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EXPERIMENTAL SCHISTOSOMAL GLOMERULOPATHY IN MICE AND ITS RELATION TO PORTO-SYSTEMIC COLLATERAL CIRCULATION. A LIGHT AND ELECTRON MICROSCOPE STUDY

by

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Summary — The light and electron microscopic findings on kidneys from mice, infected or not with *Schistosoma mansoni*, in which partial ligation of the portal vein had been performed, are reported. Only the operated and/or infected mice showed lesions of slight to moderate mesangial glomerulopathy with electron-dense deposists. No qualitative morphological differences were observed between the kidneys of mice with ligation, infection or both. The results suggest an enhancing effect of the porto-systemic collateral circulation on schistosomal glomerulopathy.

KEYWORDS : Schistosomiasis, Experimental; Schistosoma mansoni; Glomerulopathy; Portal System; Collateral Circulation; Electron Microscopy.

Observations on the glomerulopathy of humans infected with *Schistosoma mansoni* point towards liver fibrosis as a necessary factor for the induction of kidney lesions (Andrade and de Queiroz, 1968; Andrade, Andrade and Sadigurski, 1971). One of the consequences of liver fibrosis is the development of collateral porto-systemic circulation. We have attempted to evaluate the role of experimentally induced collateral circulation in the genesis of schistosomal glomerulopathy in mice.

In a previous paper (Van Marck, Deelder and Gigase, 1977) a report was given on the incidence and nature of immune glomerular deposits in mice infected or not with *Schistosoma mansoni*, in which partial ligation of the portal vein, resulting in subsequent porto-systemic collateral circulation, was performed. It was shown that such ligation enhanced the disposition in immunoglobulins and complement in infected animals. Moreover, operated uninfected animals also showed immune glomerular deposits in a substantial percentage as compared to sham operated or intact animals.

The present paper aims at characterizing the morphology of kidneys from the same groups of mice both at the light and electron microscopic level.

Material and Methods

The animals, outbred female OF 1 (Oncins-France) mice, belong to five distinct groups. Group I (IL) comprises *Schistosoma mansoni* infected animals with ligation of the portal vein (n = 23), group II (L) uninfected and

ligated animals (n = 14), group III (I) unoperated infected animals (n = 15), group IV (CS) sham operated animals (n = 15), group V (C) intact control animals (n = 12). For technical details on partial portal vein ligation and mode of infection with *Schistosoma mansoni*, see Van Marck, Deelder and Gigase (1977).

Animals were sacrificed 47-72 days after infection of groups I and III or 56-82 days after partial portal vein ligation in groups I and II. For light microscopy a longitudinal section of a kidney from each animal was available after Bouin fixation and embedding in paraffin wax. One observer (V. M.) examined P. A. S.-stained sections of 2-3 μm thickness without being aware of the group to which each section belonged. In selected cases a P. A. S. M.-silver impregnation was also done. The appearance of glomeruli from the peripheral cortex was taken as representative for the morphology of the glomeruli as a whole. A previous survey of kidneys from intact animals had shown indeed that juxtamedullary glomeruli often disclosed features on the verge of abnormality.

For electron microscopy, small cortical kidney specimens were immediately fixed in glutaraldehyde 2 per cent at sacrifice and post-fixed in osmiumtetroxide 1 per cent, both buffered with cacodylate 0.15 M at pH 7.4. Dehydration in ethanol series was followed by embedding in Epon 812. Semi-thin sections (1 μm) were stained with toluidine blue for prelocation of glomeruli in the tissue blocks. Thin sections, prepared on a LKB III Ultrotome, were mounted on uncoated copper grids, stained with uranylacetate and lead citrate (Reynolds) and examined on a Jeol 100B electron miscroscope operated at 80 KV. Specimens processed in this way were available from 18 animals of group I, 6 animals of group II, 12 animals of group III, 2 animals of group IV and 2 animals of group V. From each case studied, an average of 3 glomeruli were examined (minimum 1, maximum 6).

Results

Table 1 summarizes the results of the *light microscopical findings*. When lesions were seen almost all of the glomeruli appeared diffusely affected, but the glomerular alterations were slight and not distinctive of the groups. Characteristically, the mesangial stalk was expanded through fibrillar, P. A. S.-positive material, sometimes resulting in a distortion of the capillary pattern. This was also evident after silver impregnation (Figures 1a, b). As judged from the 2-3 μm thick sections, no definite increase in cellularity could be detected. However, small areas of focal proliferation were often seen within a given glomerulus, at the periphery of the glomerular tuft (Figure 2b, as compared to an unaltered glomerulus in figure 2a). True exudative lesions were absent. From time to time a neutrophil could be seen within a capillary lumen, but this occurred also in glomeruli which were otherwise normal. Capillary walls never displayed peculiarities such as thickening or irregularities. Capsular adhesions or sclerotic areas were not detected.

