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Ultrastructure of Foam Cell Nephropathy

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Die elektronenmikroskopischen Befunde der Schaumzellen-Nephropathie

Zusammenfassung. In unseren Untersuchungen fanden sich unter 1400 Nierenbiopsien 2 Fälle, bei denen es sich um eine neuartige Nephropathie handelte, obwohl keine klinischen Befunde auf eine solche hinwiesen. Histologisch waren beide durch Schaumzellenbildung gekennzeichnet. Elektronenmikroskopisch zeigten sich jedoch unterschiedliche Befunde, die durch Ablagerungen verschiedener Natur bedingt waren. Im ersten Falle waren die Ablagerungen osmiophil, so daß es sich wahrscheinlich um Phospholipide, die in vier verschiedenen Formen erschienen, handelte, die auch bei der Fabryschen und Tay-Sachsschen Krankheit vorliegen. Der Patient jedoch wies weder Hautveränderungen noch neurologische Symptome auf. Im zweiten Falle bestanden die Ablagerungen aus Cholesterin. Elektronenmikroskopisch erschienen die Vacuolen meist leer und nur zum Teil osmiophil.

Summary. In our study of 1400 percutaneous renal biopsies we have found a new morphological type of renal change: a nephropathy with foam cells, seen in patients without any peculiar clinical characteristics.

Two cases of this nephropathy are presented with similar light microscopic changes but widely different electron microscopic findings.

We interpret the differences of the electron microscopic studies to be produced by deposits of different nature. In Case 1, the deposits are osmiophilic, probably phospholipid, and of four morphological types. These types are similar to those described in Fabry's and Tay-Sachs's diseases, but in a patient without dermatological or neurological involvement.

In Case 2, the deposits are cholesterol. With the electron microscope the vacuoles generally appear empty and partially osmiophilic.

In 1961 we described (Jiménez Diaz, Oliva y Garrido) a glomerulopathy in a patient without dermatological symptoms whereby foam cells almost completely filled all the glomeruli. Three years later Hamburger reported on two members of a familiy with a similar renal lesion. We have also found the same pathologic changes in biopsy tissue from another patient who was later autopsied. This paper is to report our completed studies and to emphasize this process which is probably infrequent since we have observed it only twice in 1400 renal biopsies and only once in all our necropsies.

Case 1

C.R.D., a 22 year old man had complained of palpebral and facial edema, and pain in the left lumbar region for the last three years with no alteration in the urine or urinary outpot. With slight variation this situation persisted up to ten months prior to our initial examination, when the lumbar pain increased and became bilateral. The patient presented hematuria, chills, and a fever occasionally to 38°C, and complained of headache and asthenia. He was treated with a salt-free diet, diuretics and antibiotics, which improved his condition somewhat.

The physical examination revealed a well-developed man with some palpabral edema, a pulse rate of 72, and a blood pressure of 120/85 mm Hg. No tenderness was elicited on palpation of the kidney. The extremities were cold without edema.

We followed the patient for a two year period during which the laboratory investigations, summarized below, were performed. Protein was always present in the urine, varying between 0.5 and 2.5 gm/l with a urine density of 1.015—1.022. The Addis count maximum was 1,500,000 red cells and up to 275,000 casts. Urine cultures were always negative. Blood urea varied between 28 and 50 mg% with a clearance of 75%. Rose's reaction was negative and the O-antistreptolysin titer was normal. Total proteins were 5.029 gm% (albumin 2.76 gm%, α-globulin 0.691 gm%, β-globulin 0.777 gm%, γ-globulin 0.796 gm%, giving a normal spectrum). Total lipids were 610 mg%, total cholesterol 175 mg% (ester 91 and free 84). The ophthalmological examination was normal. The sedimentation rate varied between 14 and 40 mm. The WBC was normal. The hemoglobin was 97%. A plain abdominal radiogram disclosed a somewhat enlarged left kidney. During his hospital stay, the patient occasionally had a slight fever (37.3—37.5° C).

Patient was readmitted in April 1965, this time to the Foundation, five years after we had seen him and three years after his last discharge. He reported having been in good health until the last five months when the edema reappeared, this time accompanied by headache and occasional fever. An elevated blood pressure was discovered for the first time. His weakness increased, and he exhibited polydipsia, polyuria, and nycturia.

The physical examination revealed slight facial and ankle edema. The blood pressure was normal, 140/50 mm Hg.

Laboratory studies revealed a hemoglobin concentration of 55%, sedimentation rate of 110, uremia 181 mg%, blood creatinine 13.2 mg% with a creatinine clearance of 6 ml/min, total lipids 715 mg% and cholesterol 148 mg%. In the urine the protein was 1.4 gm/l, specific gravity 1.010. Addis count: 5,000 white cells, 6,000 red cells, 44,000 hyaline casts.

A second renal biopsy was performed and the patient was discharged on a symptomatic treatment. Later, we heard of his death.

Case 2

A.A.C., a 22 year old man, was first admitted in March 1964 with the following complaints: Frequent tonsillitis until the age of 8 years; two months previous when he had a dental phlegmon, which was treated with antibiotics for two days, he noted oliguria, dark urine and edema extending to the abdomen, thighs, and genitalia. Urine analysis revealed the presence of protein. He was diagnosed as having nephritis and referred to the Foundation. Physical examination showed pallor, puffy edema of the inferior parts of the body, and ascites.

The initial laboratory examination revealed proteinuria of 7 gm/l, hematuria with blood cells and casts. The electrophoretic spectrum disclosed marked hypoalbuminemia, with an increase of α_2 and β-globulin. The cholesterol was 492 mg% and total lipids were 971 mg%. Blood urea was 125 mg%.

Treatment consisted of thiazides, osmotic diuretics, and spirolactones, with little improvement. A solution of high molecular weight Dextran was therefore administered. Several paracenteses were performed. Ascites fluid was opaline, with a positive Rivalta reaction, and with 25 mg% albumin. Blood urea varied widely. We interpreted these variations as the consequence of change in blood volume, influenced negatively by the paracentesis, the diuretics, and his nephrotic syndrome, and positively, by the administration of Dextran.

Later we began a treatment with 30 mg Prednisone daily which improved the diuresis and the biochemical symptoms of the nephrotic syndrome. Two weeks later he presented melena and hematemesis with hypotension, which necessitated a transfusion and the suspension of steroid therapy. A GI X-ray examination disclosed no ulcer. At this time the first renal biopsy was performed. A new treatment with 6 Mercapto-purine up to 150 mg daily was begun, with no improvement. ACTH was then administered with equally negative results.

During this time his general condition was poor and he was given large quantities of high molecular weight Dextran (2,760 gm in total) and Sorbitol (4,050 gm in total). Whem this symptomatic treatment was discontinued, diuresis was markedly reduced, and edema and uremia developed.

A renal biopsy was repeated in October, 1964. In December, he was discharged with some mprovement of his nephrotic syndrome but with renal insufficiency and slight hypertension.

