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Kidney lesions in baboons infected with *Schistosoma mansoni*

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SUMMARY

Glomerular lesions in baboons (*Papio anubis*) infected with different dosage regimes of *Schistosoma mansoni* were studied by immunofluorescence and light microscopy on kidney sections and by countercurrent immunoelectrophoresis on kidney homogenates and tissue eluates. Mild lesions, characterized by focal and segmental deposits of immune complexes, developed in sixty-two out of 103 baboons, irrespective of the intensity and duration of the infection. Severe, diffuse lesions developed in six baboons after prolonged and heavy infections. Adult worm and soluble egg antigens, together with IgM, IgG and C3, were detected in most of the severe lesions and in some of the mild lesions. In some animals, antigens were detected in acid homogenates and eluates of kidneys which showed no deposits of immunoglobulins or complement. These observations indicate that renal lesions in *S. mansoni* infections may be attributable to the deposition of immune complexes pre-formed in the circulation. However, the demonstration of antigens alone in some animals may suggest an alternative possibility, namely that antigens are deposited first with a subsequent binding of antibody and complement.

INTRODUCTION

Nephropathies associated with *Schistosoma mansoni* infections have been described both in experimental animals and in humans (Andrade & Queiroz, 1968; Andrade, Andrade & Sadigursky, 1971; de Brito *et al.*, 1971; Cavallo *et al.*, 1974; Higashi *et al.*, 1975; Hillyer *et al.*, 1975; Queiroz *et al.*, 1973; Silva *et al.*, 1970; Rocha *et al.*, 1976). In most cases, observations by light, immunofluorescence and electron microscopy are compatible with lesions of the immune complex type, involving deposition of immunoglobulins and complement alongside the capillary walls of glomerular vessels. Schistosomal antigens, however, have not been detected until recently. Natali & Cioli (1976), using an immunofluorescence technique after removal of excess host immunoglobulins, demonstrated antigens in about 20% of Swiss mice infected with *S. mansoni*, while Hoshino-Shimizu *et al.* (1976) described the presence of antigens in glomerular lesions in two human cases (one biopsy and one necropsy specimen) out of twelve patients examined.

In our laboratories, we have studied the parasiological and immunological responses as well as the immunopathological changes in Kenyan baboons infected with a local strain of *S. mansoni*. In this paper we describe the kidney lesions at different stages of the disease in baboons infected with different doses of cercariae, with special emphasis on the identification of the antigens involved.

MATERIALS AND METHODS

Animal infections. Two series of baboons (*Papio anubis*) were exposed percutaneously to different doses of *S. mansoni* cercariae as described by Sturrock, Butterworth & Houba (1976). Unless otherwise stated, times in weeks refer to the period elapsed after primary exposure.

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Series A. This consisted of seventeen baboons. Four baboons (group A1) were infected with a single dose of 200 cercariae per baboon (c.p.b.); six baboons (A2) with a single dose of 200 c.p.b., followed at about monthly intervals by four more doses of 200 c.p.b. (1000 c.p.b. in all); and five baboons (A3) with a single dose of 1000 c.p.b. The course of infection was monitored by weekly examination of faeces and urine as described elsewhere (Sturrock *et al.*, 1976), and the animals were killed between weeks 30 and 32. A further group of six baboons was infected with 5000 c.p.b.: four of them died, however, and the remaining two (A4) were killed earlier than the groups described above, at weeks 7 and 10 respectively. In addition, three baboons matched for weight were kept as an unexposed control group throughout the experiment, and were killed at 32 weeks.

Series B. 100 baboons were treated as follows: forty were exposed to a low primary infection of 500 c.p.b.; forty were exposed to a high primary infection of 2000 c.p.b.; and the remaining twenty were not exposed. The baboons were allocated to four groups (B1 to B4) containing twenty-five animals each, comprising ten animals with a high and ten animals with a low primary infection, and five unexposed animals. Groups B1 to B4 were challenged at 8, 16, 32 and 68 weeks respectively. The original plan, to challenge with 2500 c.p.b. five of both the low and high primary exposure groups and five unexposed baboons, as a challenge control, was unavoidably modified in some cases by mortality, especially among the high primary exposure animals. The numbers of animals actually examined are shown in Table 1.

The baboons in each group were killed about 10 weeks after the challenge exposure, namely at weeks 18, 26, 42 and 78 for groups B1 to B4 respectively.