In four cases of group I and in three of group II schistosomal pigment was seen in mesangial or epithelial cells of a few glomeruli. Five cases, all from group I, showed one or two peri-ovular granulomas within the renal

TABLE 1

Summary of light microscopic findings as compared to electron microscopic findings

Groups	Light microscopic lesions present				No obvious light microscopic lesions			
	EM. performed	EM. lesions +	EM. lesions −	Total	EM. performed	EM. lesions +	EM. lesions −	Total
I (LI)	10	10	0	12	8	6	2	11
II (L)	4	4	0	4	2	1	1	10
III (I)	5	5	0	5	7	3	4	10
IV (CS)	0	0	0	0	2	0	2	15
V (C)	0	0	0	0	2	0	2	12

TABLE 2

Summary of the electron microscopic findings

Groups	Animals examined/ total	B.M. changes		Mesangial deposits			Increase of mesangial cells			Increase of mesangial matrix			Viral particles
		±	+	±	+	++	±	+	++	±	+	++	
I (LI)	18/23	3	1	4	6	5	2	9	2	1	10	5	2
II (L)	6/14	0	0	1	1	3	0	4	0	0	4	1	2
III (I)	12/15	1	2	0	6	2	0	6	1	0	7	1	1
IV (CS)	2/15	0	0	0	0	0	0	0	0	0	0	0	0
V (C)	2/12	0	0	0	0	0	0	0	0	0	0	0	0

B.M. : glomerular capillary basement membrane.
± : dubious or scanty.
+ : present.
++ : marked or heavy.

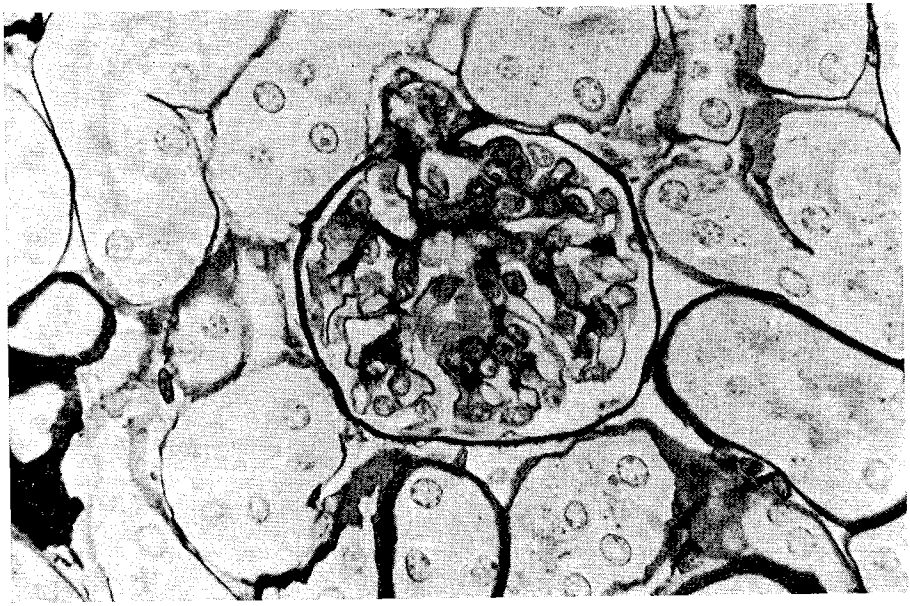

Figure 1a.

Normal glomerulus from an operated uninfected animal.
P. A. S. M.-stain. 830 ×.

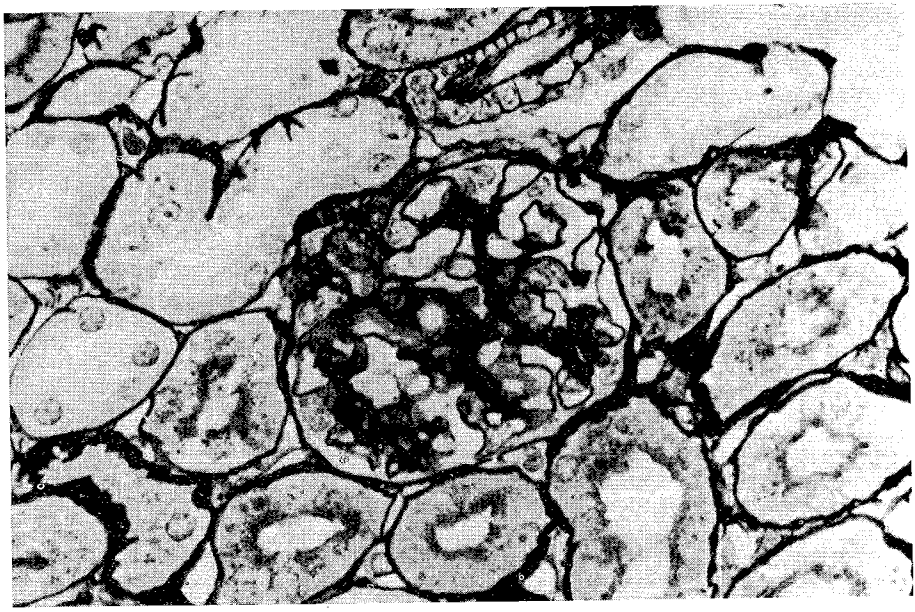

Figure 1b.

Glomerulus showing fibrillar increase of the mesangial matrix,
from an infected unoperated animal.
P. A. S. M.-stain. 830 ×.

