as the liver homogenates in one cirrhotic liver whereas it lost 85% activity in another. Thirty percent of the acetone powder MU-oleate lipase activity was butanol soluble and 70% was recovered in the butanol insoluble pellet. Tripalmitylglycerol and oleylcholesterol lipase retained 10–20% activity in butanol extracts from acetone powders, and none was recovered in the insoluble pellets. This suggested that endogenous lipids did not compete with or contribute to the MU-oleate lipase activity whereas they were essential to the activities against exogenous lipid substrates and could not be replaced by the phosphatidyl serine or nonionic detergents present in the substrate mixture.

Case report. The detailed clinical history has been previously reported (3). The parents were doubly consanguineous. This obese

girl (Fig. 1) had a height below the third percentile, tapetoretinal degeneration with decreased vision, deafness, progressive psychomotor deterioration, seizures, acanthosis nigricans, hepatomegaly, and chronic tubulointerstitial nephropathy.

She developed painful hepatomegaly, thrombocytopenia, marked jaundice, depigmentation and partial loss of hair. She expired at 10 years and 3 months of age, in a state of renal insufficiency and uncontrolled seizures.

Laboratory studies. There was glomerular dysfunction with elevated blood urea nitrogen and creatine, hypertension and proteinuria, tubular dysfunction with metabolic acidosis, aminoaciduria, glucosuria and phosphaturia, hepatic dysfunction with hyperbilirubinemia (5 mg/dl total, and 3.8 mg/dl conjugated bilirubin), and elevated SGPT (95 I.U.). Plasma neutral lipids were increased (total cholesterol 360 mg/dl, esterified cholesterol 270 mg/dl, triglycerides 402 mg/dl), phospholipids and lipoproteins were normal.

A diabetic glucose tolerance test without ketonuria was found. Cerebral spinal fluid protein was normal. The EEG showed a generalized low voltage activity. Ophthalmologic examination showed a tapetoretinal degeneration with pigmentary changes.

Audiometry showed a bilateral neurosensory hearing defect.

Autopsy. The macroscopic examination showed an unusual orange appearance of the liver, heart, and intestine and to a lesser degree of the other viscera. The heart weighed 250 g with atheromatosis of the endocardium, thoracic aorta and coronary vessels.

