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Kidney Biopsies in Human Leptospirosis: A Biochemical and Electron Microscopy Study*

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Nierenbiopsien bei Leptospirose des Menschen:

Biochemische und elektronenmikroskopische Befunde

Zusammenfassung. Die Niere von Patienten mit Leptospirose wurde mittels Elektronenmikroskopie und biochemisch-enzymatischer Bestimmungen untersucht. Verminderte enzymatische Aktivität wurde bei einer morphologischen Läsion der Tubuluszellen gefunden. Cytosome und Cytosegresome wurden mit größerer Häufigkeit in einigen veränderten Tubuluszellen nachgewiesen. Ferner werden pathologische Veränderungen von Mitochondrien, Bürstensaum, Capillaren und Glomerula beschrieben. Diese Befunde sind mit einer toxischen Wirkung auf Blutcapillaren und auf Tubuluzellen vereinbar, welche die Niereninsuffizienz der Leptospirose erklären kann.

Summary. Biochemical enzymatic assays with electron microscopic studies of biopsied human kidney were made in leptospirosis. There was decreased enzymatic activity together with morphological evidence of tubular cell injury. Cytosomes and cytosegresomes were more abundant in some of the altered cells; mitochondrial and brush border injury were present. Damage to capillaries and a mild glomerular alteration were noted. These data are consistent with a circulating toxin which, acting on capillaries and/or directly on the tubular cell, might be responsible for the kidney failure seen in leptospirosis.

Introduction

The purpose of this paper is to reevaluate and to extend some of our first morphological observations on the kidney in leptospirosis. In addition, biochemical dosages of enzymes in the biopsies were employed in an attempt to correlate morphology with functional status of the cell.

Material and Methods

Fourteen patients with definite clinical symptoms of leptospirosis and ascending serum agglutination titers to *L. icterohaemorrhagiae* were biopsied for this study. The main clinical and laboratory findings of these patients are in Table 1.

The electron microscopy study was done in thirteen biopsies as previously described (BRITO et al., 1965) except that the fragments were embedded in Araldyte and examined with a Zeiss EM 9 electron microscope. Furthermore, thick sections obtained from the blocks were stained by toluidine blue (TRUMP et al., 1961) and examined in a light microscope. The remainder of the fragments in thirteen cases were frozen and studied biochemically. The activity of alkaline and acid phosphatase was measured by the GREENSTEIN'S (1942) technique. Succinic dehydrogenase activity was evaluated through a modification of the technique proposed by KUHN and ABOOD (1949). Basically, this modification consisted in the substitution

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Table 1

Case No	Highest blood urea mg/100 ml. Between elipses ( ), the days of illness. Normal = 16 to 35	Highest total serum bilirubin mg/100 ml	Urine (+ to ++++)				At biopsy			Clinical evaluation (severity of the disease + to ++++)	Highest observed serum agglutination titer (*L. icterohaemorrhagiae*) [a]
			Protein	Red cells	Leucocytes	Casts	Days of illness	Blood urea mg/100 ml	Total serum bilirubin mg/100 ml		
1	380 (8)	58.4	4+	+	4+	+	22	127		++++	1:800
2	230 (7)	23.4	2+	+	2+	3+	11	135	15.5	++++	1:800
3	135 (7)	35.6					11			+++	1:800
4	148 (10)	24.0	4+	+	3+	3+	14	64	11.2	+++	1:1,600
5	146 (14)	33.8					16	81	18.0	+++	1:1,600
6	119 (8)	22.6	+	+	+	+	15	42	7.4	+++	1:1,600
7	77 (7)	42.6	2+	0	2+	+	9	77	42.0	++++	1:3,200
8	250 (7)	—	4+	4+	4+	4+	13	46	22.6	++++	1:800
9	66 (13)	—	2+	0	+	+	13	66	9.5	++	1:1,600
10	230 (17)	23.2	+	+	4+	+	32			++++	1:3,200
11	155 (9)	20.8					9	155	20.8	++	1:1,600
12	145 (9)	27.6					13			+++	1:3,200
13	310 (10)	22.6	3+	4+	4+	—	17	93		++++	1:800
14	405 (9)	32.8	2+	4+	2+	+	11	139		++++	1:800

[a] Not necessarily the highest titer reached during the illness.

of the triphenyltetrazolium chloride by the MTT (3-(4,5-dimethylthiazolil-2)-2,5-diphenyl tetrazolium bromide).

Statistical significance of the results was evaluated by STUDENT's test as described by SNEDECOR (1948).

Eight kidney fragments, obtained from patients with duodenal ulcer during gastrectomy and one needle biopsy from a healthy volunteer, were used as controls for the biochemical study. In four of the patients, including the volunteer, electron microscopy study was also done.

The mean age of the control group was 38 years, the oldest being 49 and the youngest 23 years old. In the pathological group the mean age was 32 the oldest being 49 and the youngest 15 years old. All the patients, except one, were males.