TABLE 1. Numbers of animals examined at the different dosage schedules

Groups	Subgroups				
	(a) 500* —†	(b) 2000* —†	(c) 500* 2500†	(d) 2000* 2500†	(e) —* 2500†
B1 (8 weeks)‡	5	5	4	3	5
B2 (16 weeks)	5	3	5	4	4
B3 (32 weeks)	5	4	5	5	5
B4 (68 weeks)	5	3	5	3	3

* Primary exposure (in c.p.b.).
† Challenge exposure (in c.p.b.).
‡ Time in weeks of challenge after primary exposure.

Serum samples. All baboons were bled before infection and at approximately 10–14 day intervals; serum was separated by centrifugation after defibrination and stored in aliquots at −20°C until use.

Termination of the experiments. At the end of the experiment the animals were heparinized (25,000 u) and killed with 100 mg of sodium pentabarbitone. The abdomen was opened and samples of the left kidney were taken for microscopic examination. The hepatic portal system was then perfused. The adult worms recovered from the aspirated perfusion fluid were counted as described elsewhere (Sturrock *et al.*, 1976). After perfusion, the remainder of left kidney and the whole of right kidney, as well as smaples of other tissues, were removed. The kidneys were frozen and kept at −70°C until homogenates and eluates were examined, as described below. Tissue egg loads were estimated for selected organs by the potassium hydroxide digestion technique (Cheever, 1968).

Kidney specimens and their processing. Specimens for light microscopy were fixed in 10% formolsaline, processed routinely, and were stained with haematoxylin–eosin and with periodic acid–Schiff's reagent (PAS).

Specimens for immunofluorescence investigation were embedded in Ames OCT compound (Wilson, Sulzer & Walls, 1974), frozen on dry ice and stored at −70°C until examined. Cryostat sections (5μ) were cut at −20°C, dried at room temperature and kept in plastic bags with silica gel at 4°C if necessary. Otherwise they were washed with saline and stained with conjugates, without any fixation, as previously described (Houba *et al.*, 1971). Evans blue (0·1%) or rhodamine-labelled BSA were used for counter-staining of preparations stained with fluorescein conjugates. Double-staining with two conjugates was carried out as described by Hijmans, Schuit & Klein (1969). After a final wash, the preparations were mounted in a 9:1 solution of glycerol–phosphate-buffered saline (PBS) and sealed with nail varnish. All preparations were examined on a Leitz Orthoplan microscope using incident light (mercury pressure lamp HBO 200) with filter blocks Ploempak I for fluorescein and Ploempak N for rhodamine conjugates. Photographs were taken with a Leitz camera using Agfacolor CT 18 film.

Antigens, antisera and conjugates. Commercial conjugates to the human immunoglobulins IgG, IgM, IgA and to the third component of complement (C3) were obtained from: Hyland Labs, Costa Mesa Inc., California; Kallestad Labs Inc., Chaska, Maine; and Cappal Labs Inc., Downingtown, U.S.A. They were labelled either with fluorescein–isothiocyanate (FITC) or tetramethyl–rhodamine–isothiocyanate (TRITC). Anti-human conjugates were used throughout the study because of their high cross-reactivity with baboon antigenic determinants (Neoh *et al.*, 1973).

Conjugates to other components were prepared from the following antisera: anti-IgE (donated by D. S. Rowe, WHO, Geneva) and anti-properdin factor B (donated by P. H. Lambert, WHO, Geneva). Antisera to soluble worm antigens (SWA) were prepared in rabbits immunized with the supernatant from the total homogenate of adult *S. mansoni* worms centrifuged at 100,000 *g* for 45 min. Two injections of SWA with Freund's complete adjuvant were given subcutaneously at an interval of 2 weeks, followed by booster doses at monthly intervals. Antisera to soluble egg antigens (SEA) were prepared in rabbits as described previously (Houba *et al.*, 1976), or donated by A. F. F. Mahmoud, Case Western Reserve University, Cleveland, U.S.A.

IgG fractions of antisera (prepared by DEAE–cellulose chromatography) were labelled by the dialysis technique (Clark & Shepard, 1963), and conjugated antisera were separated from free fluorochromes, either by dialysis or by filtration through Sephadex G-25 columns. The conjugates were then fractionated on columns of DEAE–cellulose by stepwise elution at selected molarities of sodium chloride (Brandtzaeg, 1975).

All conjugates were kept in frozen aliquots stored at −20°C and checked for free fluorochromes on Sephadex G-25 before use. The protein:fluorochrome ratio of conjugates was estimated (Brandtzaeg, 1975), and only those conjugates with ratios between 1·2 and 2·0 were used. The elutions of conjugates required for staining were estimated by chequer-board titration (Feltkamp, 1970). The specificity of staining was tested by absorbing conjugates with the appropriate antigens, or by blocking during staining. In the latter procedure, preparations were treated first with unconjugated antiserum, then with conjugated antiserum. Both of these techniques showed negative staining, confirming the specificity of the reaction.