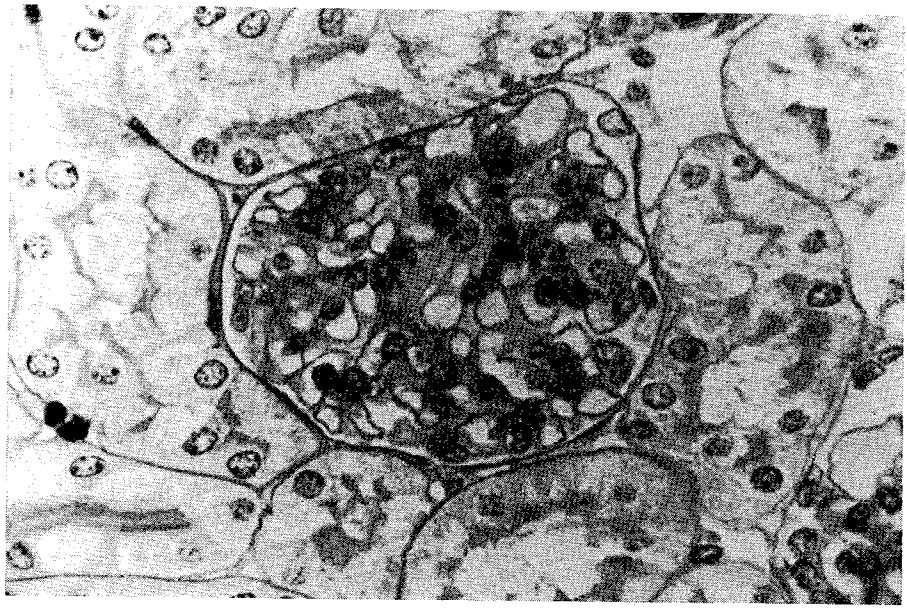

Figure 2a.

Glomerulus without obvious lesions from an operated uninfected animal.
P. A. S.-stain. 830 ×.

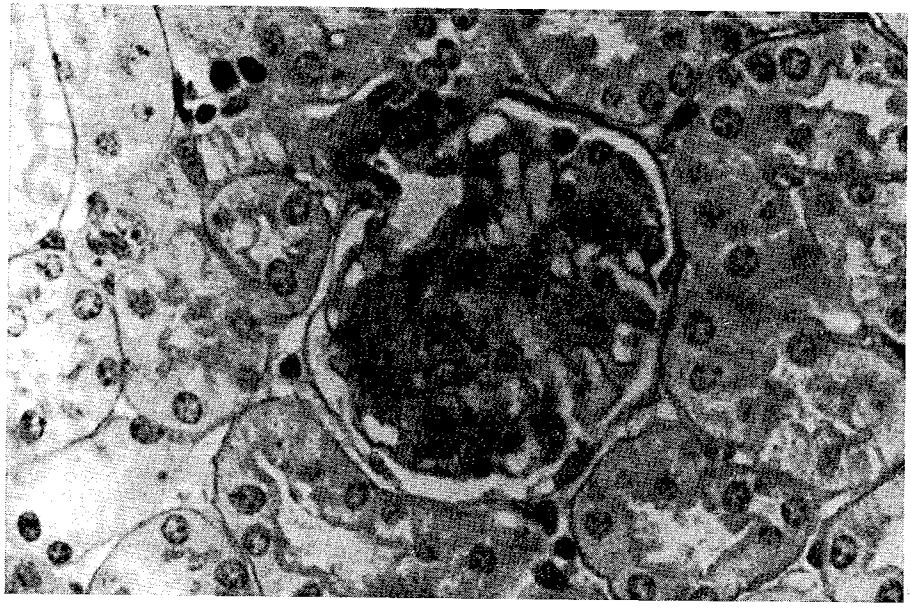

Figure 2b.

Glomerulus with increase of mesangial matrix and light focal cellular proliferation, from an infected operated animal.
P. A. S.-stain. 830 ×.