Results

a) Clinical Evaluation. The main clinical and laboratorial data of our patients are listed in Table 1. The severity of the disease in each case was graded from one to four plus. Three patients needed peritoneal dialysis to control mounting uremia.

b) Biochemical Study. The biochemical data regarding the enzymatic activity of the kidney in patients with Leptospirosis as compared to controls are in Table 2. There was a significant reduction in the activity of all enzymes studied, mainly alkaline phosphatase and succinic dehydrogenase.

Table 2. *Biochemical determinations of the enzymatic activity in normal and pathologic kidney (mean values ± standard deviation)*

Enzyme	Normal	Leptospirosis	t test. Normal versus pathologic
Succinic dehydrogenase	7.97 ± 0.48	3.83 ± 1.84	$t = 4.86$
	$N = 5$	$N = 8$	$p = < 0.001$
Alkaline phosphatase	20.32 ± 5.43	5.14 ± 1.69	$t = 8.03$
	$N = 9$	$N = 9$	$p = < 0.001$
Acid phosphatase	30.14 ± 6.13	18.54 ± 9.61	$t = 3.05$
	$N = 9$	$N = 9$	$p = < 0.005$

N = Number of patients; p = probability level.

c) Electron Microscopy. Usually focal lesions were found in the kidney, normal groups of nephrons alternating with pathological ones, without a definite pattern of distribution. The lesions were mainly tubular, injured cells being seen throughout the nephron but predominating in the proximal convoluted tubules.

Mild alterations were seen in a limited number of glomeruli, epithelial cells showing areas of irregularity and foot process fusion (Fig. 1 A). Although in most areas the basement membrane appeared unaltered, in some it was uneven in shape with thickened segments due to a prominence of the lamina rara interna.

Mesangial cells were hyperplastic and sent projections along the capillary wall, between the basement membrane and the endothelial cytoplasm (Fig. 1 A and 2).

Groups of endothelial cells appeared slightly swollen and occasionally granulocytes and/or mononuclear cell were seen occluding the glomerular capillary loop (Fig. 2). Round electron dense inclusions were seen in the cytoplasm of some endothelial cells.

