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Glomerular Lesions in Experimental Infections of *Schistosoma mansoni* in *Cebus apella* Monkeys*

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Three monkeys (Cebus apella) *experimentally infected with* Schistosoma mansoni *studied for periods of 19, 14, and 11 months showed deposits containing γ-globulin in subendothelial and subepithelial basal membranes and in basement membranes proper. The glomeruli showed mild reactivity characterized by local hypertrophy and hyperplasia of mesangial cells. Such findings were close to those observed by us in the kidney of hepatosplenic schistosomiasis patients without evidence of renal disease. The distribution of the deposits, both in human and experimental disease, are suggestive of preformed, nonglomerular antigen-antibody complexes that form in a zone of excess antigen and become trapped in the glomerular capillaries.*

The possibility exists, but has not yet been proved beyond doubt, that renal disease in schistosomiasis patients could be the end result of this pathogenetic mechanism.

Previous studies (Andrade & Queiroz, 1968; Brito et al., 1969; Silva et al., 1970) have shown early glomerular lesions in human schistosomiasis caused by *Schistosoma mansoni* without clinically manifested renal disease. In such lesions, electron microscopy revealed deposits in close contact with proliferated mesangial cells, and γ-globulin and complement could be demonstrated in them by immunofluorescence techniques.

This paper describes glomerular lesions seen in monkeys with experimental schistosomiasis that closely resemble those seen in man.

MATERIAL AND METHODS

Altogether, 3 non-splenectomized *Cebus* monkeys experimentally infected with *Schistosoma mansoni* and 4 control *Cebus* monkeys, one of them subjected to biopsy both before and after infection, were used in this study. None of the monkeys studied was found to pass *S. mansoni* eggs prior to the experimental infection.

Each animal was infected by placing a determined number of cercaria on a previously shaved area of the abdomen for 45 minutes. Albino mice exposed to 100–150 cercaria from the same batch were used as infectivity controls. The cercaria were obtained from infected snails (*Biomphalaria glabrata*) in our laboratory.

Monkeys no. 4 and 6 were exposed to primary infection with 500 and 550 cercaria, respectively, and monkey no. 11 was exposed to 120 cercaria. Monkey no. 4 was challenged with 540 and 560 cercaria in the 5th and 12th months, respectively, after the primary infection. After the 18th month, 5 successive challenges of 110 cercaria were carried out (see Table 1). Monkey no. 6 was challenged with 650 cercaria in the 12th month and 11 times with cercaria after the 18th month. Monkey no. 11 was challenged 13 times with 110 cercaria after 2 weeks (Table 1). In all the animals, the interval between challenges was 1–2 weeks.

Monthly faecal examinations began in the fifth week after exposure and egg counts were performed on 2 or 3 samples obtained from a 24-hour collection of faeces, according to the technique described by Ferreira (1966). Haemagglutination tests (Hoshino, 1970) were carried out with serum samples taken at intervals of 4–5 months.

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Table 1. Experimental infection of *Cebus apella* monkeys with *Schistosoma mansoni*

Monkey no.	Number of cercaria used					Interval between primary infection and necropsy (months)
	Primary infection	Challenges (time from primary infection)				
		2 weeks	5 months	12 months	18 months	
4	500	—	540	650	110 (× 5)	23
6	550	—	—	650	500 (× 11)	26
11	120	110 (× 13) [a]				13

[a] The interval between challenges was 1-2 weeks.

The kidney fragments were taken at necropsy and monkey no. 11 was also biopsied prior to being infected. Animals used in this experiment were part of the material described in a previous paper (Silva et al., 1969); none had been previously inoculated with *Plasmodium simium*.

Immunofluorescence staining of kidney sections was performed according to the mixed antiglobulin immunofluorescence technique (Beutner, Holborow & Johnson, 1967). Sections fixed on slides by drying were covered with diluted antimonkey globulin antiserum and incubated for 1 hour at 37°C in a wet chamber. After being washed in two changes of saline buffer solution (10 minutes in each), the sections were incubated for another hour with a dilution of fluorescein-labelled monkey globulin, washed for 1 or 2 hours, and then mounted in alkaline glycerol under a coverslip. Antimonkey globulin antiserum with a titre of 16 precipitating units (Beutner, Holborow & Johnson, 1967) was obtained by immunizing rabbits with monkey serum fractions precipitated with 1.56 M ammonium sulfate. Antiglobulin dilutions for use contained 4 units per ml. Labelled monkey globulins were prepared by conjugating fluorescein isothiocyanate [1] to the monkey serum fraction by a fluorochrome slow-addition dialysis technique (Clark & Shepard, 1963). The fluorescein : protein weight ratio was 19. For use, the labelled conjugate was diluted 1 : 80 in 1% Tween 80 in buffered saline; this dilution contained about 2 μg of fluorescein per ml. Normal rabbit serum was used as a control.