Treatment of cryostat sections with citric acid. Unfixed cryostat sections on microscope slides were dipped into citric acid (0·1 M, pH 2·5) and left for 2–24 hr at 20°C or 37°C. They were then washed five times in PBS and stained with conjugates as above.

Elution of kidney samples. The technique described elsewhere (Houba *et al.*, 1971) was used, with minor modifications. The cortex was separated from the medulla and vessels, weighed, cut into small pieces and homogenized. The homogenate was washed eight to ten times with PBS (pH 7·4) by centrifugation at 4°C until a clear supernatant was obtained. Citric acid (0·1 M, pH 2·1) was then added (1 ml for 5 g of original cortex tissue) to the washed sediments, which were stirred by vortex mixing. The pH was checked with pH papers and, where necessary, reduced to below 2·5 with a few drops of 1 M citric acid. The samples were left for 1 hr at 37°C, followed by 16 hr at 4°C. About 10% of the homogenate was then separated as the acid tissue homogenate for counter-current immunoelectrophoresis (CCI). The remaining homogenate was centrifuged and the supernatants collected as neat eluates. A proportion of each supernatant was concentrated five to ten times by using a Minicon concentration set B 15 (Amicon Corp., Lexington, U.S.A.).

Preparation of S. mansoni adult worms for immunofluorescence. Mice (Swiss white and inbred CBA strains) were infected percutaneously with *S. mansoni* cercariae. After 7–9 weeks, the adult worms were collected by perfusion with citrated saline (Sturrock *et al.*, 1976). The adult worms were washed with PBS and fixed in OCT embedding medium, frozen and kept at −70°C until cryostat sections of 5–6 μm were cut.

Estimation of antibodies by indirect immunofluorescence. Sera, diluted with PBS 1:5, 1:10 and then in three-fold dilution steps, were dropped on to cryostat sections of worms and incubated for 30 min at room temperature. The sections were then washed three times with PBS at 5 min intervals. Next the preparations were exposed to appropriate conjugates (anti-IgM or anti-IgG) for 30 min, washed three times with PBS, and counter-stained with 0·1% Evans blue solution for 5 min. After the final wash, the preparations were mounted in glycerol–PBS and examined with a u.v. microscope as described above. The brightness of fluorescence of the surface membranes and caecal (gut) linings was recorded. The reciprocals of the final serum dilutions showing definite fluorescence were calculated as the end-points. Eluates from kidneys prepared with citric acid were tested neat and after concentration.

Counter-current immunoelectrophoresis (CCI). The technique described in a previous paper (Houba *et al.*, 1976) was used for the estimation of antigens in kidney eluates. Eluates were tested neat and diluted up to 1:25. Acid tissue homogenates were examined similarly, except that the homogenates (30–50 mg) were left in the centre walls for about 2–3 hr at room temperature before running the separation. Increasing the temperature to 56°C during the run, as recommended by Milgrom, Campbell & Andres (1976), was done on samples showing negative results at room temperature. Precipitin lines were read immediately and again after washing and staining the preparations.

Cytotoxicity assays. These were carried out on sera at different times during the infection by the ^{51}Cr-release technique from schistosomula as described elsewhere (Sturrock *et al.*, in preparation).

RESULTS

Immunological observations

Series A. Deposits of immunoglobulins were detected by immunofluorescence in kidney samples from eleven out of fifteen animals (groups A1, 2 and 3) killed between weeks 30 and 32 (Table 2). All of these samples were positive for IgM; eight were positive for IgG and five for C3. In all but one there was only a focal and segmental distribution of fine or medium granular deposits alongside the glomerular capillary walls; in a few of them positive fluorescence was also detected in the mesangium. These were all classified as 'mild' lesions. The exceptional specimen (No. 263 from A2) had heavier deposits of

TABLE 2. Deposition of immunoglobulins and complement in baboons infected with *S. mansoni*

Treatment group	Baboon number	Immunofluorescence* (cryostat sections) IgG	IgM	C3
A1 (200 c.p.b.)†	206	−	−	−
	238	−	+	−
	257	−	−	−
	260‡	+	+	−
A2 (5×200 c.p.b.)	204	+	+	+
	224‡	+	+	+
	228	+	+	−
	245	−	−	−
	255	−	−	−
	263‡	++	++	++
A3 (1000 c.p.b.)	203	−	-	−
	234‡	+	+	+
	240	−	−	−
	249	+	+	−
	250	−	+	+
A4 (5000 c.p.b.)	233	−	+	−
	251	+	+	−

* Kidney samples from each animal in groups A1 to A4 were tested for deposits of IgG, IgM and C3 by immunofluorescence. (−) No staining, or a few faint spots; (+) 'mild' lesion: focal and/or segmental staining in some glomeruli; (++) 'severe' lesions: intense, diffuse, granular staining in all glomeruli.