He was readmitted in January and September, 1964 and for the last time in March, 1966 in very poor condition, with nausea, diarrhea, and hemoptysis. Laboratory analyses revealed a sedimentation rate of 140 in the first hour, a WBC of 6,000, and a normal differential count. Blood urea was 170 mg%. Total lipids were 1012 mg% and cholesterol 280 mg%. The electrophoretic spectrum showed an albumin of 1.27 gm% and an increase of α_2 and β-globuline. Proteinuria was 7.5 gm/l with granular and hyaline casts. Plasma creatinine was 13 mg% with a clearance of 3 ml/min. Response to treatment was poor. The patient died March 30, 1966, in a uremic coma.

Methods and Materials

From the first patient (Case 1) we studied two percutaneous renal biopsies obtained four years apart, a hepatic needle biopsy, and a bone marrow aspiration. In Case 2 we studied two renal biopsies, obtained five months apart, and the autopsy performed two years later.

The kidney biopsy was fixed in 10% Formalin and stained with hematoxylin-eosin, P.A.S., Mallory trichrome, Sudan Black, and Gallego for elastic fibers. The same techniques were applied to the autopsy specimens, and in addition, Sudan III and Schultz-Liebermann-Burchardt, as modified by PEARSE, were performed for cholesterol. A determination of the cholesterol content by the Windaus gravimetric method was carried out on a fragment of the fixed kidney. Each second biopsy specimen of each case was divided longitudinally and a one mm^3 fragment fixed in osmium solution according to PALADE, washed in buffers at a pH 7.4, dehydrated in progressively concentrated solutions of acetone, stained with uranile acetate and a lead hydroxide solution (REYNOLDS), mounted on Formar film and examined under the electron microscope (Tesla BS 242).

Results

Case 1

a) Light Microscope. The first biopsy (15.306) showed all the glomeruli involved but to varying degrees. The capsular spaces were filled with cells whose cytoplasm was multivacuolated, giving them a foamy, honey-comb-like appearance. These cells were identified as epithelial, mainly from the visceral layer. They produced a collapse of the vascular lumen, although some red cells were seen. With Sudan Black fine granules appeared in the cytoplasmic vacuoles. There was some endothelial proliferation and a thickening of the basement membrane of axial distribution. The foamy cells also appeared in Henle's Loop, the distal tubules, and the walls of small arteries (Fig. 1). The second biopsy specimen (26.254) showed a progressive evolution towards sclerosis but with certain polymorphism. Hyperplasia of the juxtaglomerular apparatus appeared as did an intense sclerosis of the capillary cluster, still surrounded by the foamy cells (Fig. 2), or a marked atrophy with conjunctive proliferation, pericapsular and interstitial. Foam cells were also seen among the cells of the collecting tubule (Fig. 3) and among those of the muscular layer of the arteries. Local infiltrates of lymphocytes were noticed in the interstitium.

The hepatic biopsy (15.648) presented a perfectly preserved lobular structure: only an isolated von Kupffer cell exhibited foamy cytoplasm. The bone marrow aspiration showed good cellular maturation; many reticular cells appeared foamy.

b) Electronic Microscope. The most outstanding feature was the large number of intensely osmiophilic bodies of variable structure which appeared in the glomeruli, tubules and arterioles (Figs. 4, 5, 6).

In the glomeruli, where they were most manifest and diffuse, four types of osmiophilic bodies could be distinguished: A) The irregularly oval or spherical type of 0.65 to 1.73 microns, B) those composed of thick concentric, onion-like

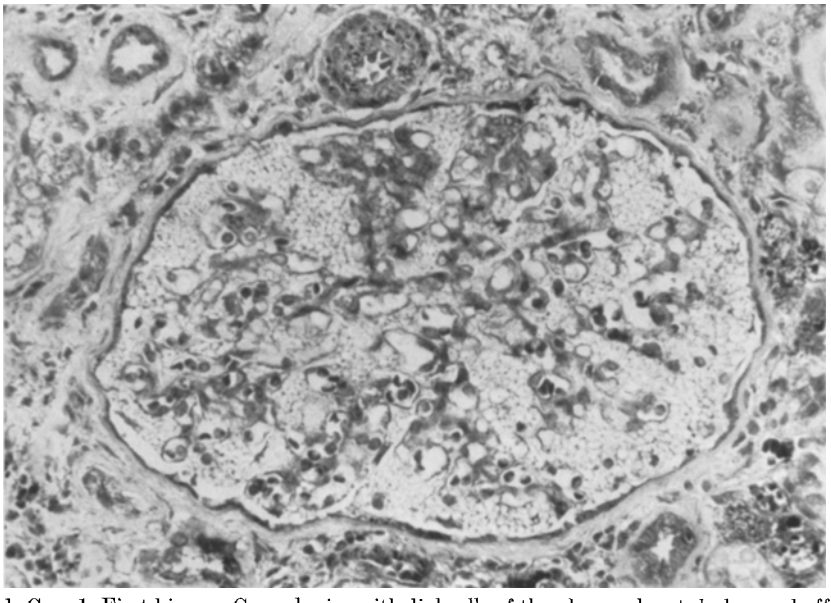

Fig. 1. Case 1. First biopsy. Granules in epithelial cells of the glomerulus, tubules, and afferent arterioles. Sudan-Black. × 190

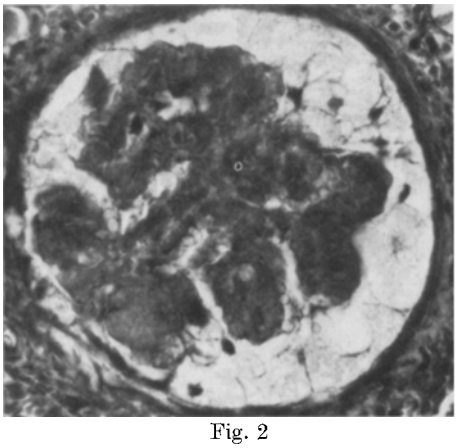

Fig. 2

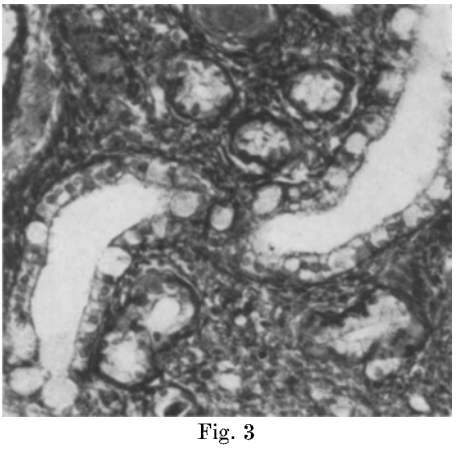

Fig. 3

Fig. 2. Case 1. Second biopsy. Sclerosis of the glomerular tuft surrounded by foamy epithelial cells, visceral as well as parietal. Thickening of Bowman's capsule. Mallory trichrome. × 320