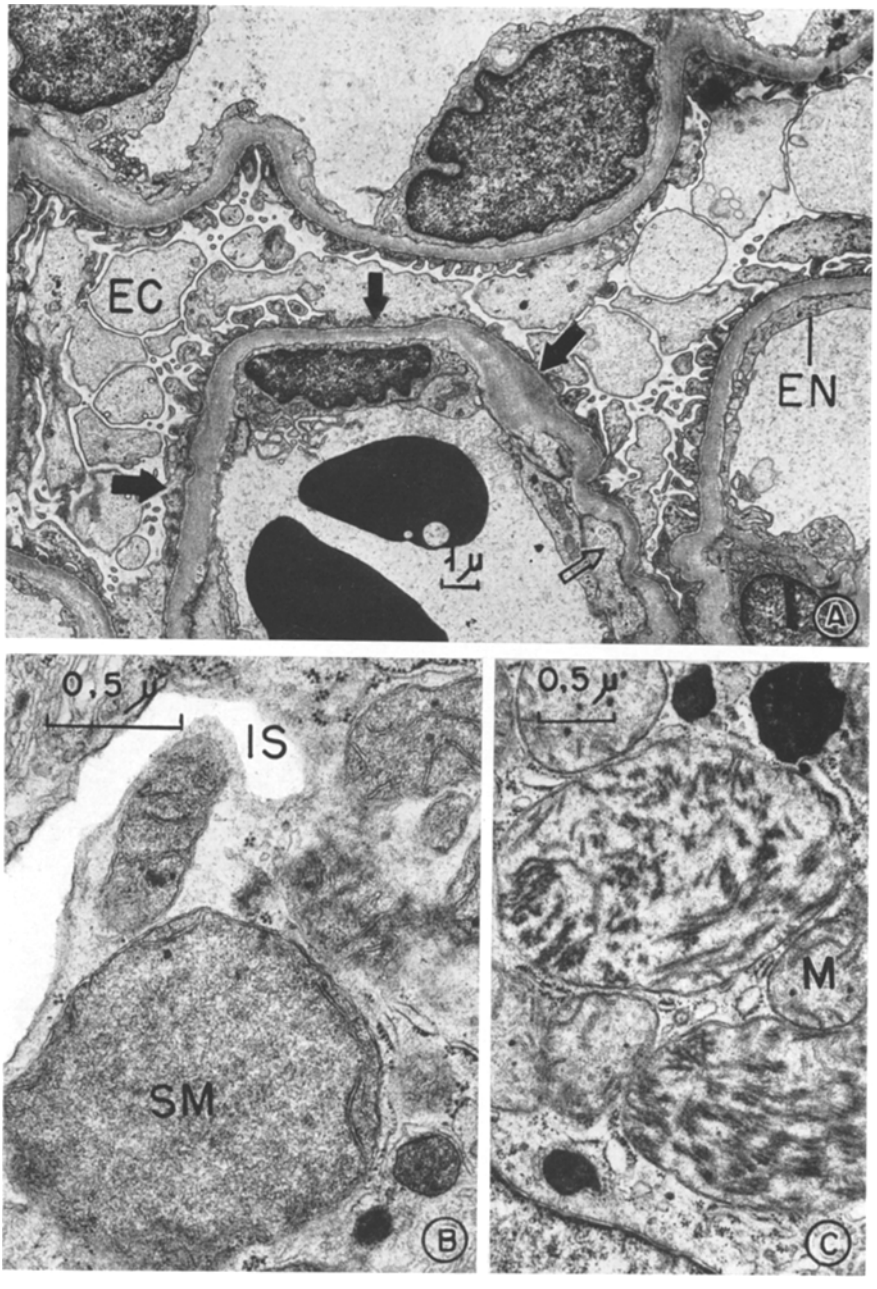

Fig. 1 A. Glomerular epithelial cells (*EC*) exhibiting areas of foot process fusion (black arrows) and slight microvilli formation. Basal membrane show focal swellings. Mesangial cells cytoplasm extended so as to surround capillary lumina partially, adding a third cellular layer to the capillary wall (light arrow); there is slight endothelial cell swelling (*EN*). 5250 ×

Fig. 1 B. Enlarged mitochondrium (*SM*) with a matrix of normal density and few peripherally located cristae. Compare its size with the normal appearing mitochondria of the tubular cell. *IS* designates the intercellular space. 36.000 ×

Fig. 1 C. Enlarged mitochondria with many abnormal cristae. Compare with normal mitochondria (*M*). 21.000 ×