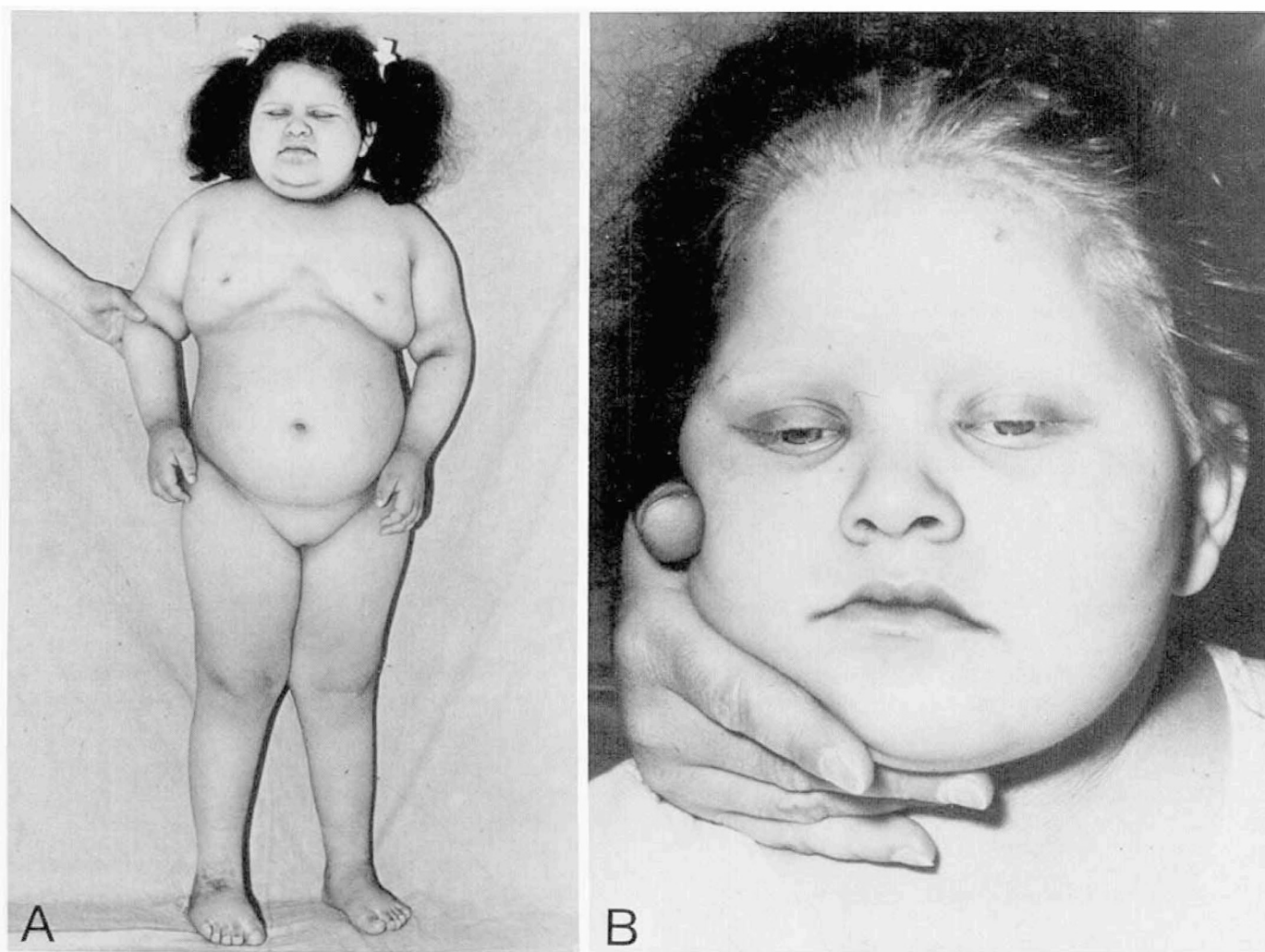


Fig. 1. (A) General appearance of the patient and (B) Moon face. Note the acanthosis nigricans near the hairline and the depigmentation of the hair roots.

The liver weighed 1850 g and spleen 250 g. Kidneys were small (left 42 g and right 52 g). The small intestine mucosa had an orange color with thick, flattened yellow villi.

RESULTS

Histochemistry. All hepatocytes contained lipid droplets (Fig. 2). The vacuoles were of variable size and did not usually displace the nuclei. In frozen sections all cells stained intensely with neutral lipid stains (Oil-Red O, Sudan III, and Sudan Black). The Schultz stain for cholesterol or its esters was negative. The periodic acid-Schiff stain was positive in small scattered aggregates. Sudanophilic droplets were found in spleen histiocytes, heart muscle fibers, lung alveolar histiocytes and interstitial tissue, and cells of the muscularis mucosa from the proximal parts of the small intestine and terminal ileum. The skin had a thickened parakeratotic stratum corneum with acanthosis. Some dermal cells stained for neutral lipids. Kidney glomeruli were markedly hyalinized with thickening of the basal membrane (Fig. 3A). Kidney tubules presented various degrees of atrophy and cystic dilation with interstitial periglomerular and peritubular fibrosis and marked arteriolar sclerosis (Fig. 3B). In frozen sections, residual tubular cells and interstitial histiocytes were stained for neutral lipids. The ultrastructural examination of a kidney biopsy showed thickening and coalescence of the glomerular basal membrane. These findings were interpreted as an endstage interstitial nephritis with arteriolar and arterial nephrosclerosis, compatible with the juvenile nephronophthisis. In the nervous system, there were focal losses and

