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Schistosoma mansoni infection in rhesus monkeys: comparison of the course of heavy and light infections *

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The course of Schistosoma mansoni *infection in rhesus monkeys exposed to 100 or 600 cercariae was compared. During the first 12 weeks, the numbers of eggs per worm pair passed in the faeces and retained in the tissues were similar in the two groups. Between weeks 12 and 27, two-thirds of the worms died in monkeys exposed to 600 cercariae, many surviving worms shifted from colonic to small intestinal venules, and the number of eggs per worm pair passed in the faeces decreased. None of these changes occurred in monkeys exposed to 100 cercariae and in these animals the number of eggs passed in the faeces consistently increased after the twelfth week. The findings indicate that study of a single level of infection is not adequate to define the host response, and that the relation between worm burden and the number of eggs in the faeces is considerably influenced by host response.*

Monkeys were also studied 6 and 7 weeks after exposure to 600 cercariae. After the seventh week of infection, inflammatory infiltrates in the colon and portal tracts became much less marked, and the granulomas surrounding mature eggs decreased in size.

Rhesus monkeys (*Macaca mulatta*) exposed to 500–1000 *Schistosoma mansoni* cercariae usually become relatively immune in a period of 3–4 months after infection (Smithers & Terry, 1965; McMullen et al., 1967). Immunity is manifested by a decrease in the number of eggs passed in the faeces, by a decrease in the number of worms, and by resistance to challenge infection. However, Ritchie et al., (1966) noted that two rhesus monkeys exposed to only 50 *S. mansoni* cercariae continued to pass relatively constant numbers of eggs in the faeces for a prolonged period after infection.

Our experiments were designed to study quantitatively the relation between the intensity of infection and the host response, as indicated by the passage of eggs in the faeces and the accumulation of eggs in the tissues after exposure to either 100 or 600 *S. mansoni* cercariae.

MATERIALS AND METHODS

The NIH Puerto Rican strain of *S. mansoni* was used throughout. Eggs in the tissues were counted after digestion of the tissues in 4% potassium hydroxide solution. The eggs in 24-hour stool collections were counted twice each week using a dilution technique. The number of eggs per worm pair passed in the faeces each day was calculated from the number of worm pairs recovered by perfusion at autopsy and the mean number of eggs passed in the 7–9 stool specimens collected in the last month before sacrifice. The details of the techniques are described by Powers & Cheever, 1972.

Monkeys exposed to 600 cercariae were killed 6, 7, 11–14, or 27 weeks after exposure. Monkeys exposed to 100 cercariae were killed either 12 or 27 weeks after exposure. One group exposed to 100 cercariae was challenged with 600 cercariae 13 weeks after the initial exposure, at which time two previously uninfected monkeys were also exposed.

RESULTS

Recovery of adult worms

The mean percentage of cercariae recovered 6–14 weeks after exposure as adult worms varied from 41% to 60%, with no evident relation to the number of cercariae applied. By 27 weeks after

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Table 1. Comparison of worm recovery, egg distribution, and egg numbers in monkeys exposed to either 100 or 600 *S. mansoni* cercariae

Weeks after exposure	Mean worm recovery		Percentage egg distribution			Eggs per worm pair in the tissues (thousands)		Eggs per worm pair per day in faeces	No. of monkeys
	No. of pairs	% [a]	Liver	Small intestine	Colon	Total	Viable		
				Monkeys exposed to 600 cercariae					
6	124	45	37	15	48	3.2	not done	not done	2 [b]
7	130 ± 20	45	34 ± 4	13 ± 6	53 ± 5	4.5 ± 0.7	3.6 ± 0.7	not done	4
11–14	147 ± 30	60	10 ± 4	30 ± 9	59 ± 12	5.7 ± 1.2	2.6 [b]	186 ± 25	6
27	36 ± 16	18	31 ± 11	51 ± 6	18 ± 7	3.9 ± 1.6	2.0 [b]	96 ± 26	4
				Monkeys exposed to 100 cercariae					
12	18 ± 1	41	13 ± 4	23 ± 7	64 ± 8	4.8 ± 0.3	2.2 ± 0.5	128 ± 40	4
27	16 ± 2	43	17 ± 5	27 ± 7	56 ± 7	4.4 ± 0.5	3.1 ± 0.5	279 ± 37	8

[a] Calculated from total worm recovery. In general, 5–10 % of the worms recovered were unpaired, i.e. males or females (immature) in excess of the opposite sex.