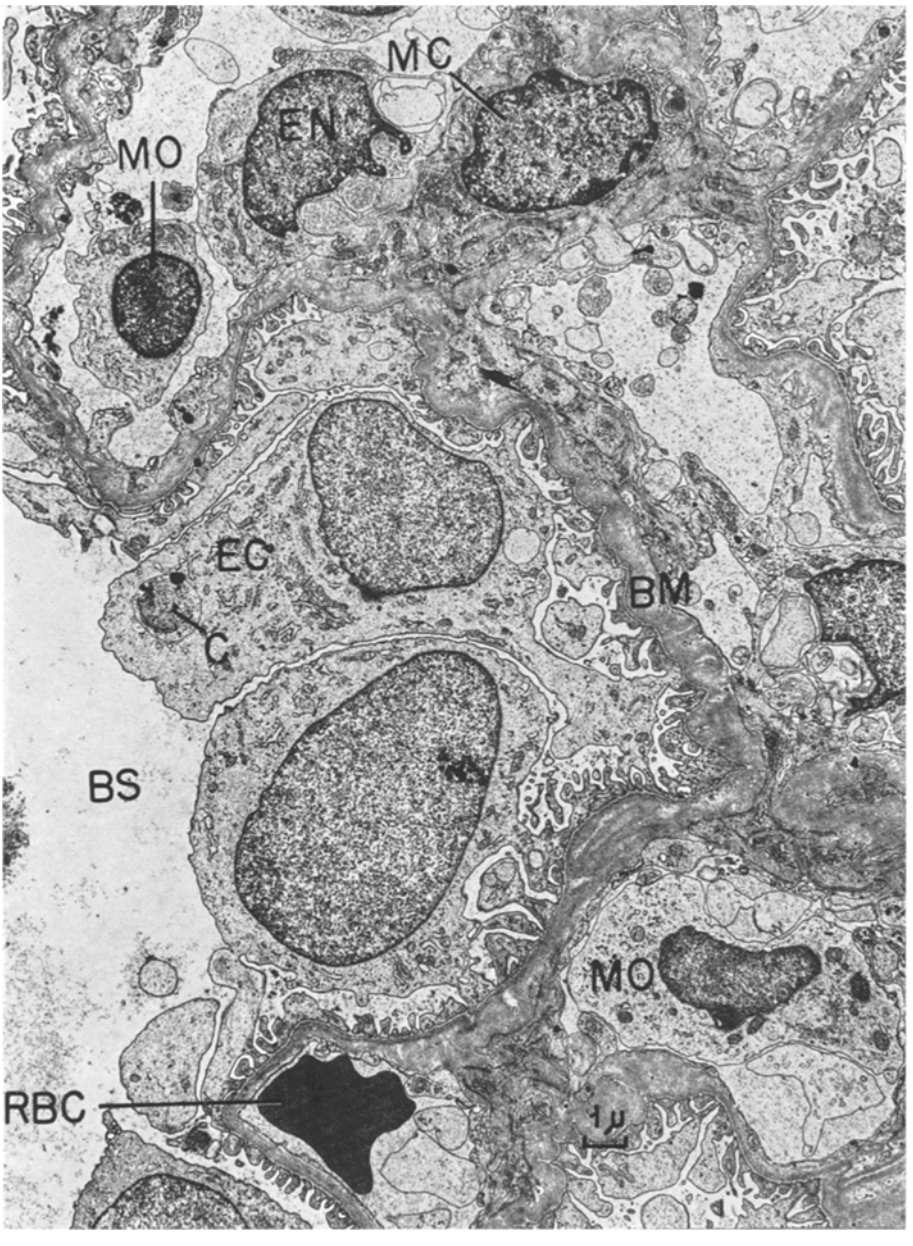

Fig. 2. Peripheral portion of a glomerulus showing impacted mononuclear cells (*MO*) in the capillary lumina. Mesangial cell (*MC*) is seen nearby the urinary space (*BS*). Epithelial cells (*EC*) appear normal. One cytosome (*C*) is seen in one of them. Basal membrane (*BM*) is irregular in thickness. Endothelium (*EN*) is slightly swollen. *RBC* designates a red blood cell. 5.250 × (reduced to $^{19}/_{20}$ for reproduction)

The proximal convoluted tubules were usually dilated (Fig. 3) despite that direct immersion of minced tissue into fixative may result in artificial closure of their lumina (ERICSSON, 1964) a fact seen by us in our controls. Cytoplasmic

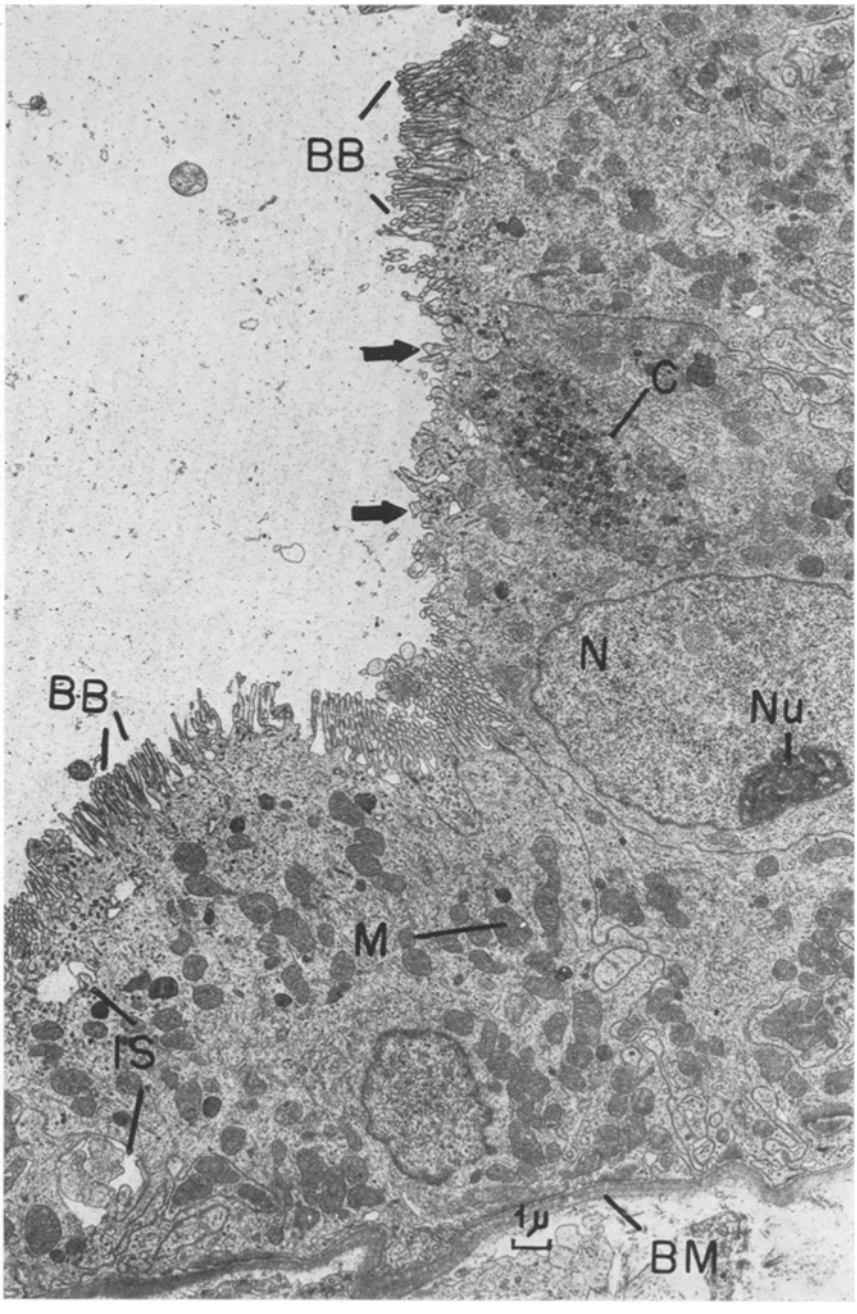

Fig. 3. Dilated proximal tubule exhibiting a cell with a shortened and distorted brush border (arrows) and a large cytosome (*C*). Normal appearing brush border (*BB*) is also seen. Nucleus (*N*) with nucleolus (*Nu*) is seen in the injured cell. Few enlarged intercellular spaces (*IS*) are also seen. Mitochondria (*M*) and basal membrane (*BM*) are normal. 5.250 ×

bulging of the cells into the lumina was considered artefactual due to short periods of anoxia prior to fixation. This artefact, however, was more common in