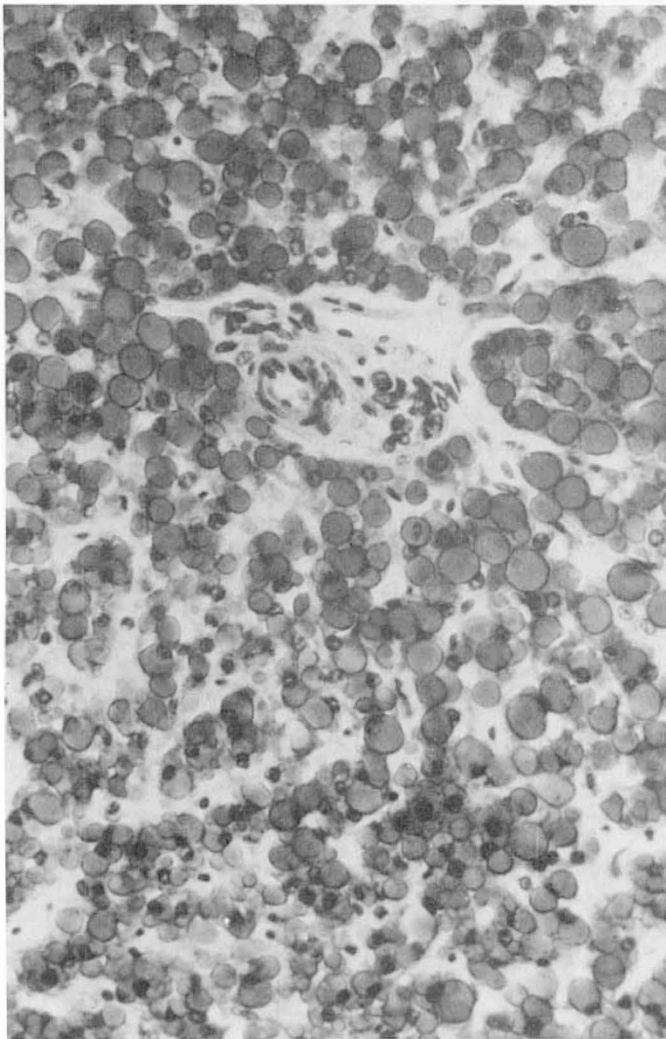


Fig. 2. Liver biopsy. Hepatocytes are filled with large lipid droplets. Oil-Red O, $\times 100$.

lipid storage in cortical neurons and transsynaptic degeneration of the optic pathways.

Lipid composition. The patient's liver contained increased total lipids (3-fold), cholesterol esters (5-fold), and triacylglycerol (8-fold), whereas total phospholipids were decreased by 50% (Table 1). The brain had a normal lipid concentration and composition (data not shown). Thin layer chromatography of brain lipids showed slightly elevated triacylglycerol; cholesterol esters were estimated to represent about 15% of the free cholesterol, whereas they were barely detectable in controls. In Wolman's disease livers, triacylglycerol was increased (5-fold) as well as cholesterol esters (25-fold) with wide variations from case to case; phospholipids were decreased by 50%.

Cirrhotic livers contained an average 3–4-fold increase in triacylglycerol and cholesterol esters. Phospholipid composition remained normal in all pathologic specimens. No more than trace amounts of lysobisphosphatidic acid were identified.

Lipase activities. The data on patient's samples were all expressed as % of the control values (Table 2). MU-oleate lipase activity was within normal limits in the cirrhotic livers. It was markedly deficient in the Wolman's and our patient's livers. In our patient's brain, however, this activity was not deficient. Trioleylglycerol and tripalmitylglycerol lipase activities in cirrhosis had an average one-third of the activity of the control livers with wide variations from case to case ranging from 7–87% of the control mean. These activities were markedly reduced in livers from Wolman's and from our patient within the range found for MU-oleate lipase. In our patient's brain trioleylglycerol lipase varied from 14–60% of the controls and tripalmitylglycerol lipase activity varied from 14–25% of the controls. Oleylcholesterol lipase activity was reduced to 20% of the controls in the only two cirrhotic livers tested. This was in the range also found for the trioleylglycerol and tripalmitylglycerol lipase from these two specimens. Oleylcholesterol lipase was undetectable in livers from our patient's and one Wolman's case. This activity was also reduced at 12–33% in our patient's brain.