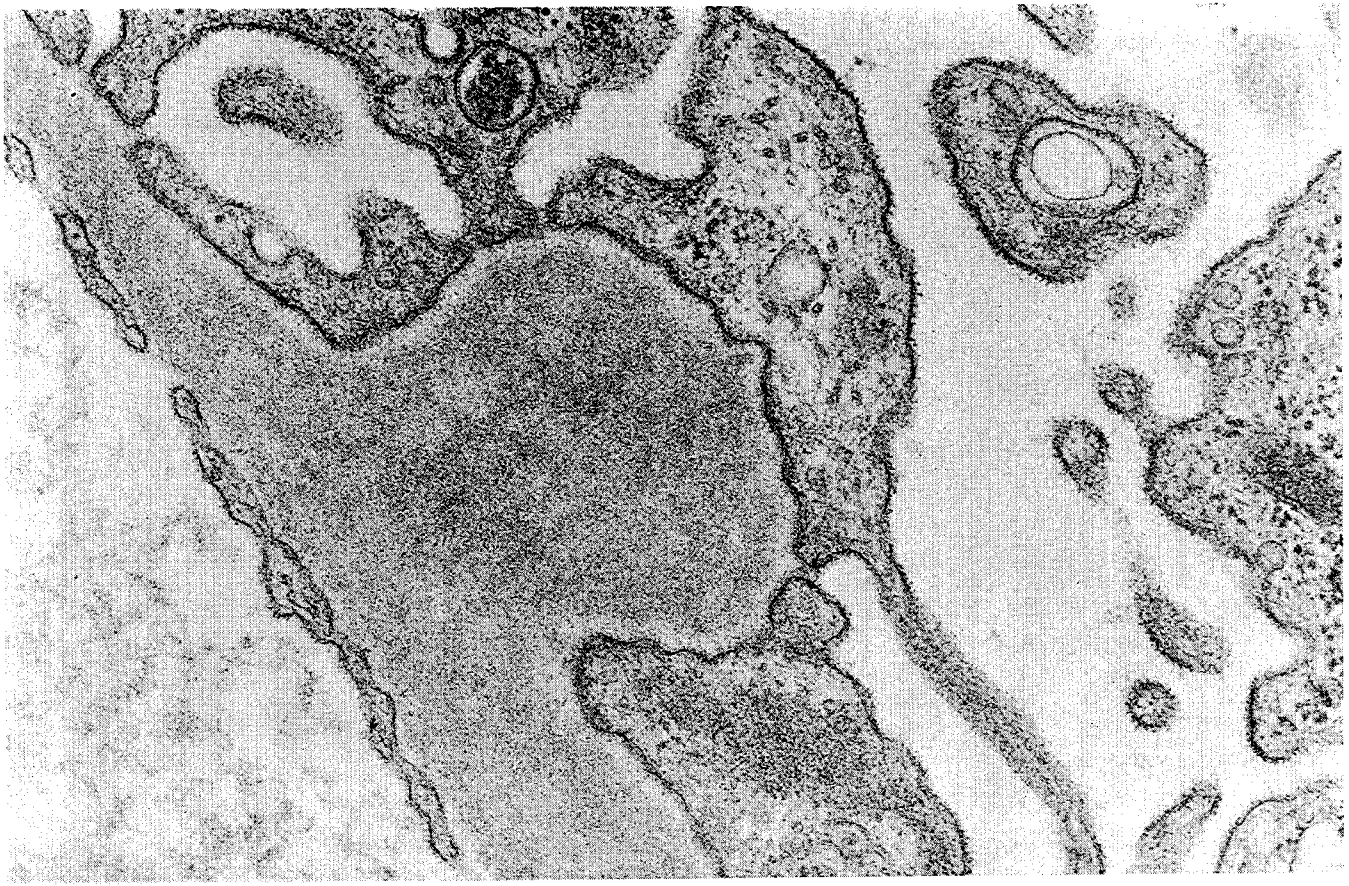

Figure 3.

Glomerular capillary basement membrane thickening in an infected unoperated animal.
Uranyl acetate and lead citrate. 50,000 ×.

