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ADVANCED KIDNEY DISEASE IN PATIENTS WITH HEPATOSPLENIC MANSON'S SCHISTOSOMIASIS

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SUMMARY

Eleven cases of hepatosplenic schistosomotic patients presenting clinical evidence of kidney disease were studied by light, electron and immunofluorescence microscopy. Clinically they exhibited a mixed nephrotic and hypertensive picture with progressive signs of chronic kidney failure. The evolution, however, is slow and no end-stage kidney could be detected among our cases so far.

Light and electron microscopy showed a uniform pattern of lesion, characterized by marked proliferation of mesangial cells with matrix deposition, and focal thickening of the basal membrane. Focal electron-dense deposits were seen corresponding to gamma globulin and complement, as demonstrated by immunofluorescence microscopy.

The pathogenesis of the lesion was discussed and the attempt to link it to a chronic manifestation of a non glomerular endogenous antigen-antibody glomerulopathy has to be considered hypothetic because so far no schistosomotic antigen was detected in the glomerular deposits.

INTRODUCTION

Previous studies [2, 5, 16] have shown early glomerular lesions in human Manson's schistosomiasis without clinically manifested renal disease. In such lesions deposits were seen by electron microscopy in close contact with proliferated mesangial cells, and in them gamma globulin and complement could be demonstrated by immunofluorescence techniques.

The purpose of this paper is to report on the lesions seen in patients with hepatosplenic form of Manson's schistosomiasis and presenting clinically advanced renal disease. The possibility of superimposed pathological findings, i.e., lobular nephritis in a patient with schistosomiasis, cannot be ruled out in our present studies mainly because, so far, no antigen was demonstrated in such lesions. However, the homogeneous pathological and clinical picture, the former suggestive of a late phase of the early lesions already described [5, 16] are certainly encouraging in drawing common pathogenetic origin for both.

MATERIAL AND METHODS

Eleven hepatosplenic schistosomotic patients with advanced clinically manifested renal disease were selected for this study. All of them came from an endemic area and had viable eggs in the stool and/or rectal biopsy examinations. No past history of repetitive sore throat or upper respiratory infections could be detected in them. The main clinical data are reported in Table I. All were submitted to kidney biopsy, three of them twice at intervals which varied from fifteen months to seven years (see Table I).

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TABLE I

Clinical data

Patient	Age (yr.)	Sex	Beginning * of symptom	Biopsy interval	B.P.	BUN (mg/100 ml)	Total serum cholesterol (mg/100 ml)	Proteins										Hematuria		Creatinine Clearence (ml/min)
								Urinary **		Serum										
								g/24 h	g/l	Album.	Alfa-1	Alfa-2	Beta	gamma	A	G	T	eritr/ field	eritr/ mmc.	
1	35	f	4 yr.	15 m	200 × 120	25	312	7.0	10.0	—	—	—	—	—	3.7	2.8	6.5	20	—	—
					180 × 110	46	347	6.4	—	2.08	0.44	0.69	0.87	2.03	—	—	6.1	12	—	80.7
2	27	m	1 yr.	—	130 × 100	76	146	3.3	4.8	—	—	—	—	—	4.6	3.1	7.7	60	393.000	—
			6 yr.	5 yr.	130 × 100	63	191	4.0	—	3.83	0.20	0.53	0.80	0.94	4.1	1.8	6.3	40	—	—
3	41	m	1 yr.	—	120 × 90	81	277	4.0	4+	0.75	0.44	1.19	0.92	1.49	—	—	4.4	50	—	22.2
4	34	m	5 yr.	—	210 × 150	45	397	3.5	3.0	1.26	0.21	0.69	0.69	1.55	—	—	4.2	80	—	44.9
5	22	m	4 m	—	150 × 100	—	246	5.8	7.2	2.31	0.51	0.70	0.84	1.64	2.4	3.5	6.0	6	9.000	49.0
6	38	m	2 yr.	—	240 × 130	70	138	3.9	—	2.27	0.35	0.52	1.06	2.08	2.7	3.4	6.1	15	—	—
7	26	f	5 m	—	150 × 90	36	299	5.0	—	—	—	—	—	—	2.1	4.3	6.4	60	256.000	73.1
8	15	m	6 m	—	110 × 60	40	158	5.2	5.8	2.87	0.35	0.26	0.83	2.52	2.9	3.7	6.7	100	—	70.9
9	21	m	1 yr.	7 yr.	140 × 100	47	—	2.2	4.4	—	—	—	—	—	—	—	—	60	—	100.0
			8 yr.		180 × 140	52	299	7.0	6.0	2.85	0.43	0.91	1.01	1.57	3.3	3.8	6.8	5	31.000	35.2
10	11	f	2 yr.	—	170 × 120	36	264	0.16	0.2	—	—	—	--	—	2.9	2.0	4.9	30	20.000	84.1
11	41	f	11 yr.	—	180 × 120	115	198	6.8	3.6	2.56	0.49	0.64	0.71	2.78	3.7	3.6	7.2	50	130.000	66.1