† c.p.b., Cercariae per baboon.

‡ Counter-current immunoelectrophoresis was carried out on acid eluates and homogenates of kidneys from four animals. Baboon 263 (with 'severe' lesions by immunofluorescence) was positive for soluble egg antigens (SEA) in the homogenate and for both soluble worm antigens (SWA) and SEA in the eluate. The remaining animals gave completely negative results.

medium to coarse granules, positive for IgM, IgG and C3, diffusely distributed in the capillary walls in all glomeruli. This specimen was classified as a 'severe' lesion. The two specimens obtained from baboons which were killed prematurely in subgroup A4 (5000 c.p.b.) showed the mild focal segmental and granular lesions described above, although one of them also showed positive linear staining for IgG alongside the capillaries. Tests for antigens by direct immunofluorescence, both on untreated cryostat sections and on sections treated with citric acid, were negative in all cases. (In a few preparations, faintly stained spots were regarded as negative.) Eluates from four kidneys were prepared after storage for 1 year at −70°C; CCI revealed the presence of SEA in the acid homogenate and SWA and SEA in the eluate from one of them (No. 263, with the 'severe' lesion).

Series B. The immunofluorescence findings are summarized in Tables 3 and 4. In eleven out of seventeen of the animals killed at week 18 (B1 subgroups a to d), there was weak to moderate granular staining for IgM alongside the glomerular vessels, irrespective of the dose of infection; all deposits were focal and segmental, and were classified as mild lesions. Staining was positive for IgG in two animals and for C3 in only one. Detection of antigens by direct immunofluorescence was positive in one specimen of subgroup B1c, showing fine granular spots in the glomeruli; indirect immunofluorescence of eluates was also positive in one case only, showing the specificity of antibody for membrane and gut-associated antigens.

TABLE 3 Numbers of baboons with glomerular depcsits in their kidneys from the subgroups of B1 to B3 infected with *S. mansoni**

Baboons (subgroups)	Numbers examined	Immunofluorescence: Direct IgG	IgM	C3	SWA†	SEA‡	Indirect, on worm cryostat sections: MAA§	GAA¶	Counter-current Immunoelectrophoresis: SWA	SEA	Number of animals with severe lesions**
B1 (8 week)††											
a‡‡	5	0	3	0	0	0	0	0	n.d.§§	n.d.	0
b	5	1	3	0	0	0	1	1	n.d.	n.d.	0
c	4	0	2	1	0	1	0	0	n.d.	n.d.	0
d	3	1	3	0	0	0	0	0	n.d.	n.d.	0
e	5	1	3	0	0	1	0	0	n.d.	n.d.	0
B2 (16 week)											
a	5	0	2	0	0	0	0	0	n.d.	n.d.	0
b	3	0	2	0	0	0	0	0	n.d.	n.d.	0
c	5	2	3	0	0	1	0	1	n.d.	n.d.	0
d	4	2	3	1	0	0	0	1	n.d.	n.d.	0
e	4	1	3	0	1	1	1	1	n.d.	n.d.	0
B3 (32 week)											
a	5	3	3	3	0	0	1	1	1	0	0
b	4	2	3	3	0	0	0	0	0	0	1
c	5	3	4	3	2	0	2	0	2	1	0
d	5	2	4	4	3	0	2	1	3	0	1
e	5	2	3	1	0	0	0	0	0	0	0

* Kidney samples from each animal in groups B1 to B3 were examined for deposits of immunoglobulins, complement and *S. mansoni* antigens by direct immunofluorescence on cryostat sections, by indirect immunofluorescence using acid eluates from whole kidneys and by counter-current immunoelectrophoresis on the same eluates.

† SWA, Soluble worm antigens.

‡ SEA, Soluble egg antigens.

§ MAA, Membrane-associated antigens.

¶ GAA, Gut-associated antigens.

†† Time of challenge in weeks after primary exposure.

** The numbers of animals with 'severe' lesions, as judged by immunofluorescence (intense, diffuse and granular staining in all glomeruli).

‡‡ Dosage schedule: a to e exposed to 500, 2000, 500+2500, 2000+2500 and 2500 c.p.b., respectively.

§§ n.d., Not done.