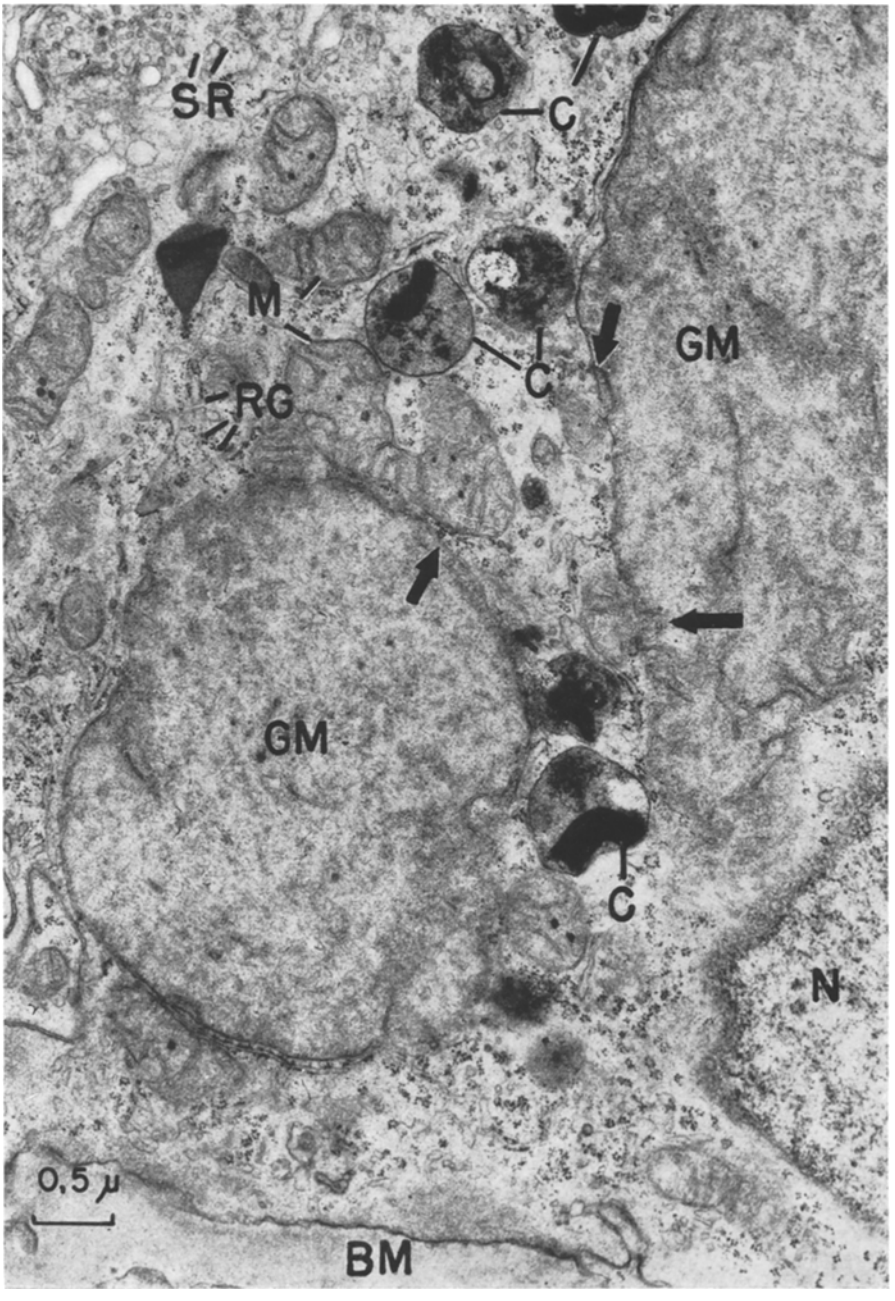

Fig. 4. Giant swollen mitochondria (*GM*) with few cristae (arrows) located at the periphery of the organelles are seen in a cell of the proximal tubule. Cytosomes (*C*), normal mitochondria (*M*), ribonuclein granules (*RG*) and smooth reticulum (*SR*) are also observed. Part of a normal appearing nucleus (*N*) and basal membrane (*BM*) are also seen. 21.000 ×

the pathologic kidney. Microvilli were normal in large areas, but groups of cells showed partial absence of these, and some appeared blunted and distorted (Fig. 3).