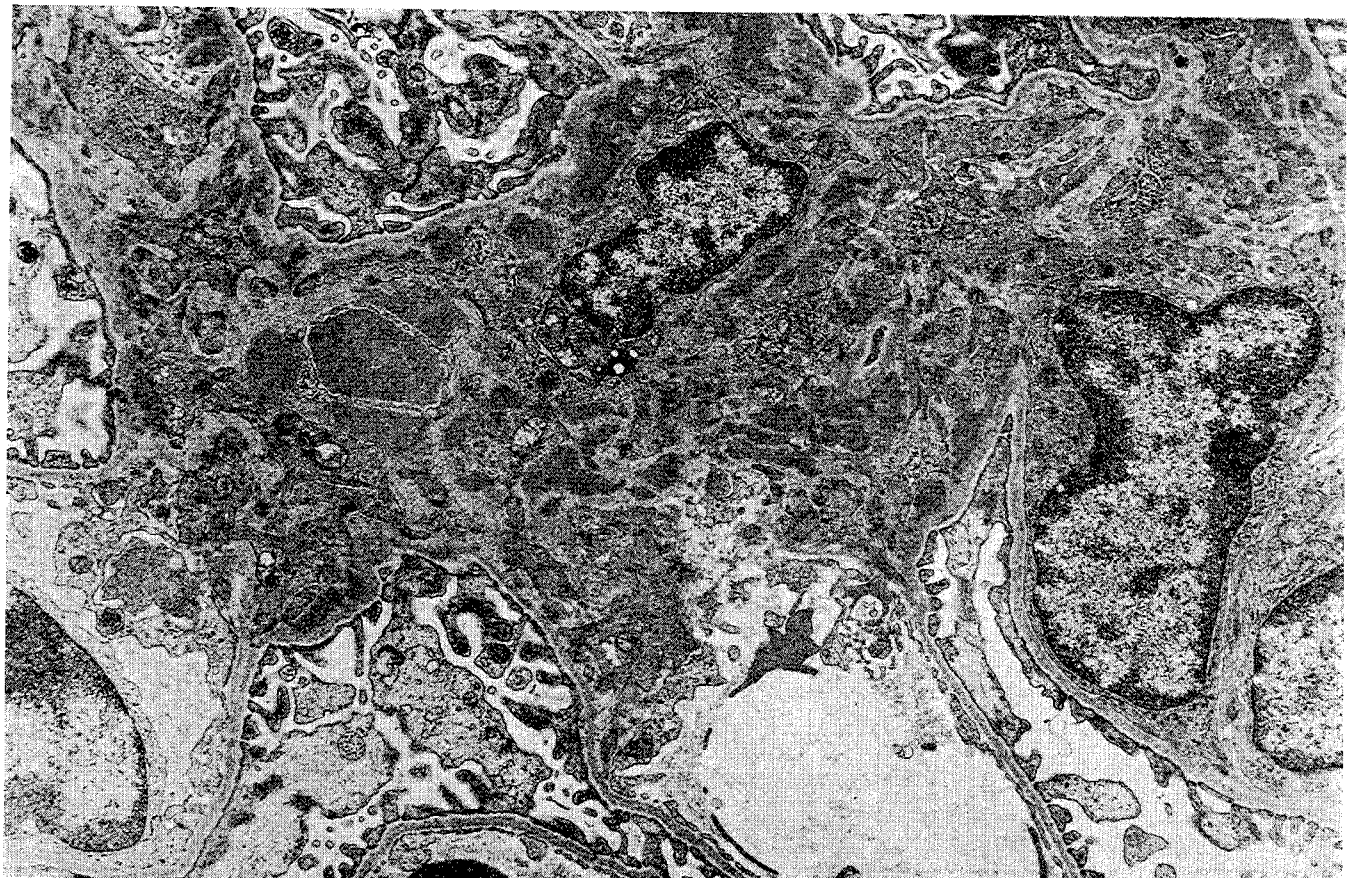

Figure 4.

Mesangial electron-dense deposits in a glomerulus from an operated uninfected animal.
Uranyl acetate and lead citrate. 12,500 ×.

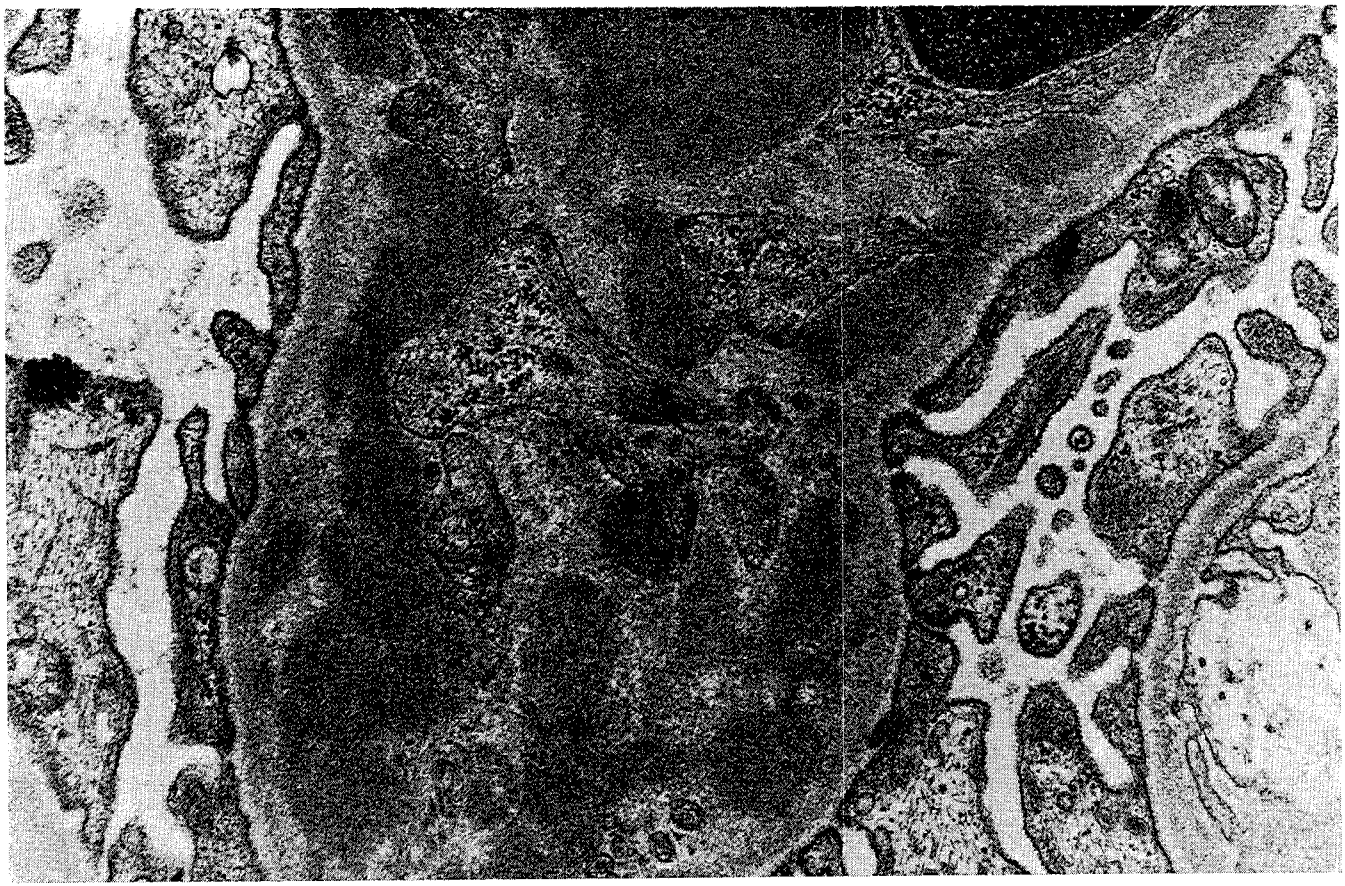

Figure 5.

Higher magnification of mesangial electron-dense deposits, from an infected and operated animal. Uranyl acetate and lead citrate. 42,000 ×.