[b] Because values were available from only 3 animals in these groups, no standard errors were calculated.

infection, the number of adult worms had decreased greatly in monkeys exposed to 600 cercariae but had not changed in those exposed to 100 cercariae (Table 1).

Number of eggs in the tissues

No difference was noted between lightly and heavily infected animals (Table 1). The number of eggs per worm pair in the tissues reached a plateau very rapidly and did not change markedly thereafter. By the sixth week after exposure, 3 200 eggs per worm pair were present in the tissues, and by the seventh week 4 600 eggs per worm pair were found. The mean values between 12 and 27 weeks after exposure were between 4 400 and 4 800 eggs per worm pair.

The concentration of eggs in the tissues (Table 2) varied between tissues and with the intensity of infection. The weights of the liver, spleen, small intestine, and colon were uniformly increased, but no relation to the intensity or duration of infection was evident.

Table 2. Concentration of eggs in the tissues of monkeys

No. of weeks after exposure	No. of cercariae	Body weight (kg)	No. of worm pairs per kg of body weight	Average no. of eggs per gram (thousands)				Organ weights as % of body weight				No. of monkeys
				Liver	Lungs	Small intestine	Colon	Liver	Spleen	Small intestine	Colon	
6	600	2.6	48	1.64	0.02	0.56	2.21	4.00	ND [a]	5.00	3.74	2
7	600	4.2	31	1.35 ± 0.18	0.03	0.90 ± 0.43	2.37 ± 0.61	3.52	0.28	2.75	4.42	5
11–14	600	3.3	44	0.57 ± 0.13	0.10	2.39 ± 0.58	4.60 ± 1.25	3.81	0.19	2.56	3.71	6
27	600	3.3	11	0.31 ± 0.19	0.01	0.65 ± 0.38	0.24 ± 0.14	3.67	0.13	3.29	3.87	4
12	100	2.5	7	0.12 ± 0.04	0.00	0.30 ± 0.10	0.42 ± 0.14	3.64	0.19	4.15	4.14	4
27	100	2.7	6	0.10 ± 0.02	0.00	0.25 ± 0.07	0.51 ± 0.11	3.81	0.21	3.17	3.01	8

[a] ND = not done.

Distribution of eggs in the tissues

In short-term infections, most eggs were in the colon, but by 27 weeks after exposure to 600 cercariae there was a marked change, eggs being most numerous in the small intestine (Table 1). This shift did not occur in monkeys exposed to only 100 cercariae, and in these animals most eggs were found in the colon at all intervals after exposure.

Passage of eggs in the faeces

During the first 3 months after infection, the number of eggs per worm pair passed in the faeces each day was similar in monkeys exposed to either 100 or 600 cercariae and averaged about 200 eggs per worm pair per day (Fig. 1). Thereafter, the number of eggs passed in the faeces of lightly infected monkeys increased to about 300 eggs per worm pair per day and remained at this level until termination of the experiment 6 months after exposure. In contrast, the number of eggs in the faeces of heavily infected monkeys fell to an average of 110 eggs per worm pair per day. The absolute decrease in passage of eggs in the faeces of heavily infected monkeys was much greater than that indicated in Fig. 1, since many worms died between 3 and 6 months after infection in these animals.

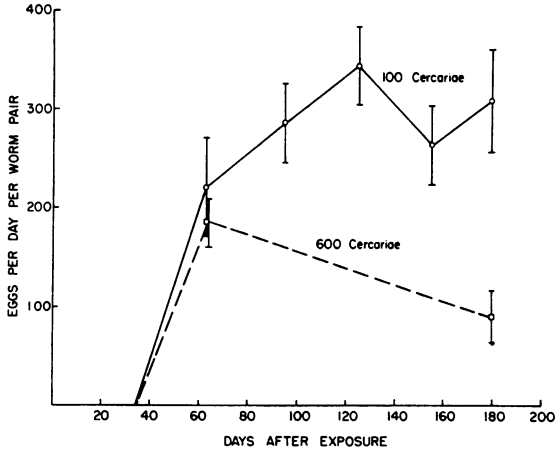


Fig. 1. Passage of eggs in the faeces at different intervals after exposure. Each point is a mean value calculated from 7 faecal collections on each of 6–8 monkeys. Thus the points shown at 65 days represent the mean values for collections between 50 and 80 days. Since many worms died in monkeys exposed to 600 cercariae, the number of eggs per worm pair is given only for those collections made during the 30 days prior to sacrifice of monkeys at about 12 and 27 weeks after infection. Intervening values were calculated for monkeys exposed to 100 cercariae, as the number of worms present in these animals did not change during the course of the experiment. The vertical lines indicate ±1 standard error of the mean.