DISCUSSION

There is a considerable disparity among patients with an oculocerebrohepatorenal syndrome. Tapetoretinal degeneration (24, 39, 30) and chronic tubulointerstitial nephropathy (Senior Syndrome) have been reported in over 20 patients. Some of those, like our case, presented hepatomegaly, progressive psychomotor deterioration, seizures, deafness, obesity, diabetes mellitus, and ichthyosis. Several were siblings and some were products of consanguineous marriages, supporting an autosomal recessive inheritance. Comparable clinical presentations have been reported (6, 13, 25, 26). The nature of the defect in Senior syndrome (30), Alstrom's syndrome (tapetoretinal degeneration, deafness, obesity, diabetes mellitus and acanthosis nigricans) (13), hepatic fibrosis and nephronophthisis (9) and other apparently related disorders (27) remains undetermined.

The deficient acid lipase activity in our patient explains the generalized triacylglycerol accumulation. TGSD and CESD represent the two extremes in the phenotypic spectrum of acid lipase deficiency. In Wolman's disease both cholesterol esters and triacylglycerols are increased in variable proportion from case to case. It would be tempting to correlate the more benign course with the extent of cholesteryl ester storage, CESD representing the more severe and TGSD the more benign phenotype. Our present case does not support this hypothesis because cholesterol esters were not markedly elevated.

Our patient presented with a generalized neutral lipid storage disorder, which was prominent in the liver and mild in most other tissues, especially the kidney. In our patient's liver, lipid composition was similar to Wolman's cases except for a lower free and esterified cholesterol content. It was also similar to cirrhotic livers except for higher triacylglycerol and lesser phospholipid content.

The demonstration of a severe acid lipase deficiency indicated that we were dealing with a new variant of Wolman's disease. Hepatic steatosis is a nonspecific manifestation of a variety of