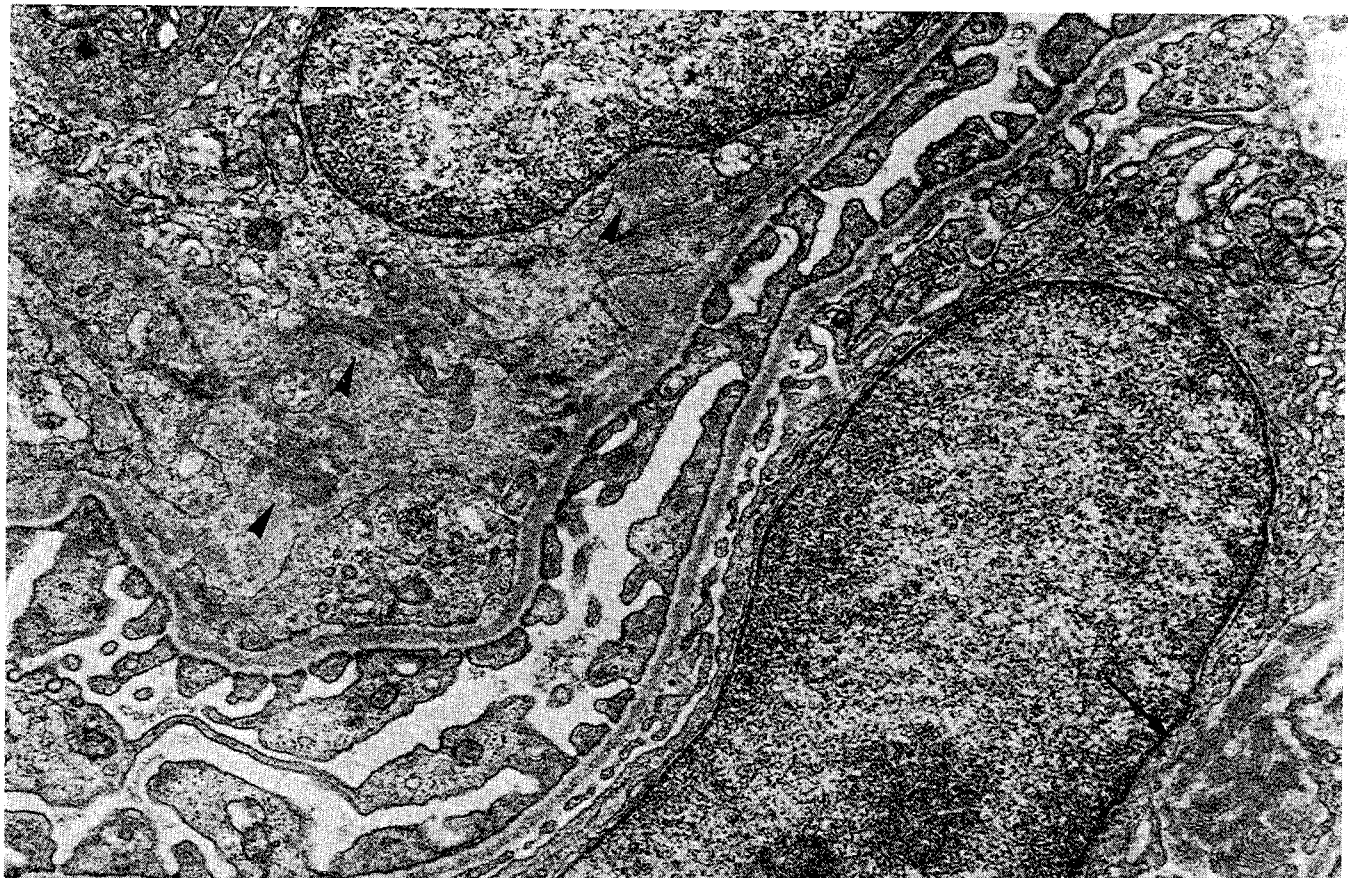

Figure 6.

Mesangial fingerprint electron-dense deposits (arrow-heads) and intracapillary deposits (arrow) in a glomerulus from an infected unoperated animal. Uranyl acetate and lead citrate. 17,400 ×.