Electron- and light-microscopic studies were carried out as previously described (Silva et al., 1970) with fragments of kidney obtained at necropsy.

[1] Crystalline, chromatographically pure isomer, supplied by Baltimore Biological Laboratories, Md., USA.

RESULTS

Light microscopy

The histopathological findings were mild and homogeneous, variations being related only to the degree of injury. Light microscopy disclosed slightly enlarged or normal glomeruli with a mild, and usually local, hyperplasia and hypertrophy of mesangial cells (Fig. 1). In some glomeruli, peripheral loops had their lumina partially or completely obliterated by endothelial cell swelling; no thickening of the basal membrane was observed. Schistosomal pigment was detected in a few glomeruli in the cytoplasm of cells but its precise nature was not evident by light microscopy.

Electron microscopy

Electron microscopy also disclosed a homogeneous pattern of lesions characterized mainly by focal, small, electron-dense deposits located in the glomerular basal membrane beneath epithelial and endothelial cells and also in the basement membrane proper (Fig. 2 and 3).

The deposits were made up of aggregates of small particles of about the size of those seen in the early lesions of schistosomiasis patients without clinical evidence of renal disease (Silva et al., 1970).

When located in the basement membrane proper, the deposits sometimes showed a peripheral clear halo of irregular thickness (Fig. 4). The subepithelial deposits were usually not lumpy and only faintly resembled the " humps " observed in acute glomerulonephritis in a man (Metcoff, 1967). One such deposit had a well-defined linear contour. The subendothelial deposits were more rarely seen and deposits near mesangial cells were even less common. Basement membranes had, in some places, a spiculated