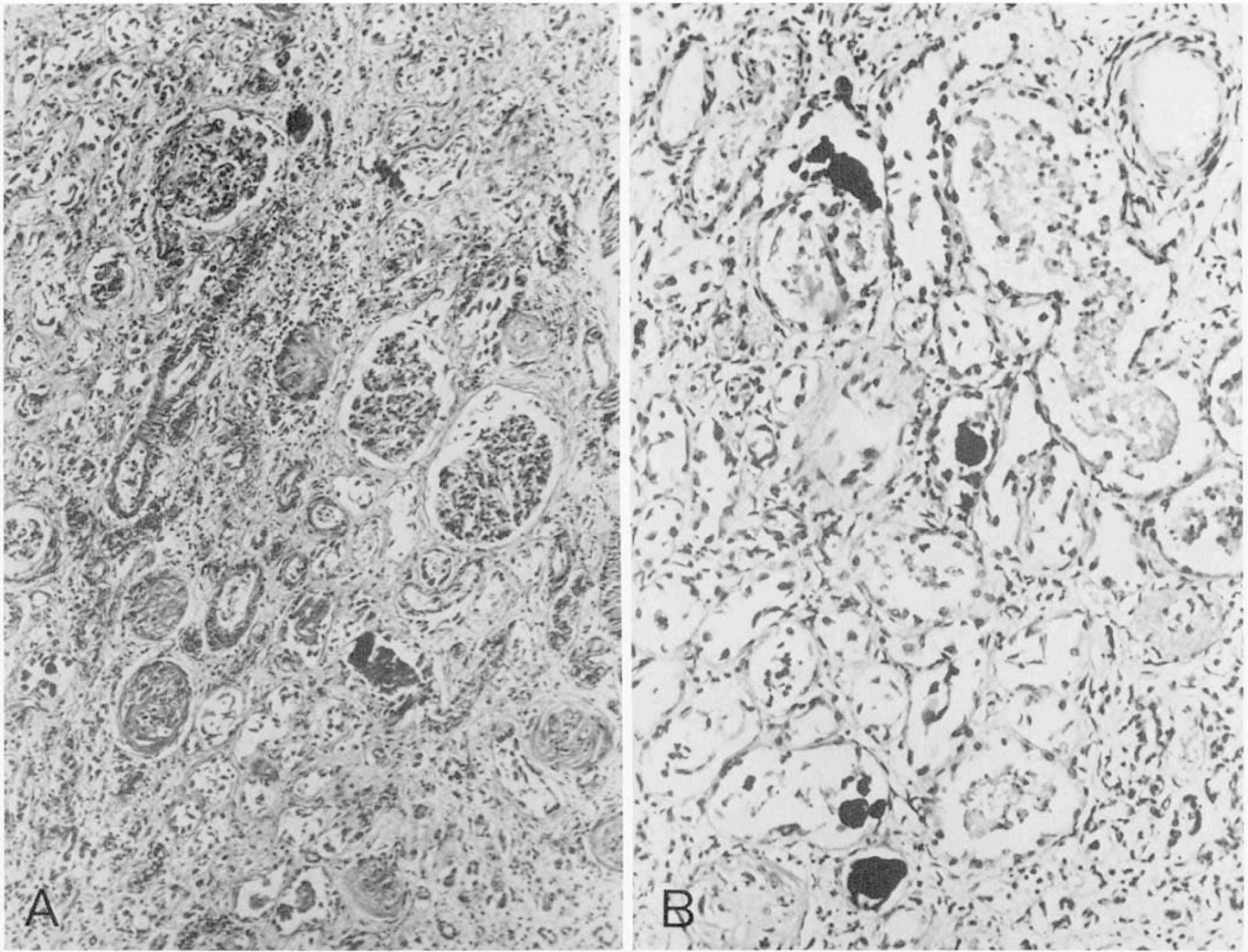


Fig. 3. (A) Kidney biopsy. Glomerular hyalinization with thickening of the basal membrane. Hematoxylin and eosin, $\times 160$. (B) Kidney biopsy, showing tubular atrophy and peritubular fibrosis. Hematoxylin and eosin, $\times 400$.

Table 1. Lipid composition (liver)

	Patient ¹	Wolman ²	Cirrhosis ²	Controls ²
Total lipids	55.3	45.4 \pm 6.2 (7)	30.8 \pm 13.4 (8)	17.7 \pm 7.0 (14)
Free cholesterol	0.4	0.9 \pm 0.5 (4)	0.4 \pm 0.1 (3)	0.4 \pm 0.3 (9)
Cholesterol esters	2.8	12.3 \pm 6.7 (5)	1.9 \pm 1.1 (7)	0.5 \pm 0.2 (8)
Triacylglycerol	37.8	22.8 \pm 15.3 (6)	15.5 \pm 13.0 (7)	4.6 \pm 5.8 (9)
Total phospholipids	3.2	2.94 \pm 0.87 (5)	5.5 \pm 1.0 (8)	6.9 \pm 1.4 (14)

¹ Data expressed as mg/100 mg of dry weight.

² Mean \pm S.D. (number of specimens).

Table 2. Acid lipase

	Methylumbelliferyl-Oleate		Trioleylglycerol		Tripalmitylglycerol		Oleylcholesterol	
	Mean \pm S.D.	Range	Mean \pm S.D.	Range	Mean \pm S.D.	Range	Mean \pm S.D.	Range
Liver (number) ¹								
Controls (7) ²	964 \pm 305	654–1560	11.4 \pm 4.2	4.5–17.4	12.7 \pm 5.8	5.1–19.3	2.8 \pm 1.5	1.1–6.1
Cirrhosis (11) ³	93.0 \pm 36.4	42.9–176.3	31.6 \pm 24.2	7.0–86.8	37.8 \pm 22.8	15.8–83.5	21.4 \pm 2.4 (2)	19.1–23.8
Wolman (7) ³	3.5 \pm 3.5	0.5–12.0	3.8 \pm 3.4 (4)		22.7 (1)		0 (1)	
Patient ³	2.4 \pm 0.2[3] ⁴	2.1–2.7	2.8 \pm 3.1 [6]	0–8.9	3.6 [1]		0 [2]	
Brain								
Controls (7) ²	524 \pm 120	375–661	4.2 \pm 2.3	1.9–8.7	1.5 \pm 0.4	1.1–2.0	2.1 \pm 0.6	1.4–3.1
Patient ³	74.2 [1]		48.2 \pm 20.0 [4]	13.6–66.0	19.3 \pm 4.1 [3]	15.8–25.0	22.5 \pm 10.9 [2]	11.6–33.4

¹ (), number of specimens.