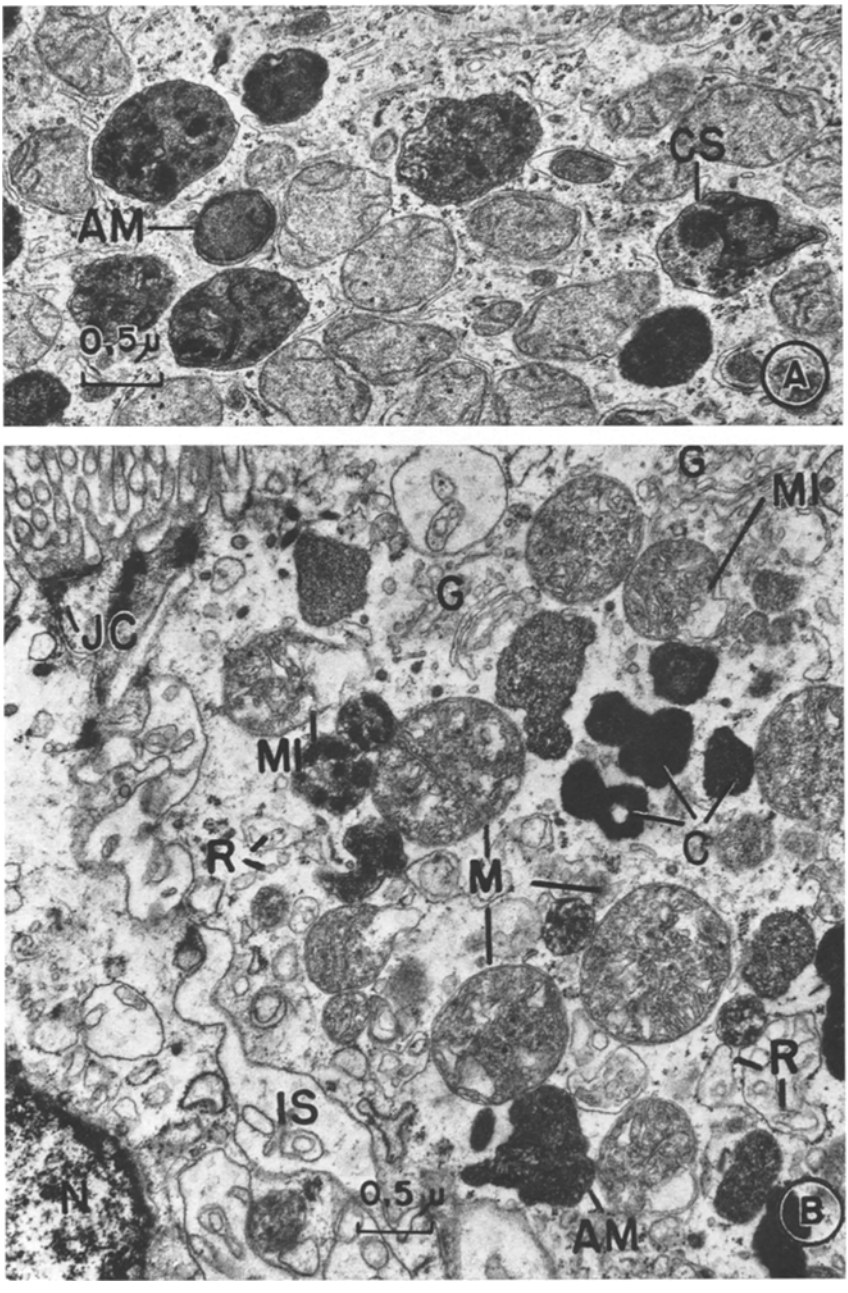

Fig. 5A. Tubular cell showing a structure with a double membrane and a dense matrix (*AM*) which could be interpreted as an altered mitochondria. A cytosegresome (*CS*), probably containing a mitochondria, is observed. 21.000 ×

Fig. 5B. Cells of a proximal tubule showing mitochondria (*M*) with disarrangement of their cristae, lightening and widening of the intermediate layers. Some of them (*MI*) show partial disappearence of the external double membrane. Cytosomes (*C*) are seen together with irregular electron dense bodies (*AM*) which could be the end result of the altered mitochondria. There is dilation of the cisternae of the reticulum (*R*). Brush border and junctional complex (*JC*) are preserved, but the intercellular space (*IS*) is dilated. *G* designates the Golgi complex. 21.000 ×