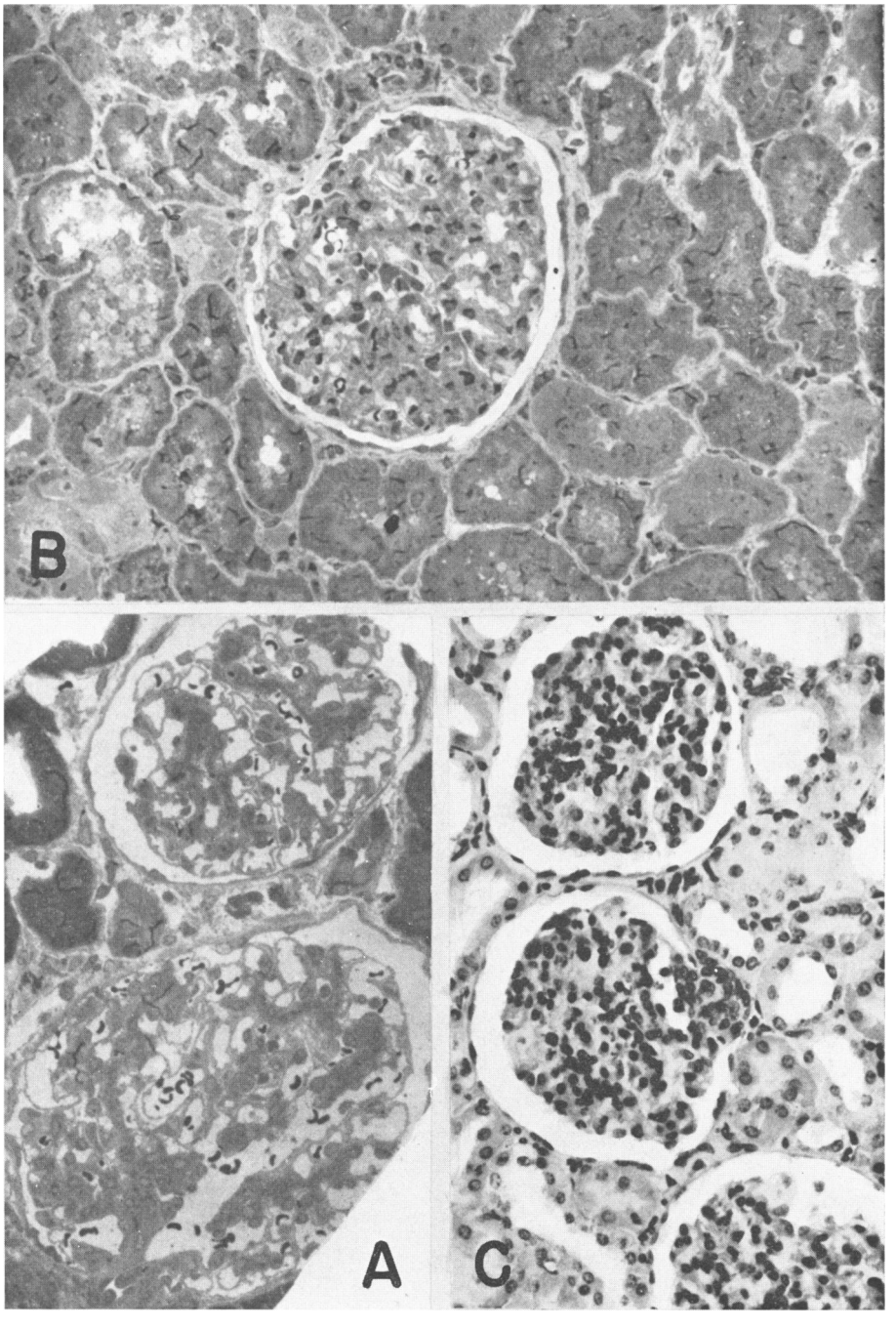

Fig. 1. A: Thin section of kidney from a monkey before infection showing normal glomeruli (×120; stained with toluidine blue). B: Thin section of kidney of the same monkey 14 months after infection with *Schistosoma mansoni*; there is local glomerular hypercellularity mostly due by axial cells (×120; stained with toluidine blue). C: Section of kidney of the same monkey after infection with *S. mansoni*; showing local glomerular hypercellularity (×100; stained with haematoxylin and eosin).

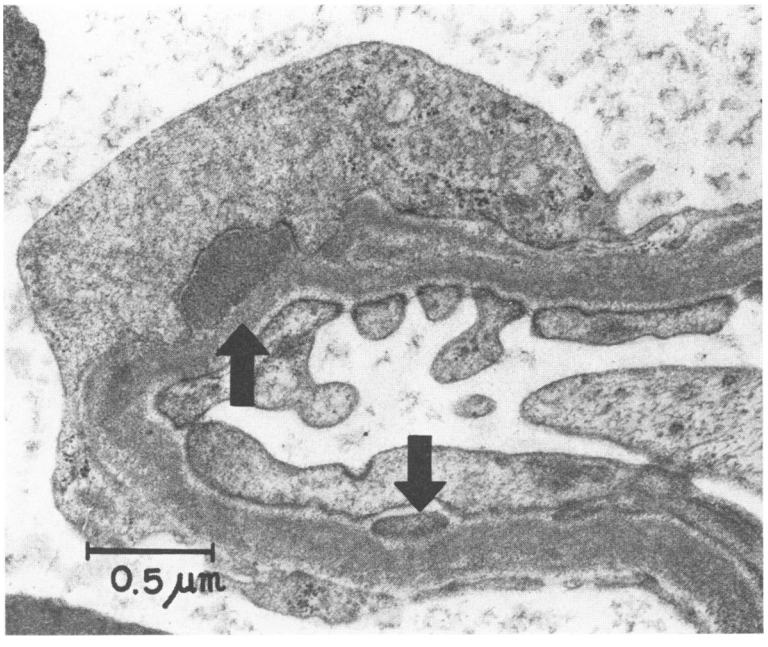

Fig. 2. Electron-dense deposits made up of aggregates of small particles located at the glomerular basement membrane beneath epithelial and endothelial cells (arrows). The epithelial cell shows fusion of the foot process, and endothelial cell cytoplasm is conspicuous.