The second group of baboons (B2 subgroups a to d), killed at week 26, showed a similar picture: ten out of seventeen were positive for IgM, while four were also positive for IgG and one for C3. All were 'mild' lesions of the focal segmental type. Only one was positive for SEA by direct immunofluorescence (in subgroup B2c), while eluted antibody from two of them (one each in B2c and B2d) showed specificity against the gut-associated antigens.

Animals killed at week 42 (B3 subgroups a to d) had a higher frequency of granular glomerular deposits (fourteen out of nineteen); all these deposits were positive for IgM and the majority also had IgG and C3 (eleven and thirteen respectively out of fourteen). As in the previous subgroups, most were 'mild' focal and segmental lesions. However, two animals, one each in B3b and B3d, had 'severe' lesions with heavy deposits, positive for IgM, IgG and C3, diffusely distributed in all glomeruli. Fine granular deposits of SWA were detected in two baboons of subgroup B3c and in three of subgroup B3d by direct immunofluorescence on untreated cryostat sections. Treatment of sections with citric acid significantly reduced the staining of immunoglobulins, but also abolished the staining for antigens in two

TABLE 4. Presence of glomerular deposits in individual baboons infected with *S. mansoni* from group B4 (challenged at week 68)*

Baboons (subgroups and numbers)	Immunofluorescence (on cryostat sections)				Counter-current immunoelectrophoresis: Acid eluates (whole kidney)		Counter-current immunoelectrophoresis: Homogenates (small piece)	
	IgG	IgM	C3	Severe lesions	SWA	SEA	SWA	SEA
B4a (500 c.p.b.)								
362	−	+	−		−	+	+	+
368	+	+	+		+	−	+	−
370	−	−	−		−	−	−	−
381	−	−	−		−	+	+	+
385	−	−	−		+	+	+	−
B4b (2000 c.p.b.)								
363	++	++	+	+	+	−	+	−
367	++	++	+	+	+	+	+	+
369	++	++	−	+	+	+	+	−
B4c (500+2500 c.p.b.)								
374	+	+	−		+	−	+	−
376	+	+	+		+	−	+	−
382	+	+	−		+	+	+	−
384	−	−	−		−	+	+	−
386	−	−	−		+	+	+	+
B4d (2000+2500 c.p.b.)								
361	+	+	−		+	+	+	−
379	+	+	−		+	−	+	−
380	−	−	−		−	+	+	+
B4e (2500 c.p.b.)								
500	+	++	+		+	−	+	−
501	+	+	−		+	+	+	−
502	−	+	−		+	+	−	−

* Kidney samples from each animal in group B4 were examined for deposits of immunoglobulins and complement by direct immunofluorescence. Immunofluorescence scoring as in Table 2: three animals (363, 367, 369) showed 'severe' lesions. Eluates and homogenates of each kidney were also examined for *S. mansoni* antigens by counter-current immunoelectrophoresis. See Table 3 for key to symbols and contractions.

animals. The specificity of antibodies eluted from these five kidneys was confirmed by indirect immunofluorescence: four of them stained mainly the membranes, one of them the gut of adult worms. In addition, counter-current immunoelectrophoresis revealed the presence of SWA in the eluates of all five kidneys and of SEA in one. One baboon in subgroup B3a had no antigens detectable by direct immunofluorescence; however, CCI detected SWA in the eluate and the specificity of antibodies in the eluate for membrane- and gut-associated antigens was confirmed by indirect immunofluorescence.

Positive glomerular deposits of immunoglobulins were found in thirteen out of sixteen animals in group B4 (subgroups a to d), killed at week 78 (Table 4). All thirteen were positive for IgM, eleven for IgG and five for C3. There were 3 'severe' lesions in subgroup B4b, with heavy deposits in all glomeruli, whereas the remainder were only focal and segmental 'mild' lesions. Antigens were not detected by direct immunofluorescence in any case; a few very faintly positive spots were regarded as negative. However, CCI of the eluates, diluted 1:25, revealed the presence of either SWA or SEA, or both, in all kidneys showing positive staining for immunoglobulins. In addition, kidneys with no detectable immunoglobulins did have antigens in the eluates (except No. 370 in B4a). CCI on the acid homogenates of kidneys revealed SWA in all but one animal (No. 370) and SEA in some.

The challenge controls for groups B1 to B4 (subgroups e, 2500 c.p.b.) were killed at week 10. The

kidneys of twelve out of seventeen showed 'mild' lesions with focal segmental deposits positive for IgM; in addition, six had IgG and two had C3 (Tables 3 and 4). Antigens were detected in some animals.

The staining of specimens from all the groups with conjugates of anti-IgA and anti-properdin factor B gave negative results; some of the specimens showed faint segmental staining with the anti-IgE conjugate.