² Controls values expressed as nmoles/h/mg protein.

³ Pathologic values expressed as % of activity in simultaneously run controls.

⁴ [], number of experiments.

liver insults. Only a minority of cases result from a genetic hydrolase deficiency. Our findings that our patient was deficient in lipase activity against a variety of natural and artificial lipid substrates raised the question whether this could be an apparent deficiency due to the competition between the exogenous substrate and the enormous quantity of endogenous substrate accumulated in the tissue. Alcoholic cirrhotic livers were chosen as easily available controls to clarify this point. The MU-oleate lipase activity was equally deficient in livers from our patient and cases of Wolman's disease, whereas it was not affected in cirrhotic livers. Using several natural neutral lipid substrates, livers from our patient and Wolman's cases were equally deficient whereas there was a wide variation in cirrhotic livers with the lowest values overlapping with Wolman's cases and the higher ones being normal. This indicated either competition with the endogenous substrate or ethanol-induced inhibition of the acid lipase. A small brain fragment of the patient provided an opportunity to study lipase activities in a tissue devoid from massive storage. Brain lipase activities were actually different compared to the liver. They were normal against the artificial substrate and reduced to about one-third of controls against natural substrates; therefore, our patient had a generalized deficiency in lipase activity against natural substrates with a variable expression in brain and liver. Detailed studies of these different lipase activities need to be conducted in different organs from the different variants of Wolman's disease before the significance of these results can be evaluated.

Another approach to study the effect of endogenous substrate on lipase activities was to use acetone powders and butanol extracts. Experiments with cirrhotic livers indicated that endogenous lipids were actually indispensable for testing lipase activities against exogenous substrates (see "Materials and Methods"). The availability of Wolman's tissues being quite limited, we were not able to explore this further.

Highly purified lysosomal lipase from rat liver was shown to require a phospholipid activator and to exhibit abnormal kinetics (16, 33). The liver enzyme was inhibited by 2 M ethanol (33), which may be the basis for our findings in alcoholic cirrhosis. Ethanol induced an accumulation of triacylglycerol (32) and cholesterol esters (17, 32), and a concomitant reduction in cholesterol esterase activity in rat liver (32). Human liver cholesterol esterase in contradistinction was stimulated by 2.0–4.0% ethanol (v/v) (34). The irregular kinetics of the interaction of enzymes with lipid micelles have been discussed in detail (10–12). The enzyme preparation may contain different amounts of activator phospholipids (33) and, in pathologic specimens, increased amounts of natural substrates or products may introduce another variable. Our patient's brain had only limited neutral lipid storage as shown by histochemistry and lipid analysis. This could be expected from the sizable acid lipase activities against natural substrates. In Wolman's disease, too, there is little lipid storage in the brain (14), but acid lipase activity has not been adequately investigated. The lack of brain lipid storage in presence of an hydrolase deficiency is also known in infantile Gaucher's disease (23) and Niemann-Pick disease type B (35). The neuronal losses might result from vascular impairment or a putative toxic factor such as that proposed for Gaucher's disease (24). Triacylglycerol and cholesterol esters are at most minor components of neurons and myelin and the traces found in normal brain probably mostly derive from non-neural elements included in the specimen, such as capillaries, meninges, and macrophages. Tissues either not apparently affected or abnormal but not involved by the storage process need to be carefully studied in lysosomal storage disorders, searching for causes of functional cellular impairment that might be more damaging than the storage itself.

Lipid storage, for example, was not prominent in our patient's kidney. A similar inconspicuous storage was reported in the biopsy from the original case from Senior's Syndrome. Because the study of patients with an oculocerebrohepatorenal syndrome has focused on the kidney, the occurrence of the lipid storage may have been

overlooked or not searched for in other organs. In the future it would be important to screen such patients for acid lipase deficiency.

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