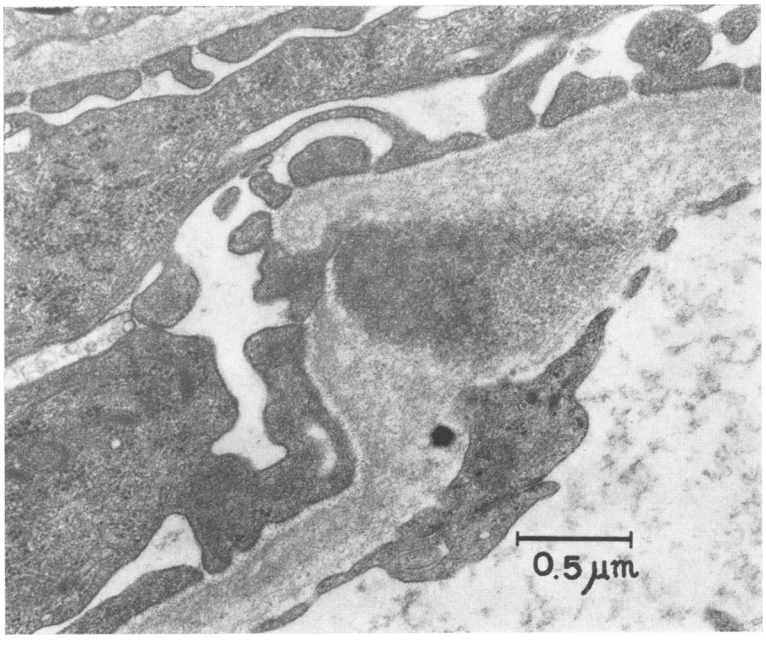

Fig. 3. Electron-dense deposit in the glomerular basement membrane proper.

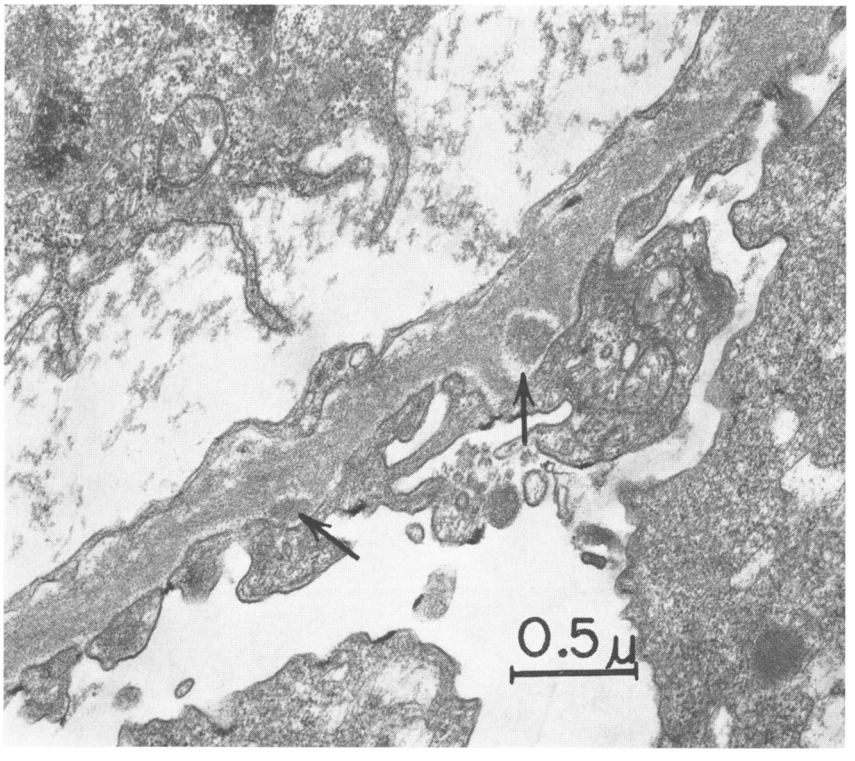

Fig. 4. Small electron-dense deposits located in the glomerular basement membrane proper close to epithelial cells. A light halo marks the periphery of the deposits.