Pathological changes

The gross and microscopic lesions were more marked in animals exposed to 600 cercariae, but the lesions were qualitatively similar in lightly and heavily infected monkeys. No jaundice, subcutaneous oedema, or ascites were seen. The colonic serosa was studded with firm 0.1–0.3-cm nodules, usually most numerous in the transverse colon. The mucosal surface of the intestine showed variable numbers of 0.1–0.2-cm petechial haemorrhages. No ulceration and no areas of marked fibrosis were seen. The small intestine was normal except for the presence of occasional petechiae. The mesenteric lymph nodes were enlarged and soft. The liver was moderately enlarged (Table 2) and grey-brown. Numerous granulomas were conspicuous on the external and cut surfaces in monkeys killed 6–7 weeks after exposure, but in animals killed more than 10 weeks after infection no granulomas, or only small numbers of tiny granulomas, were evident macroscopically. The spleen was slightly grey and enlarged and the follicles were prominent. No consistent changes related to infection were noted in the other organs.

Microscopic examination of the intestines showed variable numbers of eggs in the mucosa and eggs and granulomas in the submucosa, muscular layers, and serosa. There was moderate oedema and minimal fibrosis of the colonic submucosa (Fig. 2), which was approximately twice as thick as the submucosa of normal monkeys subjected to similar perfusion of the portal system. Phlebitis of intestinal venules was seen occasionally in infected animals but not in the six uninfected monkeys examined. Hyperplastic mesenteric lymph nodes only occasionally contained small numbers of eggs and granulomas.

Inflammatory lesions of the intrahepatic portal branches (pylephlebitis) were seen in approximately half of the animals in both the lightly and heavily infected groups. In the first type, seen only in monkeys exposed to 600 cercariae, the vein was entirely occluded by leucocytes, predominantly eosinophils (Fig. 3). Lymphocytes, macrophages, and giant cells were present in variable numbers. A delicate network of collagen fibres between the intraluminal cells was seen in sections stained with

Masson's trichrome. The inflammatory infiltrate usually extended through the vein wall (Fig. 3) and was continuous with the infiltrate in the portal space. The vein wall and elastic lamina remained intact. Remnants of dead worms were identified in these veins, or in veins elsewhere in the same liver, in two-thirds of the monkeys showing such lesions (Fig. 4 and 5). Occasional recanalized occlusive lesions were seen. Eggs were seldom present in the occluded veins. Infarcts of the parenchyma were not present. Necrosis of individual liver cells was seen occasionally in areas where the inflammatory cells infiltrated between liver cells, but more frequently the inflammatory cells were confined to the portal spaces.

The second type of pylephlebitis was "mural," characterized by involvement of portions of the wall of the vein, and most frequently involved vessels 200–600 μm in diameter, although smaller veins also showed similar lesions, and the large extrahepatic portal branches were involved in one monkey. The least severely affected veins showed a thickened, oedematous endothelium containing small numbers of lymphocytes, macrophages, and eosinophils (Fig. 6). With more marked involvement, the mass of inflammatory cells was often 100–200 μm thick. Plump endothelial cells covered the surface of such lesions. Where the endothelial thickening was focal, the lesions had a polypoid appearance. In what appeared to be later stages of the same lesions, there was slight endothelial fibrosis, moderate numbers of capillaries were present (Fig. 7), and plasma cells were present in large numbers. In all of these lesions, the most marked involvement was endothelial, but inflammatory infiltrates often extended through the vein wall. Thrombosis of veins was never seen.