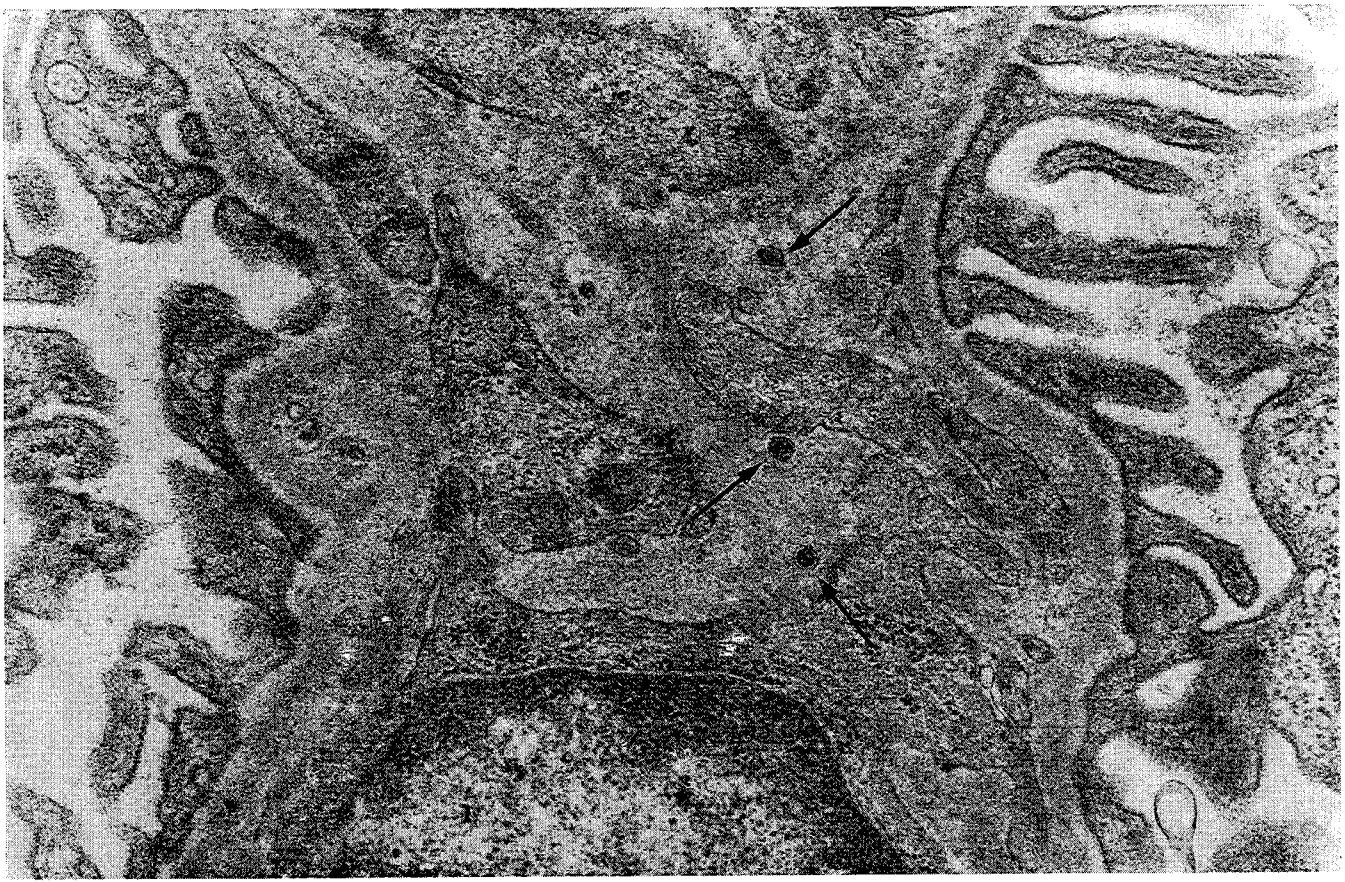

Figure 7.

Type-C viral particles (arrows) in mesangial matrix of a glomerulus from an infected operated animal. Uranyl acetate and lead citrate. 36,000 ×.

parenchyma. In another operated and infected case, with uneventful glomeruli, an adult worm was located in an interlobular vein where it had induced infarction of an extensive area of renal tissue.

Renal tubules in cases with glomerular changes were sometimes filled with hyaline protein casts, suggesting proteinuria. The interstitial tissue showed no abnormalities, apart from the above menioned granulomas. Pyelonephritis lesions were absent.

The *electron microscopic findings* are summarized in table 2. It was confirmed that alterations of the basement membrane of the glomerular capillaries were limited to an occasional small thickened zone or hump as shown in figure 3. The mesangial area was however markedly altered. There was increase of the mesangial matrix and slight but definite proliferation of mesangial cells. Their cytoplasm presented an intricate network of digitations running through the mesangial matrix up to the capillary endothelium which could be slightly lifted up. Electron-dense deposits were found scattered in the mesangial matrix (figure 4). They were rather homogeneously dense (figure 5) but sometimes of a neat fingerprint appearance. They were almost never seen beneath endothelial or epithelial cells or within the basement membrane proper. Changes in the endothelial and epithelial cells were inconspicuous.

In one infected unoperated animal extensive fingerprint electron-dense deposits were found, both within the mesangial matrix and free in the capillary lumen (figure 6). The endothelial cytoplasm of this case showed a reticular pattern thoroughly intermingled with the aforementioned deposits. The filamentous structures responsible for the fingerprint pattern were not compatible in size with fibrin and beared no resemblance to amyloid.

In five isolated cases (table II), mature type C viral particles were seen within the mesangial matrix (figure 7). No similar structures were observed in other cases of the same or other groups, though they were extensively looked for.

Discussion

It appears from the comparison of the light microscopic with the immunofluorescence findings (table 3) that immune glomerular deposits were present in all morphologically altered kidneys. Some cases, positive on immunofluorescence, were however indistinguishable from normal controls by light microscopy. All these grossly normal cases with immune deposits showed minor alterations on electron microscopic examination *, which were qualitatively comparable to those of light microscopically positive cases. It is worth noticing that the glomeruli of two mice out of the five in which schistosomal antigen was deteced by immunofluorescence, were normal on light microscopic examination and presented only minor alterations at the electron microscopic level. Light and electron microscopic findings are compared in table 1.

The occasional presence of schistosome eggs in the kidneys of five mice from group I (IL) merely reflects the bearing of the porto-systemic

(*) Two animals of the control groups showing immune desposits were normal by light microscopy but were not examined electron microscopically.