* Symptoms related to renal disease (year (yr.) or months (m.) before biopsy)

** Albuminuria

In ten cases kidney fragments were fixed in Helly's fluid and stained routinely by hematoxylin-eosin, PAS and PAMSM (periodic acid, methenamine-silver, Masson stain) [11]. This last stain gave good results only after one hour post fixation of the deparaffinized sections in buffered ten percent formalin. In one case light microscopy examination was carried in toluidine blue stained thick sections of the material embedded in Araldyte after Palade's fixation. In five cases, including this last one, small fragments 0.2-0.3 mm thick were cut from the poles of the kidney biopsies, fixed in Palade's fixative during two hours at 5°C and then carried through an ascending series of ethyl alcohol and embedded in Araldyte. Thick sections were cut from the blocks and stained with toluidine blue according to the technique of TRUMP et al. [17] and from the fragments cointaining glomeruli thin sections were cut with glass or diamond knives, stained with uranyl acetate and lead citrate and examined in a Zeiss EM 9 electron microscope.

Immunofluorescent studies were carried out in four cases. For the fluorescent staining, biopsy fragments about 5 mm in length were immediately frozen in liquid nitrogen-isopentane mixture and kept at −20°C. Sections with 4 μ were cut in a cryostat and fixed on slides by drying. Direct staining was performed by incubating sections with diluted conjugates for 1 hour at 37°C. Slides were washed for about two hours in a few changes of 0.015 M NaCl buffered to pH 7.2 with 0.01 M phosphate, mounted with alkaline glycerine pH 8.0. They were examined under a 20 × dry or a 40 × oil immersion objective, in a binocular microscope provided with dark-field, HBO-200 as the light source and BG 12 as exciter and 50 (Zeiss) as barrier filters.

Conjugates were prepared in our laboratory by labelling the globulin fraction of rabbit immunoserums with fluorescein isothiocyanate * A fluorochrome slow-adding dialysis technique [7] was used and the free fluorochrome was removed by gel-filtration in Sephadex G-25. Rabbits were immunized respectively with chromatographically purified human IgG and IgM. Immunoserums were made specific by absorptions and gave immunoelectrophoretic precipitation lines only against the corresponding immunoglobulins. Anti B_{1C} serum ** was obtained in rabbits by injecting washed complexes of zymosan and fresh human serum in a similar way to that described by MARDINEY Jr. & MULLER-EBERHARD [14]. After absorption with human immunoglobulins, this antiserum produced only one immunoelectrophoretic precipitation line against human serum, which corresponded to B_{1C}.

Conjugates had F/P weight ratios around 10 and were diluted for use, for concentrations of no less than 1/4 precipitating unit, as defined by BEUTNER, HOLBOROW & JOHNSON [4] in order to give a maximal reactivity and with a maximum of 3 mcg of fluorescein per milliliter, to avoid non specific staining.

Controls for non-specific stainings included use of conjugates absorbed respectively with IgC, IgM or fresh human serum. Also kidney sections obtained a few hours postmortem from a young accident-deceased individual were included for comparison and showed no fluorescence with any of the conjugates.