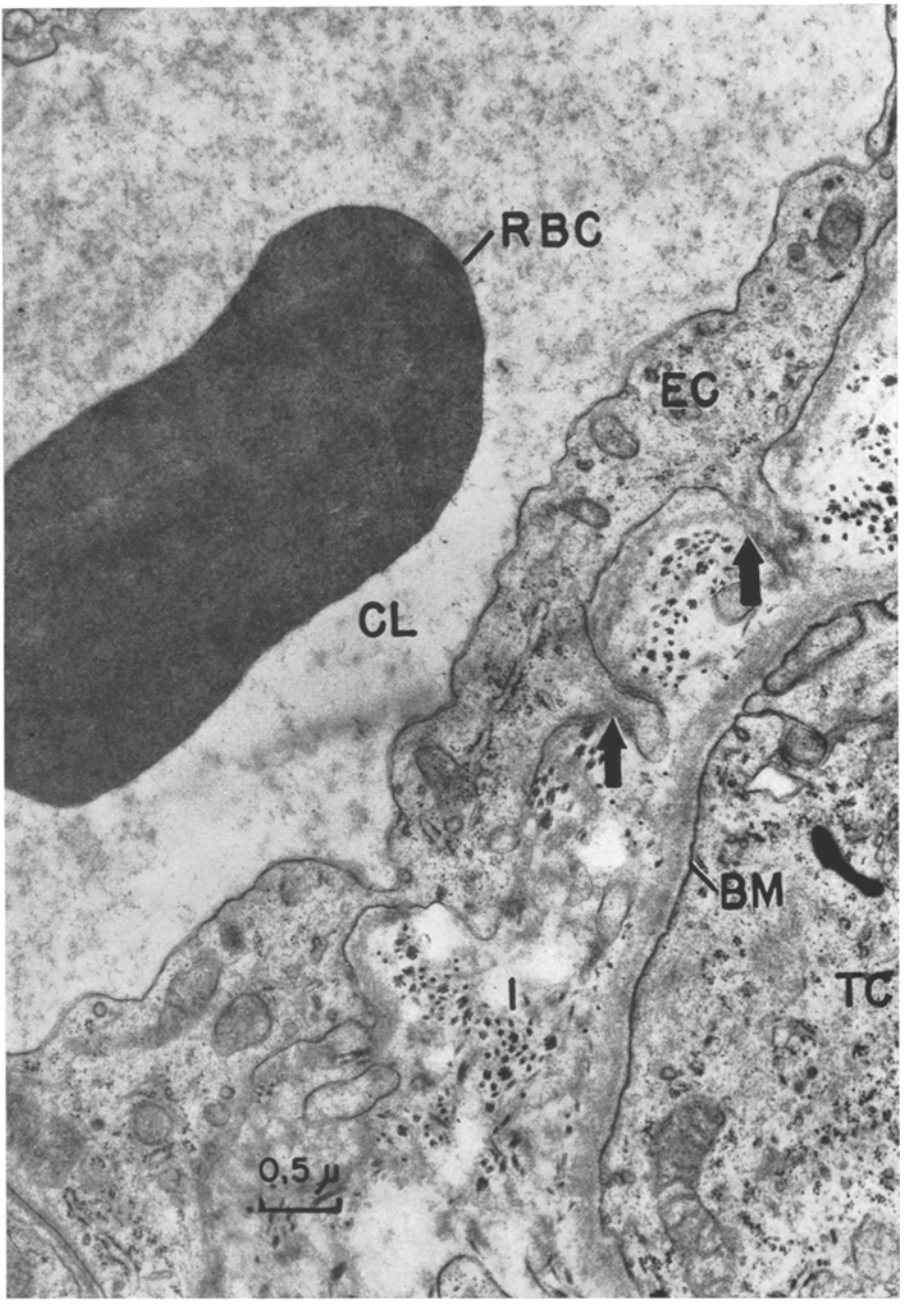

Fig. 6. Interstitial capillary exhibiting swollen endothelial cell (*EC*) with elongated finger-like cytoplasmic projections (arrows), apparently throughout the capillary basal membrane. Interstitium (*I*) is slightly edematous. Part of a tubular cell (*TC*) with an intact basal membrane (*BM*) is seen. A red blood cell (*RBC*) can be seen in the capillary lumen (*CL*). 21.000 ×

Some of the damaged cells showed an increased number of cytosomes and/or cytosegresomes.

Mitochondrial alteration was seen and grouped in four types. In the first there was organelle shrinkage with increased stromal density, but usually with preservation of the cristae and of the external double membrane. Some of these mitochondria were seen inside cytosegresomes (Fig. 5A). The second was characterized by disarrangement of their cristae with lightening and widening of the intermediate layers (Fig. 5B). Some displayed irregular deposits in the matrix of an electron dense material.

The third type is that of an enlarged swollen mitochondria with or without pallor of the matrix (Fig. 1B). The last manifestation of mitochondrial pathology was the appearence of gigantic organelles which were grouped in two types, one with a decreased matrix density and few cristae located at the periphery (Fig. 4) and the other with many abnormally disposed cristae (Fig. 1C).

Slightly swollen mitochondria were seen frequently but were linked to short periods of anoxia prior to the biopsy fixation.

The more markedly injured cells disclosed less cytoplasmic ribonuclein granules with a predominance and dilation of the cisternae of the smooth type of reticulum (Fig. 5B).