The specimens from three non-infected (control) baboons were negative with all the conjugates used, except for anti-IgM, which showed positive spots in a few glomeruli in one animal. The kidney eluates and homogenates from these animals were negative in indirect immunofluorescence and CCI tests.

Light microscopy

Light microscopy revealed a normal kidney architecture in most of the samples. Some of the 'mild' lesions had slight mesangial proliferation and localized thickening of the glomerular capillary walls in a few glomeruli, occasionally positive with PAS-staining. The 'severe' lesions had more pronounced and more diffuse thickening of the capillary walls, lobulation and proliferation of cells, with an increased frequency of PAS-positive material in the majority of glomeruli. These were classified as proliferative (two cases) and membrano-proliferative (four cases) glomerulonephritis. Eggs were found in two kidney specimens: one, from subgroup B2b, was surrounded by an infiltration of mononuclear and polymorphonuclear cells; the other, from B2e, showed no cellular reaction. The three uninfected control baboons had a normal architecture.

Relationship of other parameters to renal lesions

Serum antibodies. IgM antibodies to membrane- and gut-associated antigens appeared in the sera of infected animals soon after infection, reaching a peak between weeks 6 and 13; the titres decreased thereafter and remained low until the end of the experiment. Challenge infection did not significantly increase their levels.

IgG antibodies to the same antigens began to rise some weeks later than IgM antibodies. IgG levels exceeded those of IgM by weeks 4–8 and reached a peak between weeks 20 and 28. After that, the titres decreased slightly and in the non-challenged animals of group B4 they were about one-third of the peak value by week 78, the end of the experiment. Challenge infection markedly increased the levels of IgG antibodies to both antigens. These levels were still higher than in non-challenged groups at the end of the experiment.

Parasitological findings. The detailed parasitological findings from these two experiments are given elsewhere (Sturrock *et al.*, 1976; and in preparation). However, the findings may be summarized as follows.

Adult worm recoveries in both experiments were generally related to the number of cercariae to which each baboon was exposed. Exceptions were among the animals in experiment B, which were challenged after a primary infection. Of these thirty-four animals, fourteen (two, three, five and four from groups B1 to 4, respectively) yielded at least 30% less worms from the challenge exposure than did the appropriate controls. A composite picture of faecal egg excretion showed an initial rise between weeks 6–10 before stabilizing at a plateau level until about week 30. The counts then dropped gradually until, in B4, they fell below the limits of sensitivity of the Kato technique for about 2 months, before rising again by week 60 to a new stable level equal to, or even higher than, the original plateau. While there was some evidence, especially in group B4, that the challenge infections substantially raised the faecal egg count, this rise was by no means clear-cut. Possibly full egg production had not been achieved by the new female worms before the animals were killed. This supposition is supported by rather depressed tissue egg recoveries from the challenged animals in groups B1 to 3.

It is noteworthy that 'severe' kidney lesions occurred only in baboons with prolonged heavy infections (groups A2, B3 and B4). Otherwise there was no apparent correlation between the distribution of kidney lesions and the parasitological parameters measured.

Cytotoxicity assays. Sera taken at the time of the challenge infection from all animals in groups B3 and B4, and from animals in subgroups c, d and e in B1 and B2, were tested for cell-dependent cytotoxic activity to ^{51}Cr-labelled schistosomula. Full details of the activity of such sera, and of the relation of this

activity to resistance to superinfection, will be published elsewhere (Sturrock, *et al.*, in preparation). In the context of the present study, it may be noted that activity was detectable in some animals at week 8. By week 32, activity was observed in nine out of ten animals tested, while at week 68, six out of eight animals were positive. As described previously (Butterworth *et al.*, 1976), there was marked variation in the levels of antibody in different animals within each subgroup: there was no evidence for an association between the levels of this antibody and the development of renal lesions.

DISCUSSION

Our observations have shown that baboons infected with *S. mansoni* cercariae develop patent infections, as demonstrated by recoveries of adult worms, excretion of eggs in the faeces and recovery of eggs from tissues (Sturrock *et al.*, 1976; and in preparation). Some of these animals developed renal lesions, demonstrated by histology and immunofluorescence studies on kidney specimens taken at certain stages of the infection. Most of these lesions were 'mild', characterized by focal and segmental changes which probably do not reflect a diminution in kidney function. However, six animals (one in series A and five in series B) showed 'severe' diffuse lesions, classified histologically as proliferative and membrano-proliferative glomerulonephritis, with heavy deposition of immunoglobulins and complement in all glomeruli. These were invariably found in baboons with prolonged, heavy infections. In some of them, either schistosomal antigens themselves or a specificity of antibodies for schistosomal antigens were also demonstrated. This strongly supports the hypothesis that the 'severe' glomerular lesions in *S. mansoni* infection are due to damage to glomerular basement membranes by deposited immune complexes.