RESULTS

1) *Light microscopy* — The histopathological findings were homogeneous both by light and electron microscopy, variations being related only to the degree of injury. In all cases light microscopy disclosed enlarged glomeruli with a definite hyperplasia and hypertrophy of stalk mesangial cells. The proliferation of such cells were diffuse along the glomerular axis and in the more advanced cases a lobular pattern appeared with almost total obliteration of the capillary lumina (Fig. 1C, D). Only the glomerular capillaries located at the periphery of the lobule were patent, not dilated and hematoxylin-eosin stain disclosed a focal basal membrane thickening. Occasionally granulocytes, chie-

* Crystalline, chromatographically pure, Isomer I, BBL, U.S.A.

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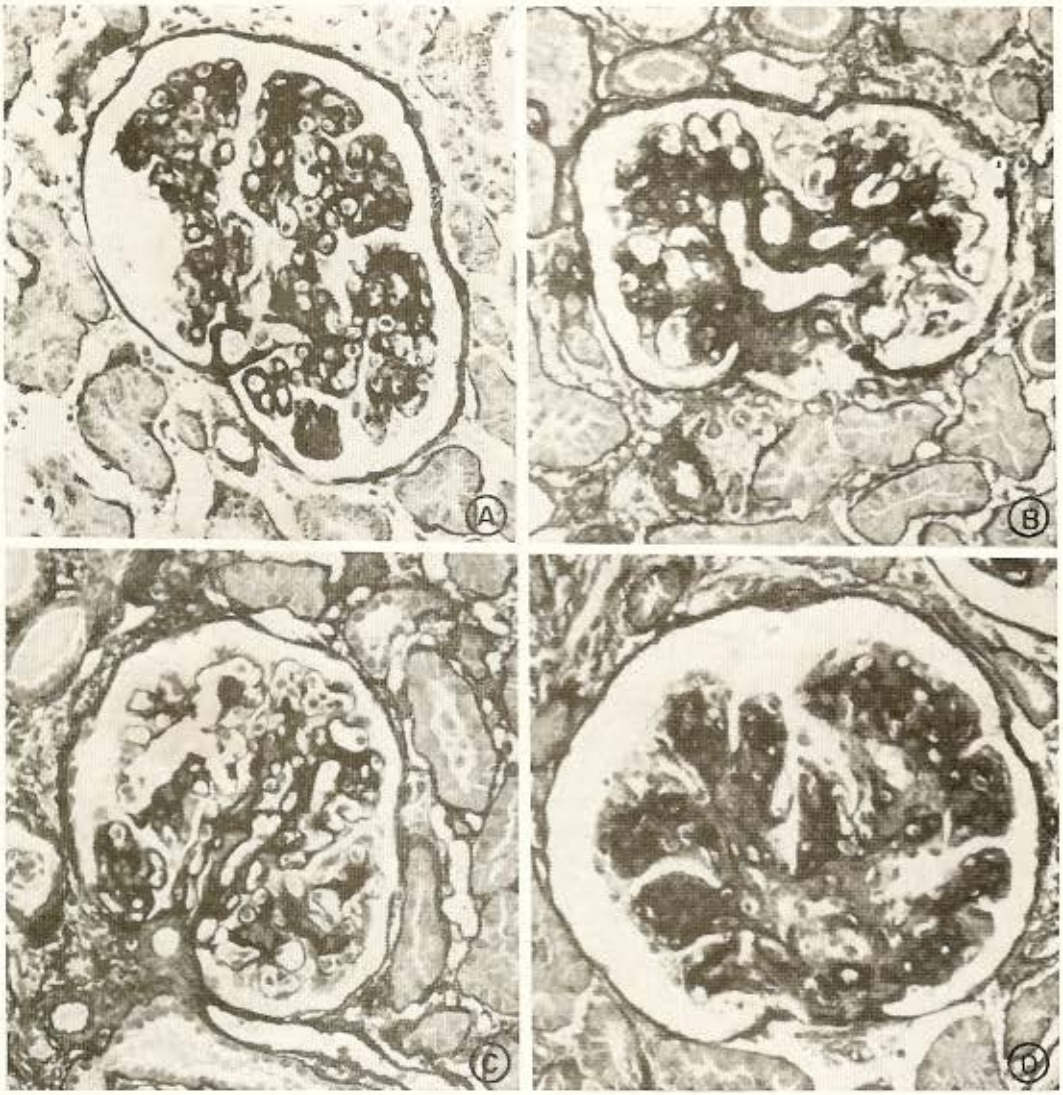