Fig. 3. Case 1. Second biopsy. Foamy cells among those of the collecting tubule. Increase in interstitial conjunctivation and tubular atrophy. Mallory trichrome. × 240

osmiophile layers, separated by osmiophobic strata, reminiscent of myelin figures, of 2.70 to 4.5 microns and generally rounded. C) Those similar to Type B, but smaller, of 1.70 to 2.40 microns and with much thinner osmiophilic bands. D) The type of 2.60 microns, representing a composite of the center of Type A and the concentric layers of Type C. At times, these bodies appeared to be outlined by membranes and were located close together but separated by

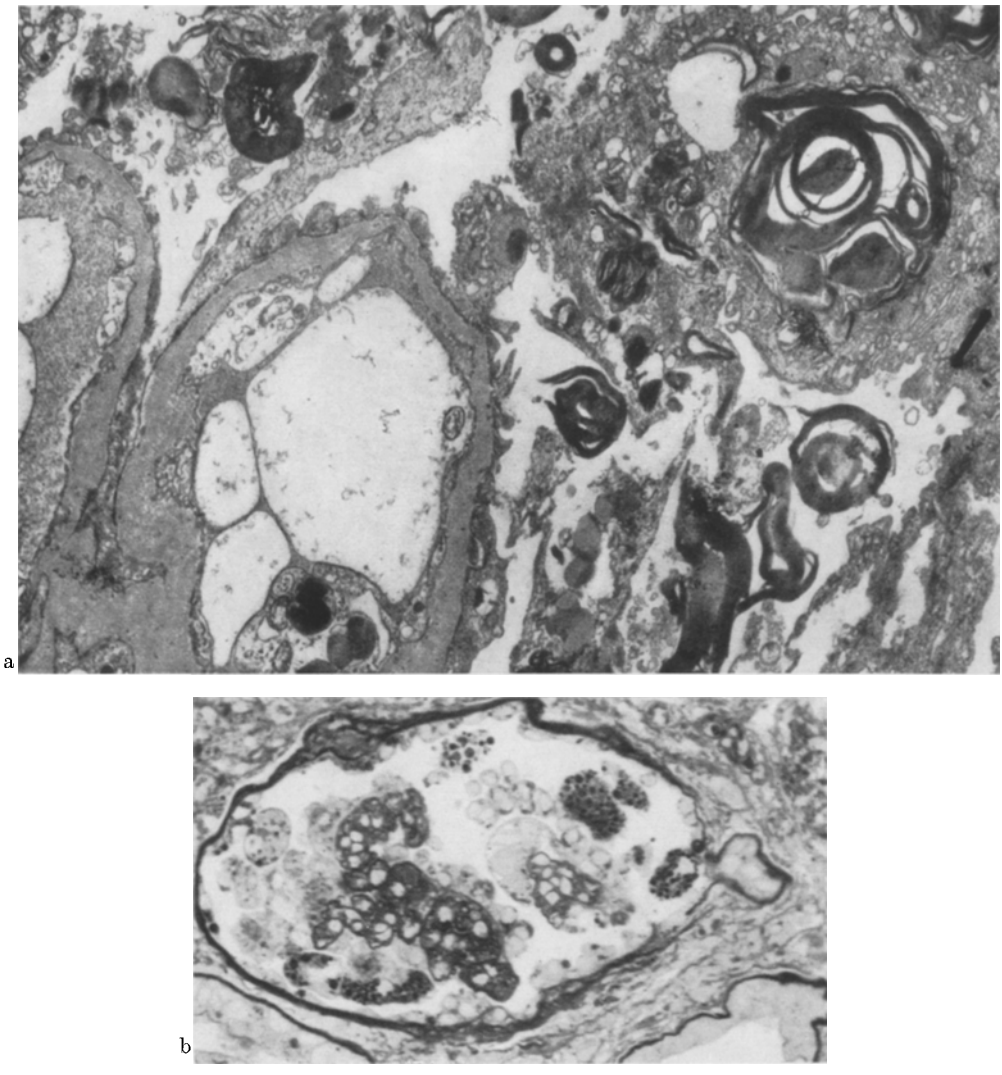

Fig. 4a and b. Case 1. a) Large osmiophilic deposits of a variable structure in podocytes. Basement membrane widened in several areas. Osmiophilic deposit in the endothelium. × 9,200. b) Control, 1 micron, stained with Giemsa, showing a cut of the glomerulus with many foam cells. × 320

cytoplasmic bands. Frequently they were in the capsular space, the result of cytoplasmic fragmentation of epithelial cells. The podocytes also showed a large number of microvilli, a zonal fusion of the foot processes, and numerous small vacuoles.

The width of the basement membrane was normal in many regions, whereas in others its thickness increased up to threefold, at which time the membrane became undulated. This occurred principally near the vascular pole where small dense deposits of a grumous appearance lay within the membrane. Some red cells were seen in the capillaries although the lumina had collapsed. In the endothelium