If we accept this as likely, at least two pathogenic mechanisms should be considered. In the first mechanism, immune complexes in the circulation are not removed by physiological mechanisms and are trapped in the glomerular basement membranes (Dixon, 1965; Mannik, Haakenstad & Arend, 1974). In the second mechanism, some of the antigens or antibodies circulating freely at certain stages of the disease may deposit in the glomerular vessels, with the corresponding antibodies or antigens binding to them at some later stage. Thus, in this mechanism, the presence of soluble immune complexes in the circulation need not be a prerequisite for their deposition in tissues (Lambert & Houba, 1974).

Several studies have demonstrated the presence of soluble schistosomal antigens, free or bound to antibodies, in the sera of patients or animals infected with schistosomiasis (Berggren & Weller, 1967; Bout, Santos & Capron, 1975; Deelder *et al.*, 1976; Gold, Rosen & Weller, 1969; von Lichtenberg, Bawden & Shealey, 1974; Madwar & Voller, 1975; Nash, 1974; Nash, Prescott & Neva, 1974; Phillips & Draper, 1975; Santos, Bout & Capron, 1976; Santos *et al.*, 1976; WHO memorandum, 1974). We have shown that the first antigens to appear in the serum of baboons infected with *S. mansoni* are gut-associated antigens, followed by membrane-associated antigens and, later, by soluble egg antigens (Houba *et al.*, 1976). Corresponding serum antibodies were detected 1–2 weeks later, so that the possibility of an initial deposition of antigens in the glomeruli should be considered. This possibility may be further supported by the demonstration of schistosomal antigens in the urine (WHO memorandum, 1974; Carlier *et al.*, 1975).

Our findings of immunoglobulins, complement and antigens in the renal lesions of some of the infected baboons are in accord with the first mechanism of deposition of circulating immune complexes, as suggested by Tada *et al.* (1975) for *S. japonicum* infections. They do not, however, exclude the second mechanism of a local formation of immune complexes in the kidney, as suggested by others (Lambert & Houba, 1974; Natali & Cioli, 1976). Our data of baboons with no immunoglobulins, complement or antigens detectable by immunofluorescence in the glomeruli, but with antigens demonstrable by CCI in the eluates and/or homogenates of kidney tissue, strongly support the possibility of an initial deposition of antigens in the renal glomeruli. Direct evidence, however, is still lacking, since the antigens may come from other parts of the cortical kidney tissue (the medulla was removed before homogenization). One question is why we have not been able to detect antigens by direct immunofluorescence. An obvious possible answer is the sensitivity of the techniques used: immunofluorescence, although sensitive, has limited applicability, because of the low concentration of antigens in cryostat sections. In contrast,

homogenates from 35–50 mg of tissue and, especially, eluates prepared from the whole kidney have a higher concentration of antigens. These antigens are concentrated further in the agarose at the zone of precipitation with antibody during the CCI separation. The fact that positive precipitin lines were sometimes obtained only with diluted eluates confirms this situation; neat eluates probably formed complexes in high antigen excess which did not precipitate.

Failure to demonstrate antigens in glomerular lesions by direct immunofluorescence is well documented in several studies on different types of nephropathy, and depends on the kinetics of the development of the lesion. During the early stages the deposited complexes are in antigen excess, and the chance of detecting antigens is much greater. This is true of the early stages of nephropathies associated with acute malaria, such as *P. falciparum* in man (Bhamarapravati *et al.*, 1973), *P. berghei* in mice (Boonpucknavig, Boonpucknavig & Bhamarapravati, 1972; Ehrich & Voller, 1972) and *P. cynomolgi* in Rhesus monkeys (Ward & Conran, 1969), as well as with quartan malaria in man (Houba, 1976). A similar observation was recorded in monkeys infected with *S. japonicum* (Tada *et al.*, 1975). In the later stages of infection, the formation of larger complexes by further binding of antibodies (Lambert *et al.*, 1973), or the covering of complexes by rheumatoid factors or by immunoconglutinins, produced in response to circulating immune complexes (Houba, 1976), makes the detection of antigens more difficult or even impossible. Different techniques of elution from tissue sections of antibodies bound in the lesion have brought partial success in some situations (Natali & Cioli, 1976). The degree of success obviously depends on the technique used; in our experience, elution with citric acid may remove the whole complex from the lesion.