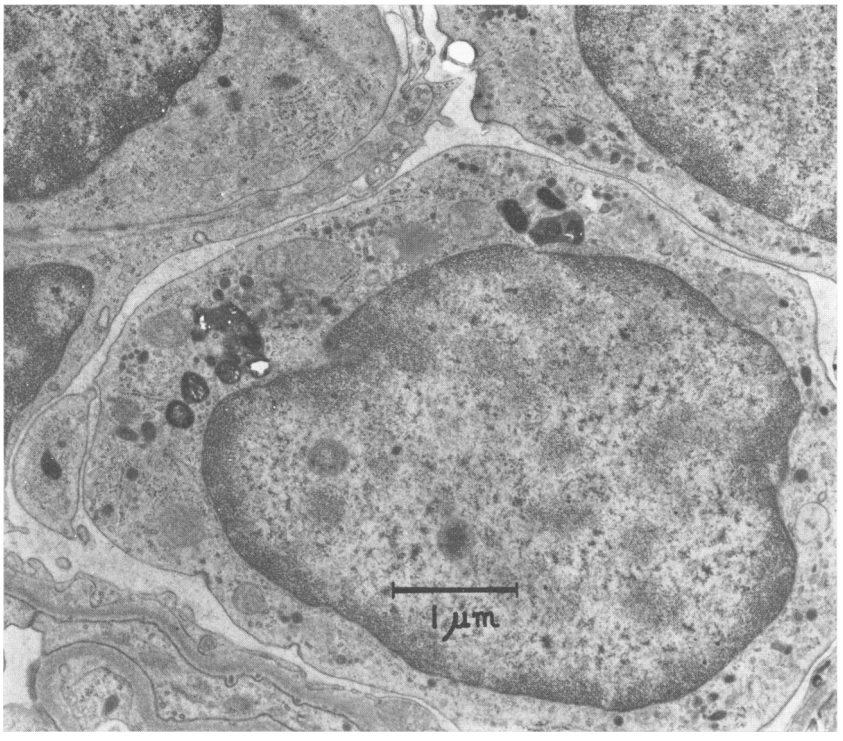

Fig. 5. Wandering mononuclear cell containing schistosomal pigment trapped in a glomerular capillary.

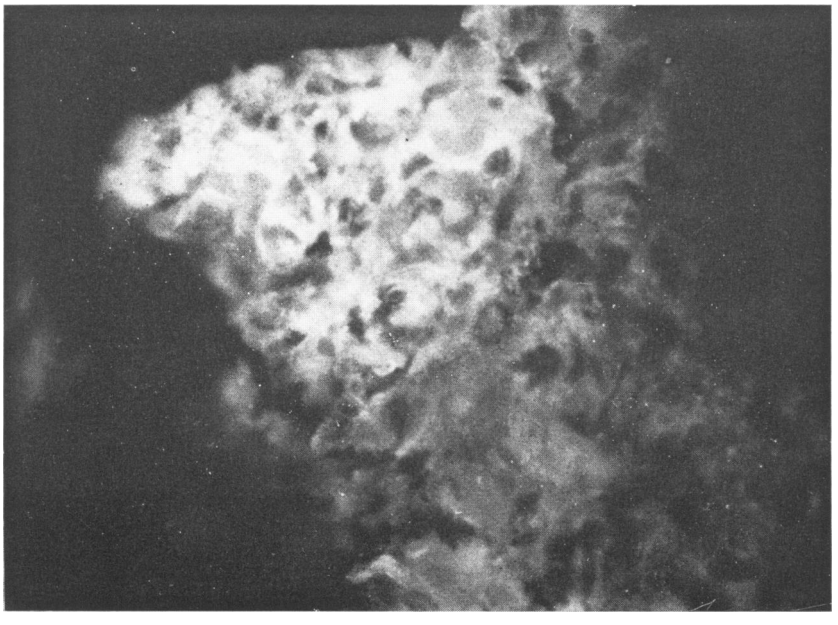

Fig. 6. Irregular immunofluorescent deposits in a glomerulus of an infected monkey (× 300).

pattern, chiefly on the epithelial side. Focal swellings of the inner lamina rara were sometimes observed.

The endothelial layer was usually normal; hypertrophy and hyperplasia of mesangial cells were not usually conspicuous. Inside the glomerular capillary lumen, granulocytes and, chiefly, mononuclear cells were visible; the latter were sometimes seen to be engulfing schistosomal pigment, which has a close morphological resemblance to malarial pigment (Fig. 5) (Rosen et al., 1967, 1968). Schistosomal pigment was also, though less frequently, observed in the cytoplasm of endothelial and mesangial cells.