Fig. 1 — (A and B — Case no. 9) Evolution after seven years of a predominant membranous to lobular nephropathy. Even in this case basal membrane thickening is not diffuse in the first biopsy and there is a slight mesangial cell proliferation. (C — case no. 7) Early mesangial cell proliferation and slight local basal membrane thickening. (D — Case no. 10) Definite "lobular nephritis" with marked mesangial cell proliferation and silver positive fibrils deposition. PAMSM stain. 400 ×

fly eosinophils could be seen inside the capillary lumina. The mesangial cells proliferation was distributed evenly along the glomerular axis without a predominance at the vascular pole, and, as seen by PAMSM stain, followed by the deposition of argyrophil fibrils. PAMSM stain failed to show a diffuse glomerular basal membrane thickening, as seen in some cases with HE stain.

A few glomeruli in the more advanced cases also showed hyaline droplets over the peripheral capillary loops. Also, fibrotic focal adhesions were seen. The lesion above described envolved to a diminished scarred glomeruli, with multiple peripheral fibrotic adhesions. PAMSM stain showed, even in such glomeruli, a marked network of silver positive fibrils and a few collagen deposition.

It is worth mentioning that in both cases with two biopsies done in a large time interval, one exhibited an evolution of a predominant membranous type of lesion to a definite lobular pattern after seven years (Fig. 1A, B). Also in this case definite arteriolar hyaline changes appeared following the increase of the sistemic hypertensive state, albuminuria, hematuria and decrease of the serum protein and creatinine clearence. The other cases followed during years had a lobular or membranous and focal proliferative pattern which suffered slow histopathological progression.

Hyalin droplets were seen inside tubular cells. The more advanced glomerular changes were accompanied by definite focal tubular atrophy.

2) *Electron microscopy* — also disclosed a homogeneous pattern, characterized chiefly by mesangial cells proliferation and marked matrix deposition which corresponds to the staining fibrils seen by light microscopy [12] (Fig. 2).

Electron-dense deposits were seen not only over the basement membrane-like material but also beneath endothelial and epithelial cells, and, occasionally, in the glomerular basal membrane proper (Figs. 3, 4). Seldomly a continuous subendothelial ring-like deposit was observed. They were made up usually of small particles about the size described in the deposits seen in the early lesions of schistosomotic patients without clinical evidence of renal disease [5, 16]. Only one case showed a single deposit formed by an aggregate of coarse electron-dense particles.

The mesangial cells proliferation extended peripherally with partial delamination of the glomerular basal membrane which appeared irregular with focal but definite thickenings, sometimes made up by the inner lamina rara. The endothelial layer was either normal or markedly swollen. Inside the capillary lumen granulocytes and mononuclear cells could be seen. Enlarged epithelial cells foot process were focally obliterated or elongated and flattened. Pseudovillous processes of glomerular visceral epithelial cells were common in all cases (Fig. 3). Cytosomes containing electron-dense ovalar material were seen in some epithelial cells.

The more advanced lesions were formed almost exclusively by the mesangial proliferation and basal membrane-like material deposition with almost complete glomerular capillary obstruction (Fig. 5).

In only one case laminar bodies were observed, similar to ones described in hepatic glomerulosclerosis and early glomerular lesions of schistosomotic patients [5, 10, 15, 16] (Fig. 3, insert).

3) Lumpy deposits were seen by immunofluorescence along the capillary walls as well as along the mesangial stalk of the glomerulus, corresponding to immuneglobulins and complement deposition.