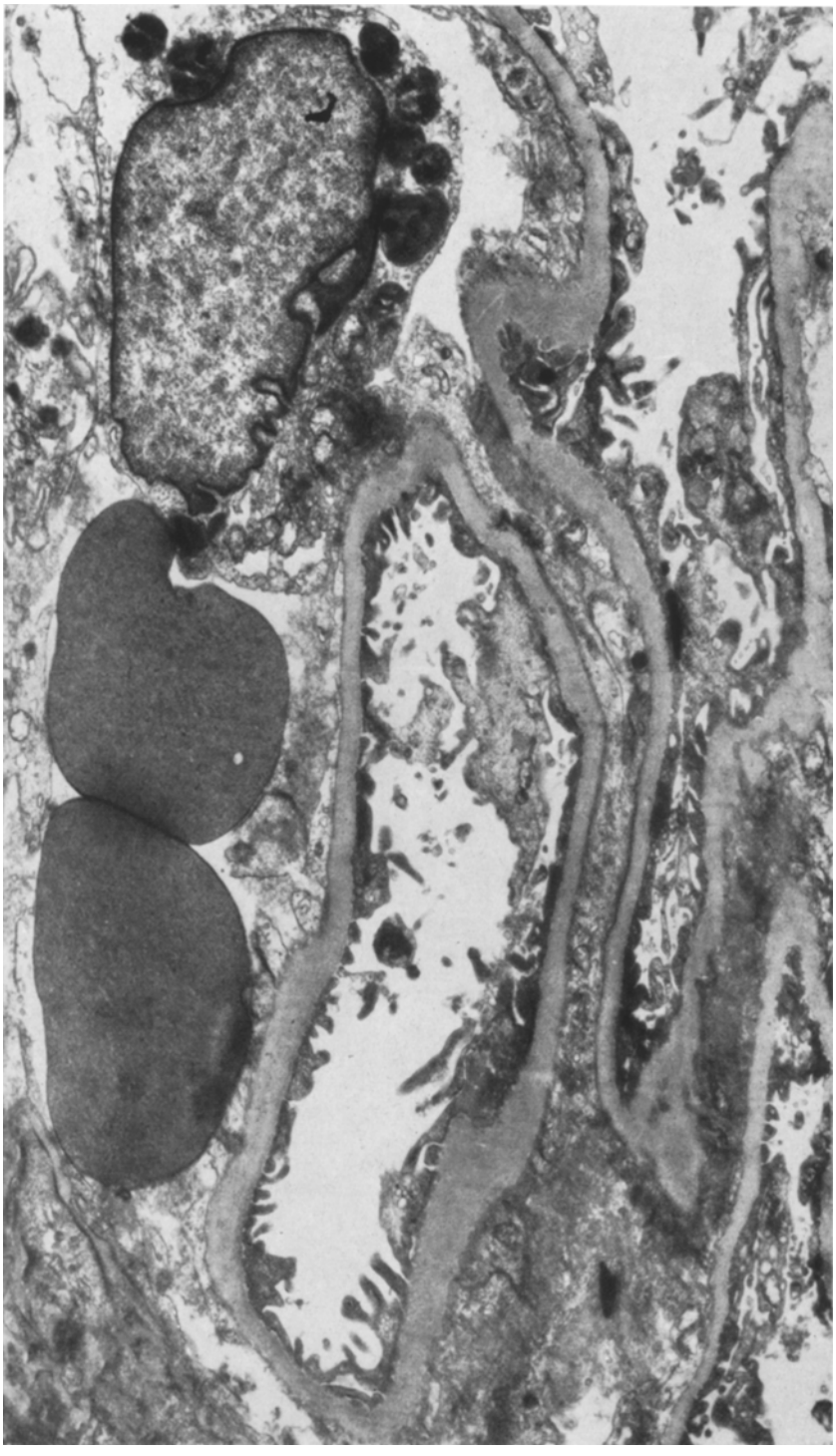

Fig. 5. Case 1. From near vascular pole of glomerulus showing irregularities of the basement membrane, zonal fusion of foot processes and numerous osmiophilic granules of the endothelium. × 9,200

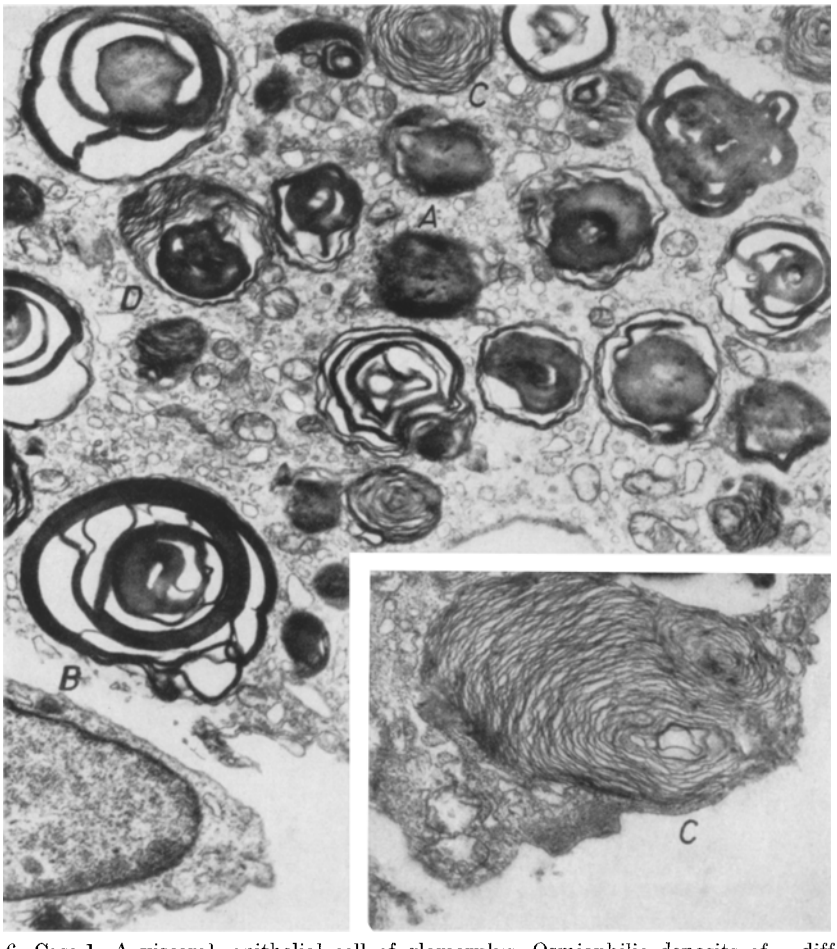

Fig. 6. Case 1. A visceral, epithelial cell of glomerulus. Osmiophilic deposits of a different configuration (see text). × 9,200. Insert: concentric, laminar deposit localized in an epithelial cell. × 9,200

some lipoid deposits were present, but were fewer than in the epithelium and found only in Type A.

Tubular deposits of Types A and B were present (Figs. 7, 8) throughout the canalicular system except in the main segment. In addition, there was mitochondrial swelling and vacuolar degeneration. Many cells, ladened with osmiophilic granules, were present in the interstitium.

The small arteries and arterioles showed Type A bodies, especially in the muscle cells of the media where they compressed and disfigured the nucleus: the endothelium showed them to a lesser degree.

Case 2

a) Light microscope. Both biopsy specimens (24.163 and 25.060) showed similar changes (Fig. 10): a diffuse glomerular process consisting of enlarged epithelial cells, mostly visceral but occasionally parietal cells as well. This change

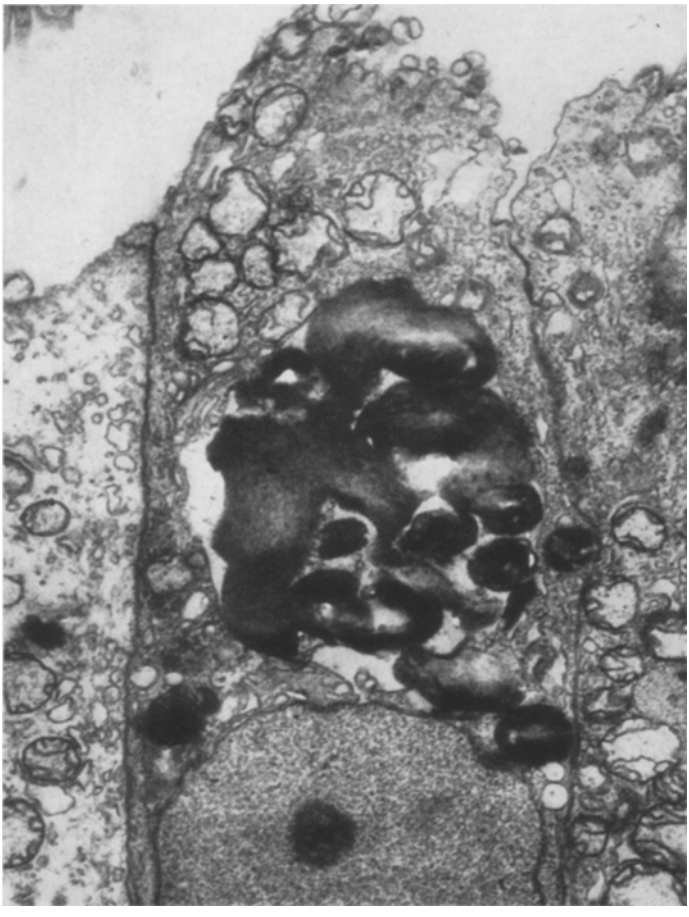

Fig. 7. Case 1. Collecting tube with a large osmiophilic deposit. × 14,000

was brought about by a multivacuolated, foamy cytoplasm. Differing from the previous case, there was a marked thickening of the basement membrane and greater ischemia. There was no proliferation. The glomerulus appeared papillary. Foam cells were also present in the tubulus (Fig. 11) and arteries. Many albuminous casts were evident and there was some secondary interstitial reaction.