TABLE 3

Comparison of immunofluorescence and light microscopic findings

Groups	Total examined	Animals *with* immune deposits		Animals *without* immune deposits	
		LM. lesions present	LM. lesions not obvious	LM. lesions present	LM. lesions not obvious
I (LI)	23	12	9	0	2
II (L)	14	4	6	0	4
III (I)	15	5	3	0	7
IV (CS)	15	0	1	0	14
V (C)	12	0	1	0	11

collateral circulation on the ectopic deposition of eggs. Their presence was not related to the magnitude of the glomerular lesions in our series.

The finding of type C viral particles was incidental, unlike the situation in NZB mice where their presence seems to be closely related to the lupus-like glomerulonephritis (Yoshiki *et al.*, 1974). Further work is needed in order to evaluate precisely the significance of this observation.

The above described lesions, though they were absent in sham operated or in intact control mice, appear to be unspecific in the sense that it was impossible to differentiate between infected unoperated mice, operated uninfected mice or operated infected mice, although the lesions were, quantitatively speaking, most frequently seen in this last group. The alterations were always predominantly mesangial. The light microscopic examination was done on thin sections (2-3 μm) which we consider to be of paramount importance for a correct evaluation. In these conditions we could find no overall increase in glomerular cellularity. We also feel that the few glomerular basement membrane changes on electron microscopy were too scanty for being of any pathological significance.

Reports on the morphology of experimental schistosomal glomerulopathy in the literature are rather conflicting. In part this could be ascribed to the use of different animal species or even strains of the same species. Anyway, no uniform pattern of lesions is described. Studying kidneys of *Schistosoma mansoni* or *Schistosoma japonicum* infected hamsters (Hillyer and Lewert, 1974), rabbits (von Lichtenberg, Sadun and Bruce 1972; Jones *et al.*, 1977), *Cebus apella* monkeys (Brito *et al.*, 1971), *Macaca fascicularis* monkeys (Tada *et al.*, 1975), baboons (Brack *et al.*, 1972; Houba, Sturrock and Butterworth, 1977) or chimpanzees (Cavallo *et al.*, 1974), the authors describe a broad variety of lesions, sometimes even questioning (Brack *et al.*, 1972) the causal relationship between the infection and the glomerular changes.

Our light microscopic results are in agreement with those of Natali and Cioli (1976) in mice, but we never saw the glomerular capillary wall alterations and increased cellularity which they described in a minority of their cases. On the other hand, Andrade and Susin (1974) did not find light microscopical features different from those of the intact control mice. They found, as we did, dense deposits located in the mesangial area on electron microscopic examination. We were unable to demonstrate in our material glomerular basement membrane changes of the magnitude shown in their paper.

Cavallo *et al.* (1974), using chimpanzees infected with *Schistosoma japonicum*, were able to demonstrate a positive correlation between the occurrence of glomerular lesions and portal fibrosis as a result of infection. They hypothesized that the porto-systemic collateral circulation in their animals with portal fibrosis could possibly be the main factor in the causation of the glomerulopathy. In this regard our previous findings on the role of partial portal vein ligation with porto-systemic collateral circulation in glomerular immune deposition are confirmed here on a morphological basis. Partial portal vein ligation in the mouse apparently enhances the nephropathogenicity of *Schistosoma mansoni* infection but is also able by itself to induce glomerular changes indistinguishable, both at the light and at the electron microscopic level, from those seen in infected animals. This strenghtens our impression that porto-systemic collateral circulation which appears in heavy *Schistosoma mansoni* infection plays a role in the genesis

of the glomerulopathy. The presence of similar lesions and deposits in the glomeruli of mice with heavy unisexual infections with *Schistosoma mansoni* (unpublished results) demonstrates however that collateral circulation is not an absolute requirement for the glomerular lesions to develop. Further investigations will have to clarify the intricate set of mechanims leading to the glomerulopathy in schistosomal infections.

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La glomérulopathie bilharzienne expérimentale de la souris et sa relation avec la circulation collatérale porto-systémique. Une étude en microscopie optique et électronique.

Résumé — Les observations en microscopie optique et électronique sur les reins de souris infectées ou non par *Schistosoma mansoni,* chez lesquelles une ligature partielle de la veine porte avait été faite, sont décrites. Seules les souris opérées et/ou infectées montraient des lésions légères à modérées de glomérulopathie mésangiale à dépôts denses. Des différences morphologiques qualitatives entre les animaux ligaturés, infectés ou ligaturés et infectés, n'ont pas été observées. Ces résultats sont suggestifs pour une influence aggravante de la circulation porto-systémique collatérale sur la glomérulopathie bilharzienne.

Glomerulopathie als gevolg van experimentele schistosomiase bij muizen en het verband met de porto-systemische collaterale circulatie. Een licht- en electronenmicroscopische studie.

Samenvatting — De licht- en electronenmicroscopische bevindingen op nieren van muizen, al dan niet besmet met *Schistosoma mansoni,* bij dewelke een partiële ligatuur van de vena porta werd uitgevoerd, worden beschreven. Slechts geopereerde en/of geinfecteerde muizen toonden letsels van een lichte tot matig zware mesangiale glomerulopathie, met electrondense neerslagen. Er werden geen kwalitatieve verschillen gevonden tussen de nieren van muizen met ligatuur, met besmetting of met beide. De resultaten wijzen op een mogelijke verergerende invloed van de porto-systemische collaterale circulatie op de glomerulopathie door schistosomiase.

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