Pylephlebitic lesions were not seen in the 6 monkeys killed 6 or 7 weeks after infection. Pylephlebitis was most frequent and most marked from 11 to 14 weeks after infection in monkeys exposed to 600 cercariae. Less extensive mural lesions were present in monkeys exposed to 100 cercariae and killed 12 or 27 weeks after exposure and lesions of both types were found in those exposed to 600 cercariae and killed 27 weeks after infection. The pylephlebitic lesions were qualitatively similar in monkeys killed 11–27 weeks after infection, i.e., no evident progression of lesions was seen. Pylephlebitic lesions were not seen in uninfected monkeys.

Portal inflammation was moderate 6 weeks after exposure and very marked 7 weeks after exposure to 600 cercariae. By 11–14 weeks, portal infiltrates were less marked and by 27 weeks after exposure were much diminished. There was moderate inflammation around large (200–500 μm) veins 49 days after exposure, but these portal spaces contained few inflammatory cells in the remaining monkeys, except when local pylephlebitis was seen. Along the terminal portal triads, where inflammation was most conspicuous, the leucocytes infiltrated between periportal parenchymal cells. Eosinophils comprised about half the cells present in the portal infiltrates 6–7 weeks after exposure, but thereafter an increasing proportion of lymphocytes, plasma cells, and large mononuclear cells was present. Monkeys exposed to 100 cercariae showed only slight to moderate portal infiltrates.

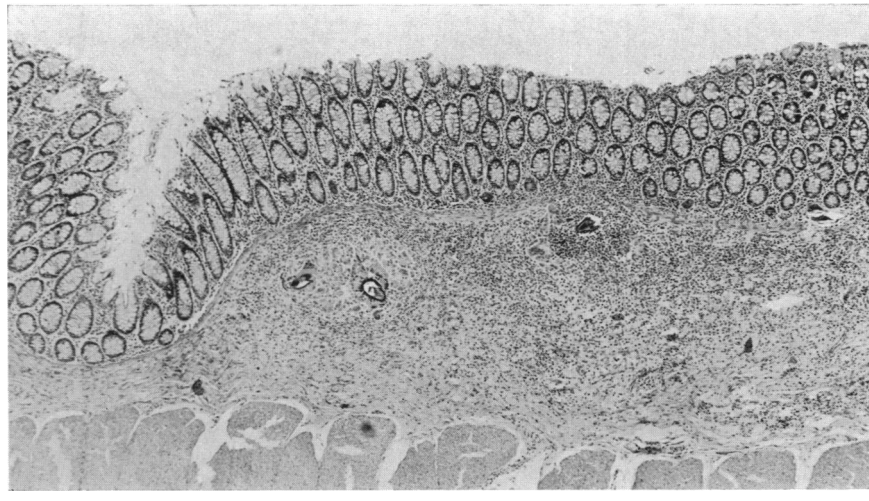
Portal fibrosis was seldom seen. When present it was predominantly periductal and did not differ in

Fig. 2. Several eggs are present in the colonic submucosa, which is thickened, oedematous, and infiltrated by eosinophils, lymphocytes, and plasma cells. There is also moderate fibrosis (× 40).

Photomicrographs of pylephlebitic lesions. Unless otherwise noted, the photomicrographs (sections stained with haematoxylin and eosin) are all taken from monkeys killed 12 weeks after exposure to 600 cercariae of the Puerto Rican strain. However, since the strain of worm used did not influence the nature of the lesions, material from monkeys infected with schistosome strains from Belo Horizonte, Brazil, and from St Lucia has sometimes been used to illustrate the lesions.

Fig. 3. Occlusive pylephlebitic lesion in a portal branch 1000 μm in diameter. The central portion of the lesion is composed of eosinophils and the peripheral portion by a mixture of macrophages and eosinophils. Arrows indicate the vein wall (× 65).

Fig. 4. Occlusive pylephlebitic lesion containing a remnant of a dead worm (arrow) in a portal branch 500 μm in diameter. Monkey 12 weeks after exposure to 600 cercariae of Belo Horizonte strain (× 160).