At autopsy: the right kidney weighed 130 and the left 100 g. Their capsules could shallow be easily stripped leaving a fairly smooth pinkish surface with very few irregular or lineal scars. The right excretory system appeared hydronephrotic and there was also a right megaureter. Section of the kidney showed a narrowing of the cortex, with normal shaped pyramids that were flecked with yellowish spots. Histologically, in spite of the marked sclerosis, foam cells stained with Sudan III were still seen in the tubules, arteries, and interstitium. In the glomerulus there were fewer foam cells than were in the biopsy specimen; the cells lay free in the capsular space. Marked polymorphism was also noticed characterized by: focal lesions, thickening of Bowman's capsule, conjunctive pericapsular proliferation,

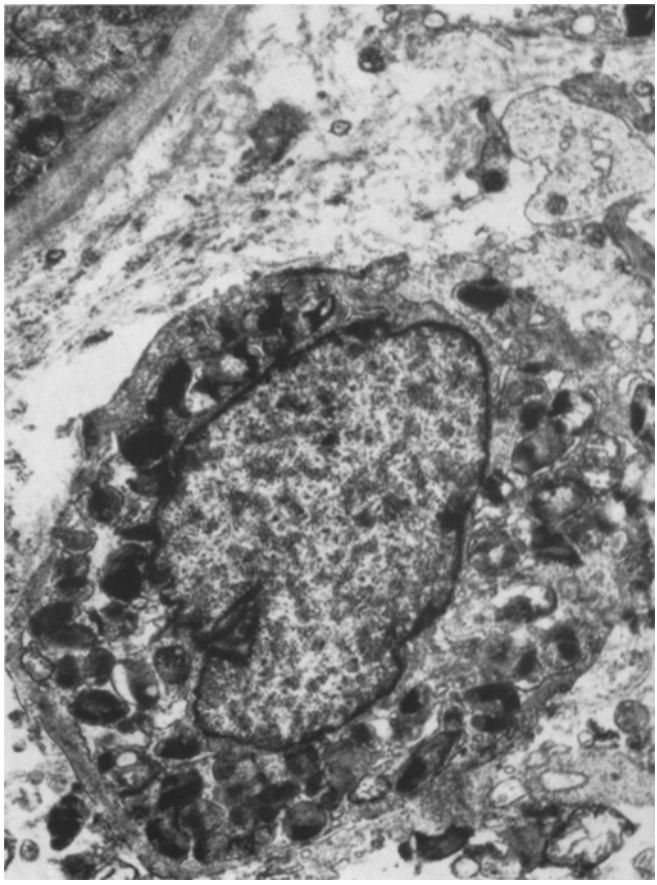

Fig. 8. Case 1. Cell from the renal interstitium, ladden with osmiophilic granules. × 9,200

marked thickening of the basement membrane with a wire-loop appearance and thrombotic, fibrinoid deposits (Fig. 12), epithelial crescents (Fig. 13), and marked atrophy of the tuft. The remaining organs presented the following alterations: moderate hypertrophy of the left cardiac ventricle, a moderate degree of arteriolosclerosis, bilateral lower lobe pneumonia, pulmonary congestion and edema, hemorrhagic gastritis, acute pancreatitis, slight splenomegaly, diffuse adenopathies and no follicular hyperplasia.

Histologically, the foam cells, whose deposits stained with Sudan III, were positive for Schultz's reaction and occasionally birefringent, were found in the bone marrow, lymph nodes, spleen, liver, and lungs.

Chemical analysis of the fixed kidney demonstrated that each gram of kidney tissue contained 310 mg cholesterol. These same deposits appeared in the histiocytes of the proliferated interstitial connective tissue.

b) Electronic Microscope. The vacuoles responsible for the foamy appearance of the glomeruli (Figs. 14, 15, 16) measured 0.65 to 1.20 microns, and were enclosed by a single membrane. At times the vacuoles touched one another and even