In some areas, wandering mononuclear cells were seen in the intracellular spaces. This permeation of the epithelium by inflammatory cells was seen both in the normal and the pathologic kidney but it was more frequent in the latter. In all cases the junctional complexes appeared preserved (Fig. 5B).

The interstitium was edematous and the capillaries dilated, their endothelial cells showing a decrease in the relative density of the cytoplasmic ground substance and a reduced concentration of ribonuclein granules and/or organelles. This combination of findings was believed to represent cellular swelling. Some endothelial cells exhibit cytoplasmic projections into the lumen and away from it, apparently throughout the capillary basement membrane (Fig. 6). More commonly than in the normal they exhibited round electron dense structures in their cytoplasm.

Golgi complexes were usually unaltered. In a few cells there were dilated vesicles and cisternae, which may countain a deposit of an electron dense material in their lumina.

Comments

Some of our previous data on the human kidney in leptospirosis (Brito et al., 1965) were confirmed and extended, chiefly those regarding mitochondria, brush borders and glomeruli. Furthermore, previously we had noticed an increased number of "dense bodies" in the injured cells. This actually corresponds to cytosomes and cytosegresomes which are often augmented in pathologically altered tissue.

Previously, the brush border lesion was overemphasized in comparison to other aspects of cell injury. Various types of altered mitochondria are now described. The first three are close to those observed by Suzuki and Mostofi (1966) in the experimental acute tubular necrosis of the rat kidney.

Gigantic mitochondria with decreased matrical density and a few peripheral cristae were interpreted as swollen degenerate organelles. On the other hand, enlarged mitochondria, with an abnormal number and disposition of cristae resemble the "fibrillary degeneration" (crystaline structures of mitochondria) seen

in the liver in human leptospirosis (SANDBORN et al., 1966; BRITO et al., 1967) but are closer to so-called "mitochondrial hypertrophy" (VOLK and SCARPELLI, 1966) described in the adrenal gland. This may be the result of increased demands upon mitochondrial function. Such possibilities could take place in human leptospirosis, where mitochondrial injury could be followed by hypertrophy of the non-damaged organelles, in an attempt to replace them functionally.

The glomerular findings were slight and except for mesangial cell hyperplasia, inflammatory cells obstructing glomerular capillary loops and a mild endothelial cell swelling, they were as previously described and commented (BRITO et al., 1965.)

Evidence of interstitial capillary injury, as observed in human kidney and that of experimental animals (BRITO et al., 1966), was probably a local representation of more widespread damage, which is in accordance with the clinical features of the disease. The dilation of proximal tubules observed in leptospirosis is also seen in the acute tubular failure of many causes (HEPTINSTALL, 1966) and has not been satisfactorily explained.

Previously (BRITO et al., 1965), too much significance was placed in the dilation of the extracellular spaces (so called cellular disjunction) which, as demonstrated by KAYE et al. (1966) and DIAMOND et al. (1966), has a physiological importance in fluid transport across the epithelium. However, in this study, considerable variation was found in their width, both in controls and pathological kidneys. Junctional complexes were preserved and we admit that the hypothesis of a shunt between the glomerular filtrate and the interstitium through the intercellular space, to explain the kidney failure in leptospirosis, cannot be sustained on a pure morphological basis (TISHER et al., 1966).

A certain correlation can be established between the deficient enzyme activity and the morphological findings, in spite of the fact that the biochemistry pointed to more severe damage than was disclosed morphologically. Mitochondrial injury is in accordance with a decreased succinic dehydrogenase and tubular dilation and/or brush border lesions correlates roughly with the low level of alkaline phosphatase activity. In few human cases, which are not part of this series, the reduction of the enzymatic activity of alkaline phosphatase was seen histochemically, a fact not observed with acid phosphatase.

Previous investigations (ERICSSON, 1964, et al., 1965; TRUMP, 1965) have demonstrated the occurence of acid phosphatase in cytosomes and cytosegresomes, which appeared in increased number in groups of damaged cells. Acid phosphatase and other lysosomal enzymes may have pre-existed in the areas to be segregated by the cell or, alternatively, may have been transported to these areas subsequent to their segregation. There is strong reason to believe that lysosomal enzymes, like other proteins, are synthesized on the ribosomes. The decrease of acid phosphatase here seen may be the result of a direct injury in its formative site, a fact having morphological support in the ribosomal depletion observed in the more injured cells.

Conclusions

Recent work of SEFER (1966) and AREAN et al. (1964) pointed to a toxin as the mechanism of leptospiral pathogenicity. This circulating toxin probably acts on capillaries (AUSTONI and CORÁ, 1961), providing support for some clinical features of the disease. The morphological and functional injury of the tubular cell

probably is the result of a direct action of the toxin filtered through the glomerulus and/or a diminished oxygen supply due to the capillary pathology. However, none of the manifestations of cell injury here described can be regarded as specific of leptospirosis.

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