None of these lesions was seen in the controls.

Immunofluorescence staining

Kidney sections from only two of the animals were studied by immunofluorescence staining, and in both cases irregular fluorescent deposits were seen in most glomeruli (Fig. 6). No staining resulted from the use of normal rabbit serum.

Parasitological and immunological data

All the monkeys passed viable schistosome eggs after and throughout the experimental infection. Haemagglutination tests were negative prior to infection but produced progressively higher titres after infection and challenge.

DISCUSSION

Early glomerular lesions characterized by the proliferation of mesangial cells and focal thickening of the basement membrane were seen by Andrade & Queiroz (1968) in schistosomiasis patients. Electron-dense deposits in the glomeruli of hepatosplenic schistosomiasis patients without evidence of renal disease have been shown to occur in a pattern very similar to that described in cirrhotic glomerulosclerosis (Sakaguchi et al., 1965; Fisher & Pérez-Stable, 1968). Immunofluorescent studies of such patients showed that the deposits were made up not only of γ-globulin but also of complement; however, no antigen was demonstrated in the human cases. Presently glomerular electron-dense deposits, in which γ-globulin could be demonstrated, were seen beneath the endothelium in the basement membrane proper, and faintly resembled the " humps " in long-term-experimental infections with *S. mansoni* in monkeys. The distribution of the deposits suggests, as in serum sickness, that antigen-antibody complexes have been formed and trapped in the glomerular capillaries.

Previous studies by Dixon (1967, 1968) 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, resulting in the formation of circulating antigen–antibody complexes that are subsequently trapped in the glomerular capillary walls or filter. This could be the mechanism of renal injury in infectious diseases where soluble circulating antigen–antibody complexes, formed in a zone of excess antigen, become, under certain circumstances, available in large quantities to the kidney and eventually produce glomerular disease. It is possible that these events occur in certain clinical forms of human schistosomiasis.

The glomerular injury we noted in monkeys probably has a similar pathogenetic mechanism. The trapped antigen–antibody complexes would induce the mild hypertrophy and hyperplasia of mesangial cells, regarded as a nonspecific reactive glomerulitis. If the proportion of trapped complex is maintained, the experimental lesion bears a close resemblance to human glomerular injury seen in patients without evidence of clinical disease. Schistosomal pigment engulfed by mesangial and wandering mononuclear cells, the latter also being trapped in the glomerular capillary lumina, are features seen only in experimental infections, in which the amount of pigment is usually conspicuous (Silva et al., 1969).

The evolution of the kidney lesion to an end stage cannot yet be predicted. It is possible that factors other than the continuous availability of antigen–antibody complexes may be involved, preventing the reversion of the lesion.

Schistosomiasis patients with advanced kidney disease usually have the hepatosplenic form of the disease. The lesion is a lobular nephritis and any attempt to link it with a chronic manifestation of a non-glomerular endogenous antigen–antibody glomerulopathy must be considered hypothetical because no schistosomal antigen has yet been detected in the glomerular deposits (Brito et al., 1970).

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RÉSUMÉ

LÉSIONS GLOMÉRULAIRES DANS L'INFECTION EXPÉRIMENTALE DU SINGE *CEBUS APELLA* PAR *SCHISTOSOMA MANSONI*

On a étudié les lésions glomérulaires provoquées chez trois singes *Cebus apella* par une infection expérimentale de longue durée à *Schistosoma mansoni.*

Chez tous les animaux, l'atteinte glomérulaire, discrète, a consisté en une prolifération localisée des cellules du mésangiome. On notait aussi la présence dans la membrane basale des cellules endothéliales et épithéliales et dans la couche sous-épithéliale de dépôts opaques aux électrons et contenant de la gammaglobuline. D'après leur répartition, il semble que ces dépôts soient constitués d'immunocomplexes préformés fixés sur les capillaires du glomérule. On a décelé la présence de pigment bilharzien dans le cytoplasme des mononucléaires visibles dans la lumière des capillaires, ainsi que plus rarement dans les cellules endothéliales et les cellules du mésangiome.

La morphologie de ces lésions rappelle celle qui a été décrite chez des patients souffrant de schistosomiase sans signes cliniques d'atteinte rénale.

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