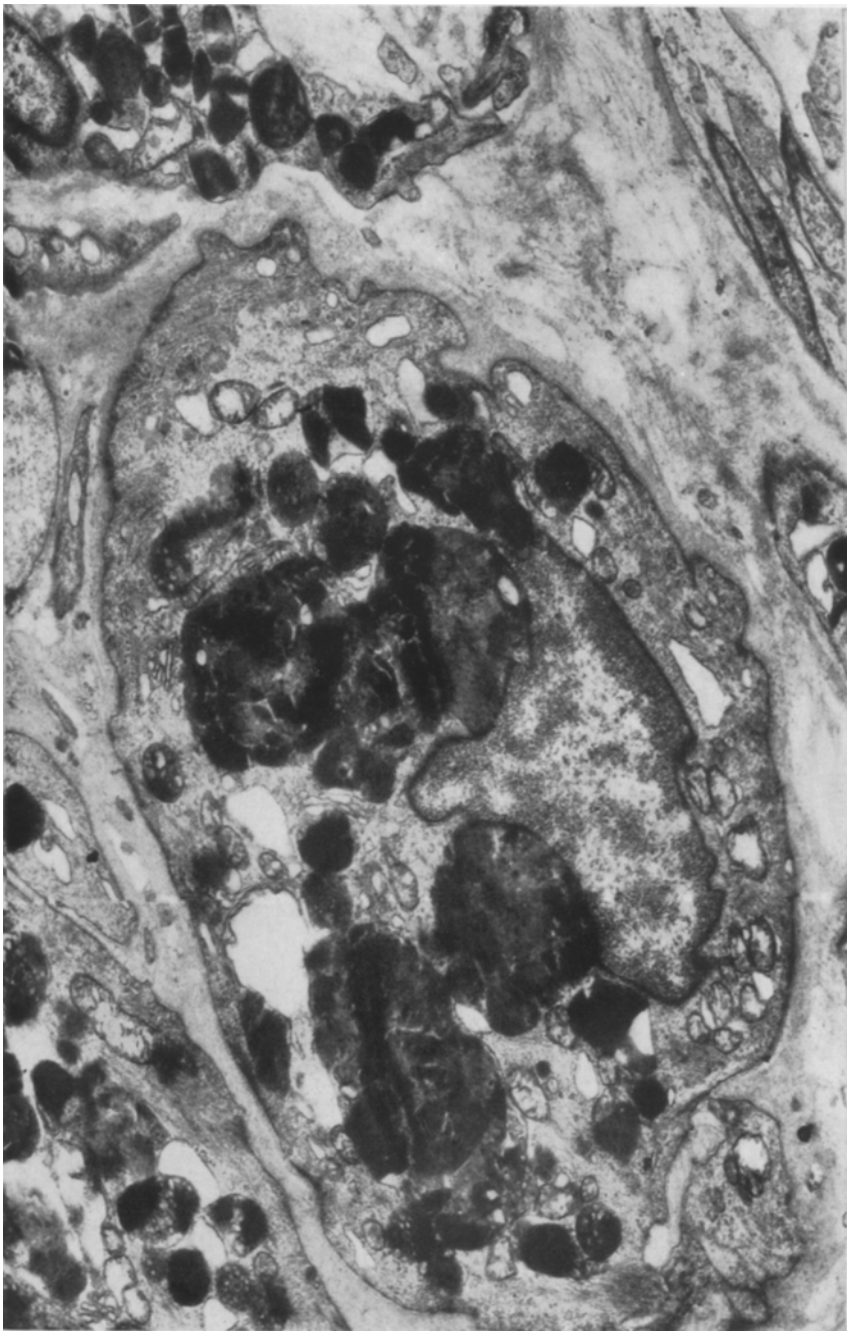

Fig. 9. Case 1. Renal artery, with smooth muscular cells of the media, ladden with osmiophilic granules compressing the nucleus. × 14,300

appeared to fuse. Most vacuoles seemed empty, but some contained a markedly osmiophilic substance in their center with ragged edges, at times threadlike and

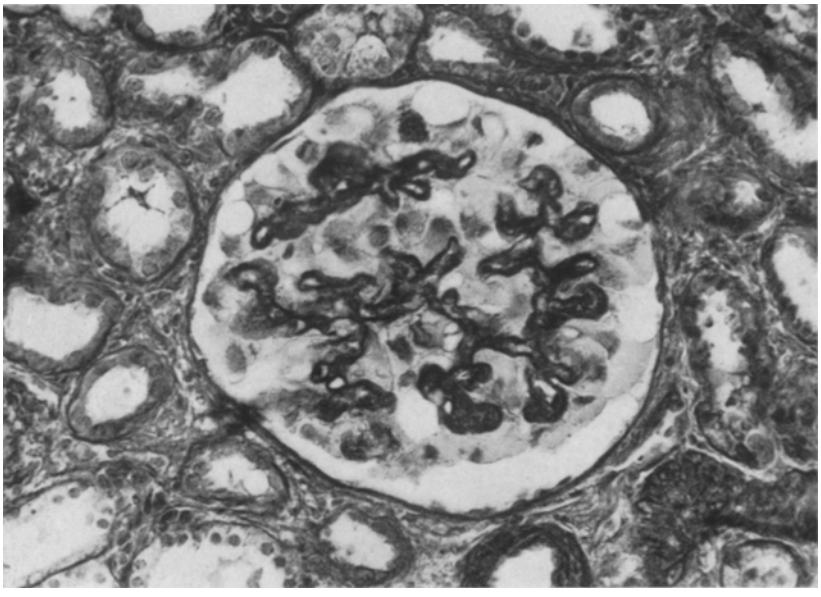

Fig. 10. Glomerulus with epithelial cells of a foamy appearance. The visceral layer is most involved, intense thickening of the basement membrane. Mallory trichrome. × 190

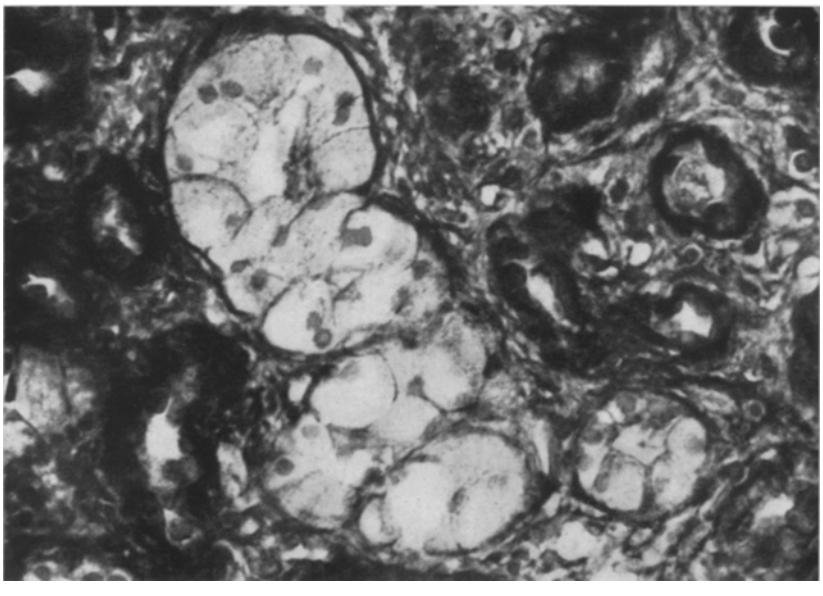

Fig. 11. Case 2. Biopsy specimen. Collapsed tube filled with foam cells surrounded by many atrophied tubules. Mallory trichrome. × 320

at other times excentric, crescent-like. A completely osmiophilic granule was occasionally observed. Almost all the podocytes were completely filled with these vacuoles, rarely un-vacuolized mitochondria were seen. We were unable to show any picture of transitional forms.

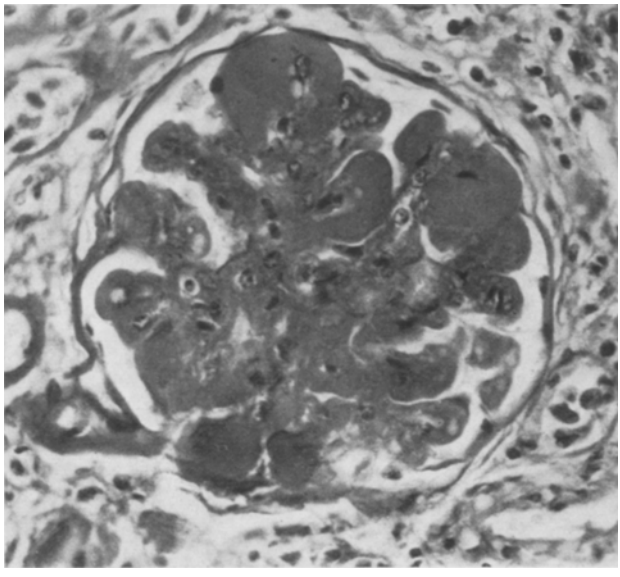

Fig. 12. Case 2. Autopsy specimen. Intense thickening of basement membrane and thrombotic lesions. P.A.S. × 240