DISCUSSION

The study of patients with hepatosplenic form of Manson's schistosomiasis and presenting advanced renal disease showed a suprisingly uniform pattern as seen both clinically and pathologically. Except for one case (no. 10) showing no albuminuria they were patients with nephrotic syndrome of variable duration (from 4 months to 11 years) and usually exhibiting progressive hypertension, subjected to therapeutic control in five cases at the time of biopsy (no. 1, 2, 3, 7 and 8).

Total serum cholesterol was normal in five cases, a fact linked to the hepatosplenic form of schistosomiasis. Schistosomiasis is also probably responsible for the high gamma globulin levels seen in five patients. No correlation was found between clinical, laboratorial and the histopathological degree of lesions. Both nephrotic syndrome and the hepatosplenic form of schistosomiasis contribute for the hypoalbuminemia seen in all but one patient. The glomerular filtration rate as measured by the creatinine clearence was low and without quantitative correlation with the other investigated aspects. In case no. 9 a progressive lowering of the glomerular filtration rate was observed in accordance with the duration of the disease.

The kidney lesion was of a mixed proliferative and membranous nephropathy evolving to the pattern called by BELL [3], ALLEN [1] and JONES [11] lobular nephritis. Arteriolar sclerosis was seen in cases with a long stand-

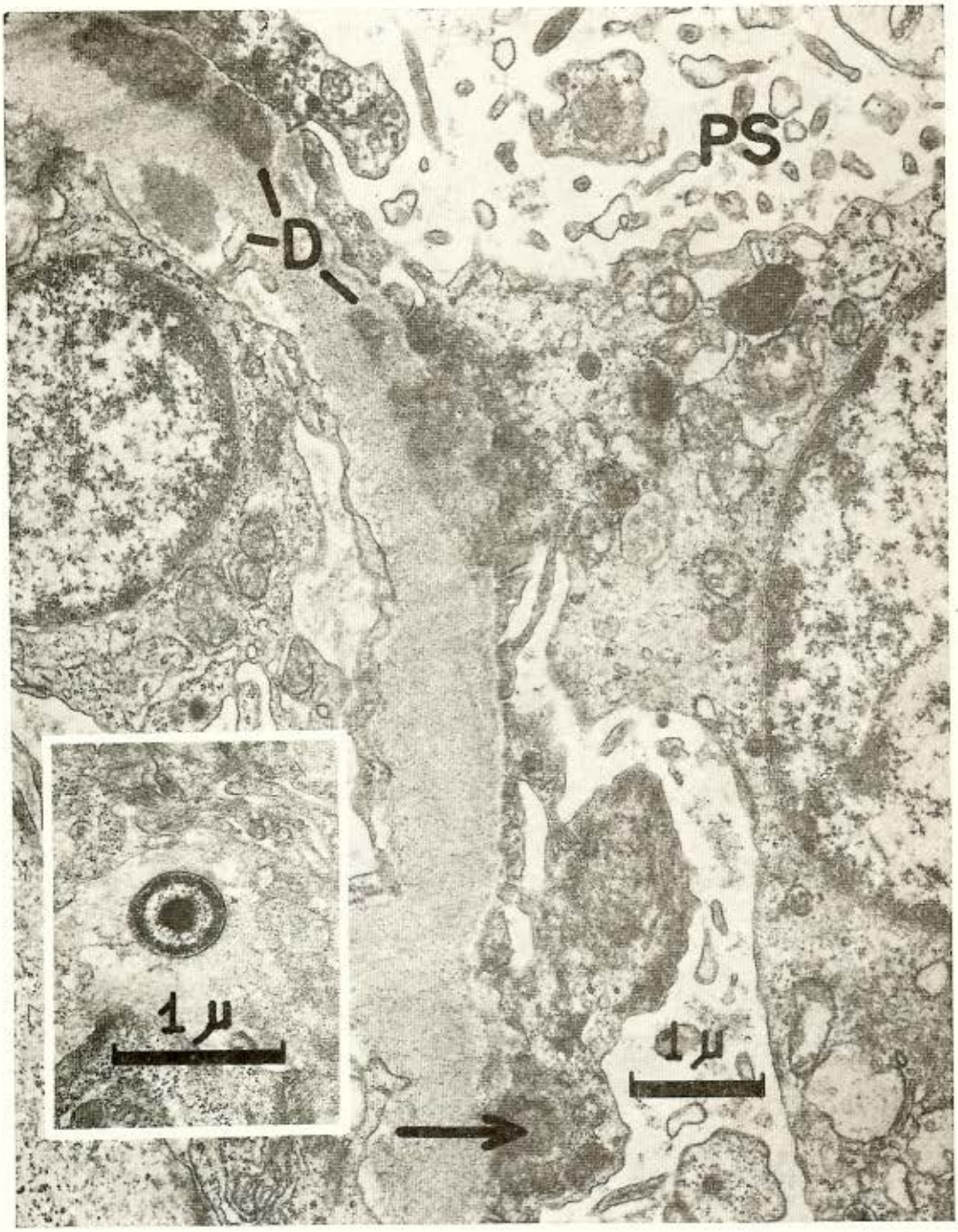

Fig. 3 — (Case no. 11) Electron dense particulate deposits (D) are seen in the basal membrane, one of them as a small lump on the epithelial side (arrows). Visceral glomerular epithelial cells show pseudovillous processes (PS) and ovalar electron dense structures in the cytoplasm. Insert designates a laminar body.