The range of antigens involved in the pathogenesis of renal lesions has not been studied in detail. All the antigens detected in the circulation and urine should be considered as possible candidates. These would include the membrane- and gut-associated antigens of the adult worms, and soluble egg antigens released from living eggs (Houba *et al.*, 1976). Antigens circulating for long periods, such as polysaccharide antigens (Deelder *et al.*, 1976) and those antigens which are excreted in the urine (Carlier *et al.*, 1975), are possibly the most likely candidates. DNA antigens of schistosomal origin have been also implicated in renal pathology (Hillyer, 1971; Hillyer & Lewart, 1974; Hillyer *et al.*, 1975). It is conceivable that parasitic DNA may initiate the lesion, which is then perpetuated by the cross-reactivity of schistosomal DNA with host DNA (Houba, 1976). In this case, the glomerular damage could be due either to depositions of DNA–anti-DNA complexes similar to those in systemic lupus erythematosus (Koffler, Schur & Kunkel, 1967; Nydegger *et al.*, 1974), or to an initial deposition of DNA attributable to its physical affinity for collagen substrates of the glomerular basement membrane (Izui, Lambert & Miecher, 1976).

Another important aspect is the presence of SEA in renal lesions. In the past, the release of SEA from eggs deposited in tissues has mainly been considered in the context of granuloma formation (Warren, 1974). We have shown in a previous communication (Houba, 1976) that SEA released in granulomas in the liver and intestine combines with antibodies, derived either from the circulation in the early stages of infection or from plasma cells present in older granulomas in the later stages. We have suggested that the local formation of immune complexes within the granuloma may explain the diminution of granuloma size, since locally produced antibodies may compete with receptor-bearing T cells for the SEA (Houba, 1976). Most of the locally formed immune complexes are phagocytosed by eosinophils, neutrophils, macrophages and Kupffer cells present in the granuloma: but some of these complexes may escape into the circulation or may be released from these cells later on. They may, therefore, take part in the development of renal lesions. This possibility is supported by the findings of Natali & Cioli (1976), using mice with single sex infections in which no eggs were produced, that the development of 'glomerular immunopathology and proteinuria was drastically reduced', despite the presence of circulating antibodies to adult worm antigens. The observation that female worms do not mature fully in unisexual infections may also be important. The effect of SEA produced by eggs present in kidney is unlikely to be responsible for the development of glomerular lesions, since it was very unusual to find eggs in the kidney: individual eggs were found in the kidneys of only two out of the 103 baboons we examined, and in two out of twelve human patients (Hoshino-Shimizu *et al.*, 1976).

The detection of IgM in all baboon kidney lesions, both mild and severe, associated with *S. mansoni* agrees with findings in mice (Natali & Cioli, 1976) and in man (Hoshino-Shimizu *et al.*, 1976), but contrasts with the serological findings, which show a switch from IgM to IgG antibodies. The persistence of IgM in these lesions may be related to its non-specific deposition. In our baboons, the severe lesions always had IgG deposits and all except one were positive for C3. The negative staining for properdin factor B indicates that complement-induced injury is mediated by the classical pathway. In contrast to observations in man (Hoshino-Shimizu *et al.*, 1976), we have not been able to detect any IgA in baboon specimens and evidence for the deposition of IgE in some animals was equivocal.

Our distinction between mild and severe lesions in *S. mansoni*-infected baboons is worth stressing. Mild lesions, characterized by focal segmental deposits predominantly of IgM, are possibly not associated with loss of renal function. They appear early in the infection and are apparently not otherwise related to intensity or duration. Somewhat similar lesions with IgM were seen in one of the uninfected baboons, and the same has been reported in man (Hoshino-Shimizu *et al.*, 1976). Severe lesions are characterized by diffuse deposits of immunoglobulins, including IgG, complement and antigens alongside glomerular capillary vessels. The pathogenic mechanism involved in their formation, as already discussed, is compatible with the deposition of either pre-formed immune complexes or of antigen followed by the corresponding antibody, producing locally formed complexes. In baboons, they developed only after prolonged heavy infection: a similar finding has been reported in mice (Natali & Cioli, 1976). Failure to distinguish between these two types of lesions could mislead investigators into overlooking a correlation between *S. mansoni* and renal lesions. Thus, Brack *et al.* (1972) concluded that there was no such correlation in baboons.

Although, in our study, they were far less common than mild and, possibly, non-specific lesions, the frequency of severe lesions was still quite high: 6% among all the baboons examined and 17% among the thirty-six baboons with heavy infections of 30 weeks or more duration. Such a prevalence in man would give rise to considerable concern.

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