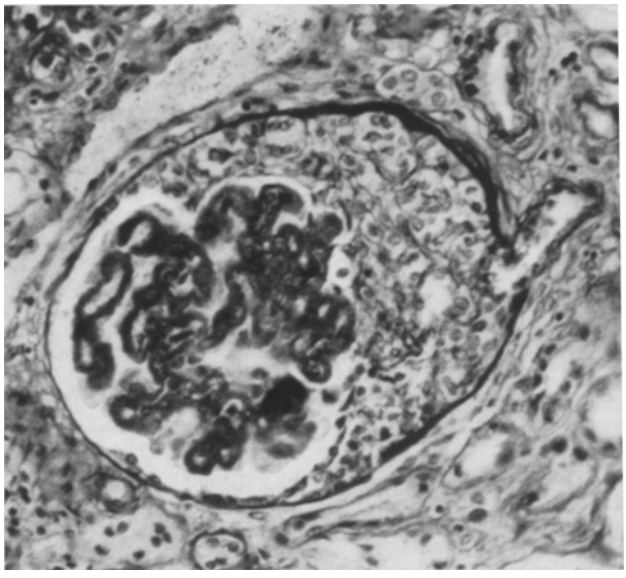

Fig. 13. Case 2. Autopsy specimen. Glomerulus with epithelial crescent. P.A.S. × 160

The podocytes had many microvilli. There was a greater incidence of fusion here than in Case 1. In the glomerular endothelium the mitochondria were swollen and the reticular endoplasmic cisterns well-developed. The basement membrane was thicker than in the previous case, the result of a dense material lying on the epithelial surface. The basement membrane contained osmiophilic inclusions and

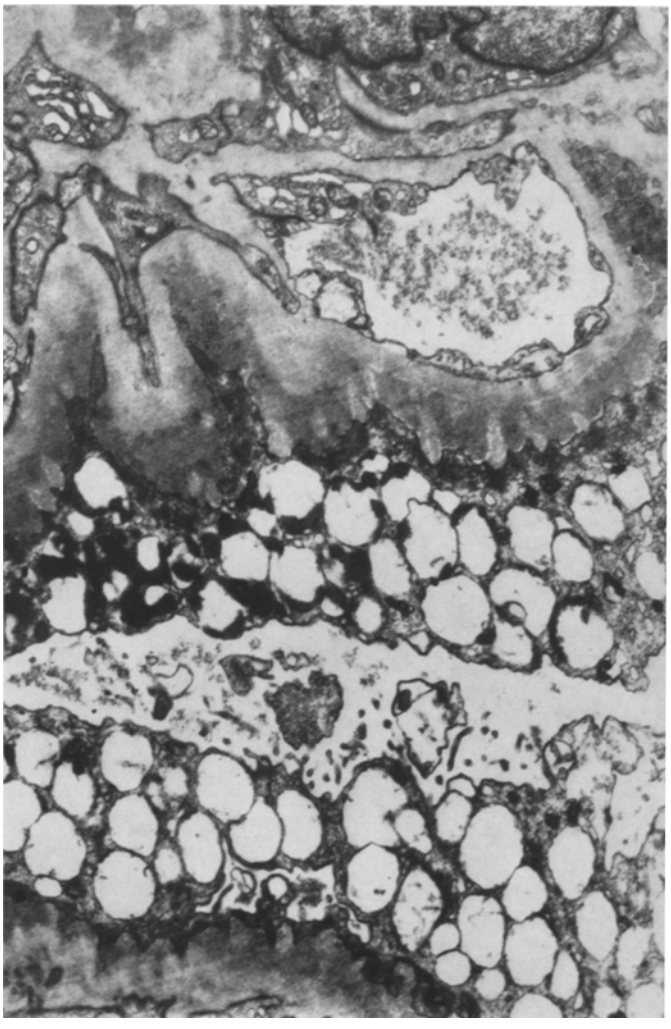

Fig. 14. Case 2. Glomerulus. Two epithelial cells filled with vacuoles, the majority empty but some contain an osmiophilic substance forming dense granules. Basement membranes are tortuous, unfolded, and thickened by a denser material deposited on the epithelial side. × 9,200.

frequently appeared unfolded. In the convoluted tubules there were numerous vacuoles similar to those observed in other types of nephropathies but without any osmiophilic content. Swollen and vacuolized mitochondria were seen.

Discussion

From a clinical point of view, neither of the patients whose pathology we are describing presented any special clinical characteristics. The first case evolved into chronic glomerulo-nephritis, initially presenting as a nephrotic syndrome, progressing later to renal sclerosis.

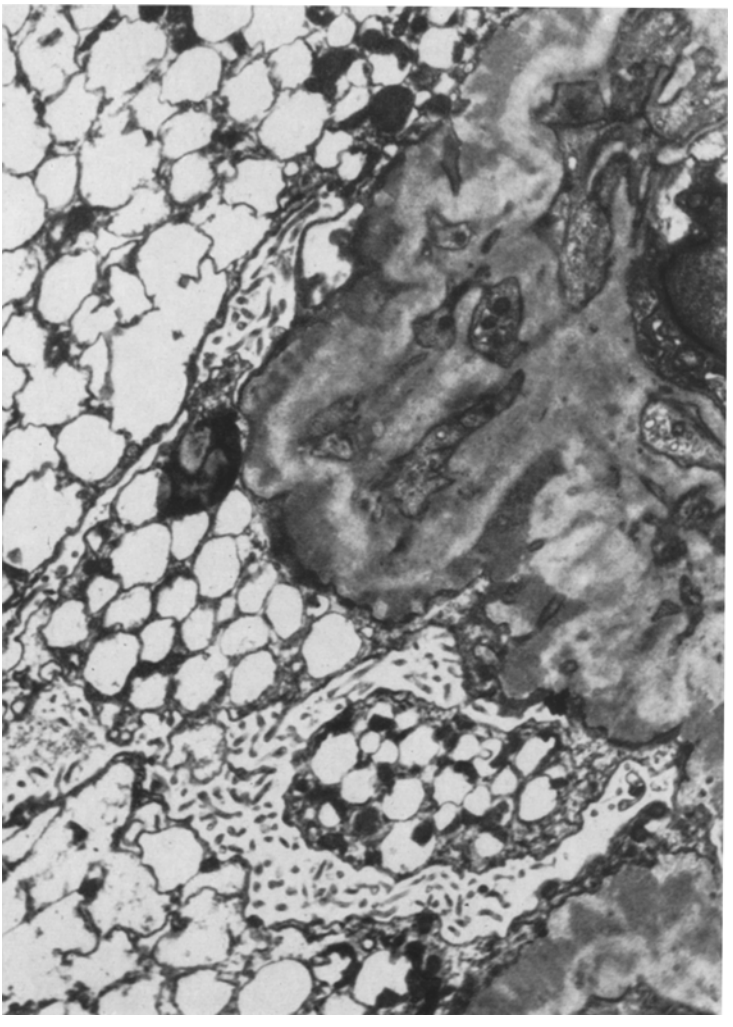

Fig. 15. Case 2. Another appearance of a podocyte with larger vacuoles, occasionally fragmented, with many microvilli. Also fusion of foot processes and extramembranaceous deposits. × 9,200

The second case began acutely and progressed rapidly as florid nephrosis to terminal renal sclerosis.

Neither patient had a familial history of renal disease or of any skin disorder.