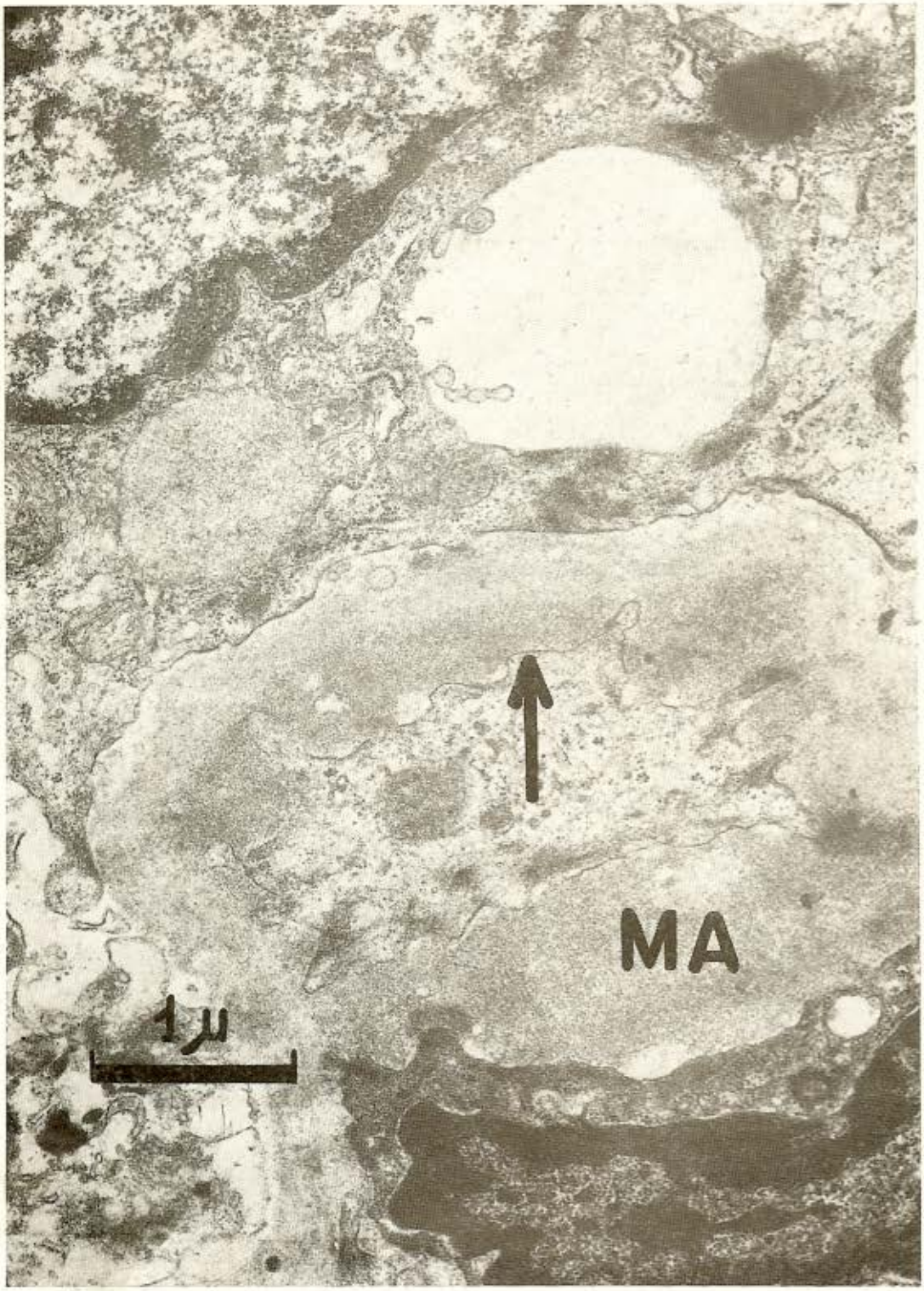

Fig. 4 — (Case no. 9) A focal ring-like particulate electron dense deposit (arrow) in the basal membrane nearby a mesangial cell. Deposit is also seen in the mesangial cell matrix (MA)