The pathological studies revealed an extraordinarily rare type of nephropathy with widespread systematic involvement. In the first case we demonstrated renal, hepatic, and bone marrow involvement; in the second patient the renal, bone marrow, splenic, lymph node, hepatic, and lung tissues were involved. With the light microscope both processes appeared similar, reminiscent of observations of some nephrotic syndromes, as in Niemann-Pick's disease or in tubular sclerosis (Wolfe), although to a much greater degree.

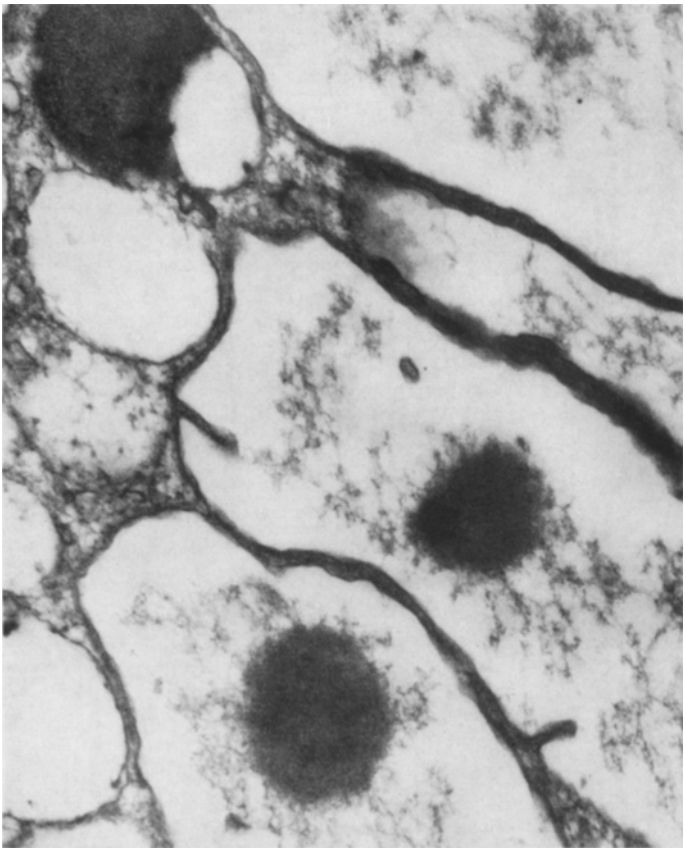

Fig. 16. Case 2. Vacuoles of the podocytes. × 27,500

In Case 1, some granules were seen in the vacuoles with Sudan Black, confirming its phospholipid nature. In the autopsy material of Case 2 the deposits stained with Sudan III and Schultz, thereby proving its cholesterol content.

Both of our cases were characterized by foamy cells in the arteries and nephrons, in the media of the artery in the visceral and parietal epithelium of the glomerulus, and in all levels of the tubular system, though mainly in the distal parts. In the arteries and tubules foam cells appeared singly, but in the glomerulus they formed a continuous covering over the tuft and even filled out the capsular space. There was cellular proliferation of the glomerulus, whereas there was thickening of the basement membrane in Case 2. In both cases, the evolution to sclerosis was similar. It began with interstitial *conjunctivation*, with lymphocytic foci but without those elements usually seen in acute inflammation. The conjunctivation produced a pericapsular belting of the glomeruli, sclerosis of the vascular pole and atrophy of the tuft and tubules. With disappearance of the basement membranes foam cells appeared in the interstitium. The interstitial activation is neither ascending nor reactive to the nephron lesion. We believe it is of an ischemic nature secondary to the vascular involvement.

Although these light microscopic studies were similar, the electron microscopic studies were widely different principally as related to the structure of the accumulated material. The electronic image of Case 1 has been described only in Fabry's disease — angioqueratoma corporis diffusum, coined by that author in 1898. As in our case, there is an unsaturated fat deposit, probably phospholipidic, in the media and intima of arteries of different organs. This deposit is also present in the ganglionic cells of the myenteric plexus (Ruiter, Dubach and Gloor), which permits a diagnostic biopsy of a more accessible region. This substance is stained with Sudan Black and Luxol-Fast-Blue, and shows granular birefringency, sometimes even of the Cross of Malta type (Bethune et al.), but for this, frozen sections previously treated with potassium bichromate are necessary. Jensen's chemical tests performed in three families showed this disease is glycolipidosis with ceroid deposits, to which are added two or three molecules of carbohydrates.

The first description of the renal involvement in Fabry's disease was made by Pompen, Ruiter and Wyers in 1947 (cit. by Hartley). Later, autopsies were reported by Ruiter, Colley et al. and Wallace, in which hypertension and fibrinoid degeneration of arterioles were present. The characteristic foamy aspect of epithelial cells, giving the glomerulus a honeycomb-like appearance, and the presence of multivacuolated cells in the tubulus and arterioles were described. These results were later confirmed in Funk-Brentano's and Bethune's biopsy studies. Bethune reported evidence of thickening of the basement membrane. Later, using the electronic microscope, Hartley et al., Dubach, Gloor, McNary and Lawenstein, and Frost et al. found lipoid deposits in cases with angioqueratoma, of diagnostic importance. The difficulty arises from Colley's description of the necropsy of a female, related to one of the other cases with renal lesions without any dermatological involvement. This probably is what happened in our case and in two cases reported by Hamburger. In Tay-Sachs disease similar, concentric, laminar structures appeared in the neurons (Terry and Weiss). Samuel first observed these structures with a morphologic similarity to that represented in the insert of Fig. 11. The chemical study of this case demonstrated the presence of gangliosides, cholesterol, phospholipids, cerebrosides, and amino acids, listed in descending quantitative order. However, in Tay-Sachs' as well as in Niemann-Pick's disease, two closely related conditions, renal involvement with foam cells in the glomeruli is very rare. The total absence of neurological symptoms in our case rules out these possibilities.

The clinical data of cold extremities might relate our first case to Fabry's disease, even if the pathognomonic sign, angioquerotoma, were not present. The exact nosological position of this case remains obscure, awaiting new cases that might permit its classification.

The micrograph of Case 2 is quite different. There is a predominance of osmiophobic vacuolization, even if some vacuoles are partially occupied by an electron dense material. The cholesterol increase in the blood, in tissues and in the histologic stains of frozen sections, however, may have their morphological counterpart in the foamy cells. The emptiness of the biopsy cells may be atributed to the solubility of cholesterol in alcohol, benzol and xylol needed for light microscope studies, and in acetone used for electron microscope studies. We also know that cholesterol does not blacken with osmium tetroxide (Lillie). Biana has

observed empty cholesterol crystals surrounded by a dense membrane. In relation to this, one must remember that superfixation is sometimes necessary (THOMAS and HALLY) to demonstrate the distribution of the Golgi's apparatus. This is the explanation given by WORTMANN to account for the partial or total emptiness of vacuoles observed by him in milk-fat.

For these reasons we believe the deposits are cholesterol. As the patient was treated with large quantities of macromolecule, we think that perhaps this material may contribute to the foamy character of the cells. With these same substances PARDO and SHAPIRO obtained endothelial and axial vacuolization, and JAMES and ASHWORTH, epithelial vacuolization.

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