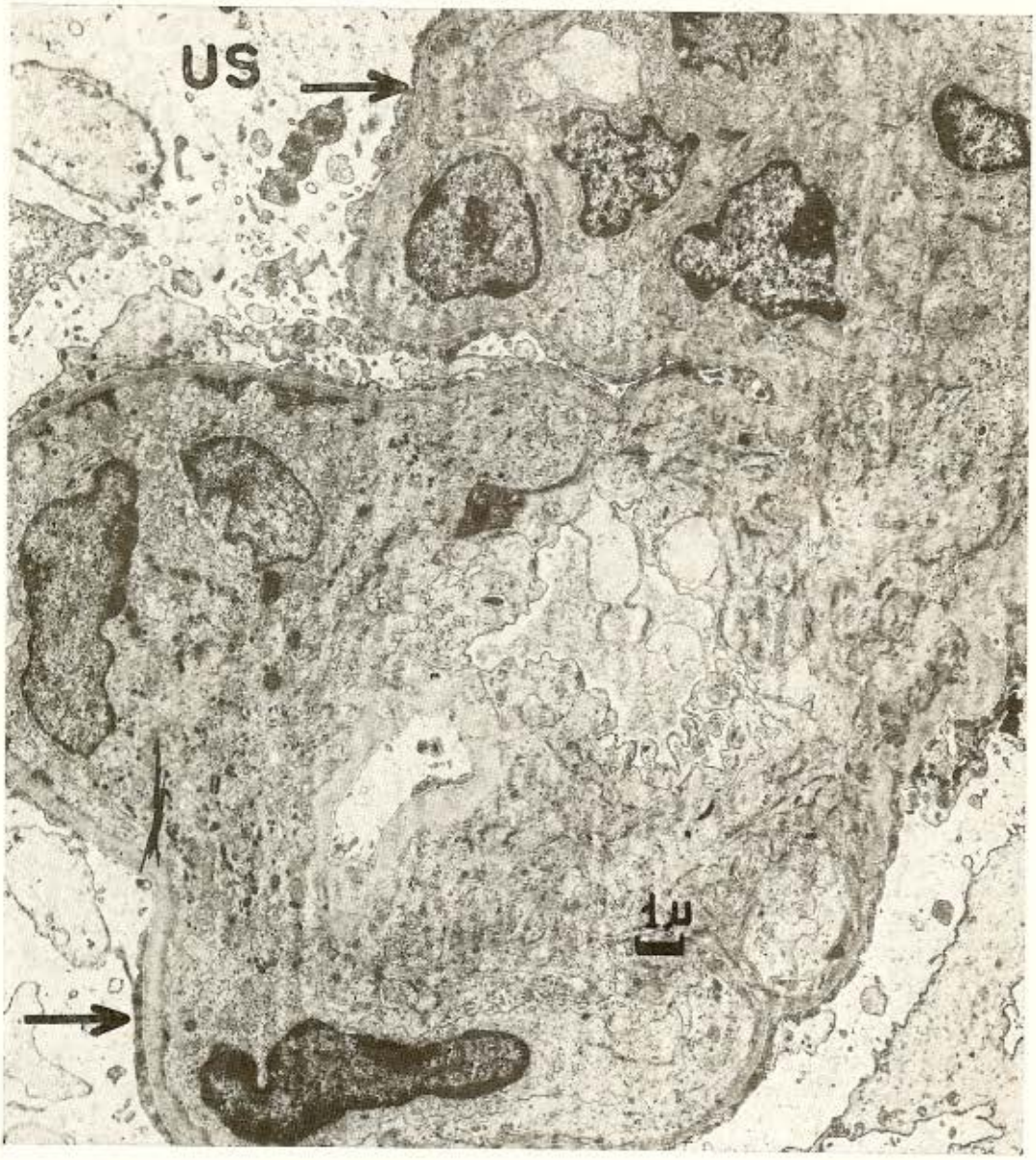

Fig. 5 — (Case no. 9) — Lobular pattern seen in an advanced lesion, due to marked mesangial cell proliferation with matrix deposition, extending to the periphery of the glomerular capillary loop. Epithelial cells foot processes are flattened (arrows). US designates the urinary space.

sits seen by electron microscopy. No schistosomotic antigen could be demonstrated so far.

Early glomerular lesions characterized by mesangial cells proliferation and basal membrane focal thickenings were seen by ANDRADE & QUEIROZ [2] in schistosomotic patients. We had shown electron-dense deposits in the glomeruli of hepatosplenic schistosomotic patients without evidence of renal disease in a pattern close to that described in cirrhotic glomerulosclerosis [10, 15]. However, immunofluorescent studies showed in such patients that the deposits were made up not only of

gamma globulin (IgG and in one case IgM) but also of complement [16] and accompanied by mesangial cells proliferation. No antigen, however, was demonstrated in these cases.

Previous studies of DIXON [8, 9] had shown that one of the mechanisms of glomerular disease depends upon the patient's production of antibodies capable of reacting with non glomerular antigens in his circulation with the resultant formation of circulating antigen-antibody complexes which are subsequently trapped in the glomerular capillary walls or filter. Such could be the mechanism of renal injury in infectious diseases where soluble circulating antigen-antibody complexes, made up in a zone of antigen excess, are offered in determined circumstances in large quantities to the kidney, eventually producing glomerular disease. It is tempting to imagine that such are the events in certain cases and forms of schistosomiasis, because for a long time it has been a clinical impression that kidney disease is more common in such patients [13].

However, a definite link between the early lesions seen by light [2] and electron microscopy [5, 16] and the advanced ones now described must await the antigen demonstration and careful follow-up studies. Without such data the possibility that we are dealing with coincidental findings such as lobular nephritis of other etiologies in schistosomotic patients cannot be discarded.

The homogeneous pattern of the advanced lesion in schistosomotic patients and the characteristics of the early lesions as previously seen make the search for the antigen a very promising track to be followed in human kidney disease in schistosomiasis.

RESUMO

Doença renal avançada em pacientes com a forma hepatesplênica da esquistossomose mansônica

Onze pacientes com esquistossomose hepatesplênica mostrando quadro misto hipertensivo e nefrótico, foram estudados pela microscopia de luz, eletrônica e pela imunofluorescência. A evolução da lesão é lenta e em nossa casuística não encontramos rins contraidos em fase terminal.

A lesão vista pela microscopia de luz e eletrônica é relativamente uniforme, caracterizada por proliferação de células mesangiais com deposição de matriz e espessamento local de membrana basal. Depósitos onde podem ser demonstradas imuneglobulinas e complemento foram vistos pela microscopia eletrônica e fluorescente.

A patogênese da lesão foi discutida e qualquer tentativa para ligá-la a manifestação crônica de doença produzida pelo depósito crônico no glomérulo de complexos antígeno-anticorpo solúveis preformados na circulação deve esperar a demonstração de antígeno, o que não foi possível até o presente momento.

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