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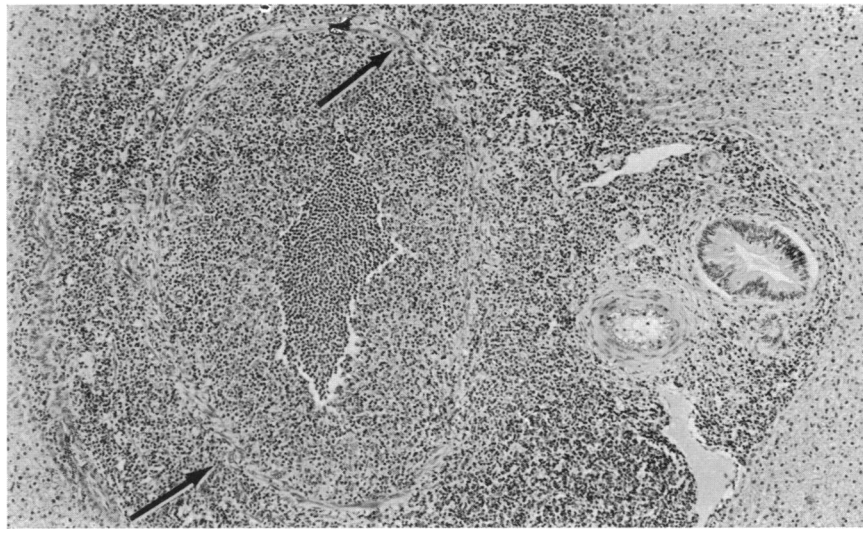

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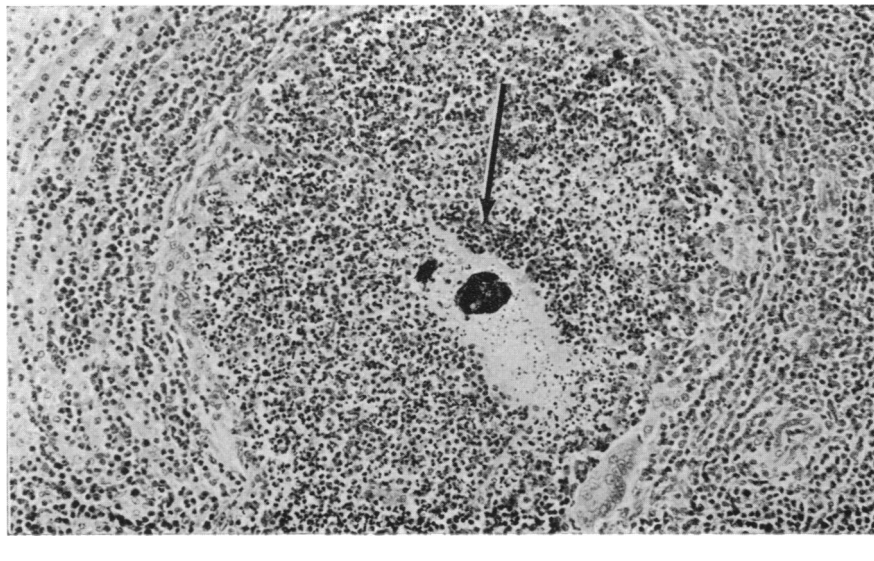

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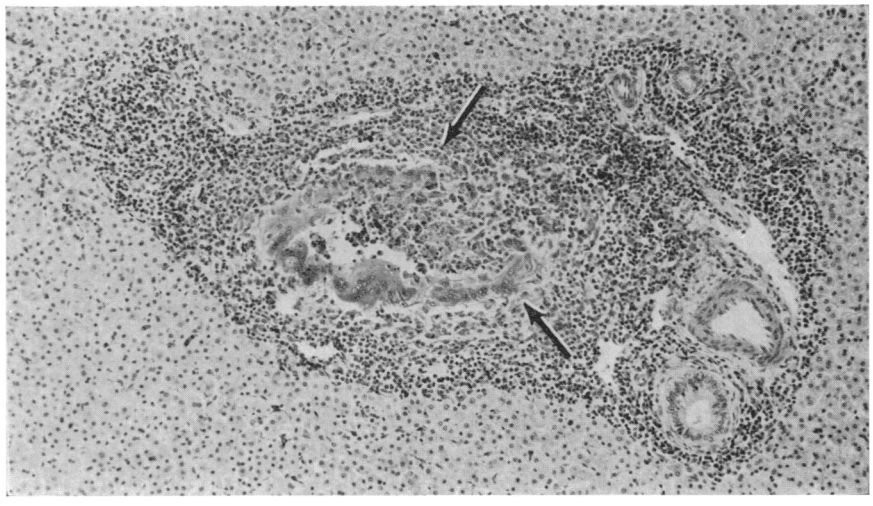

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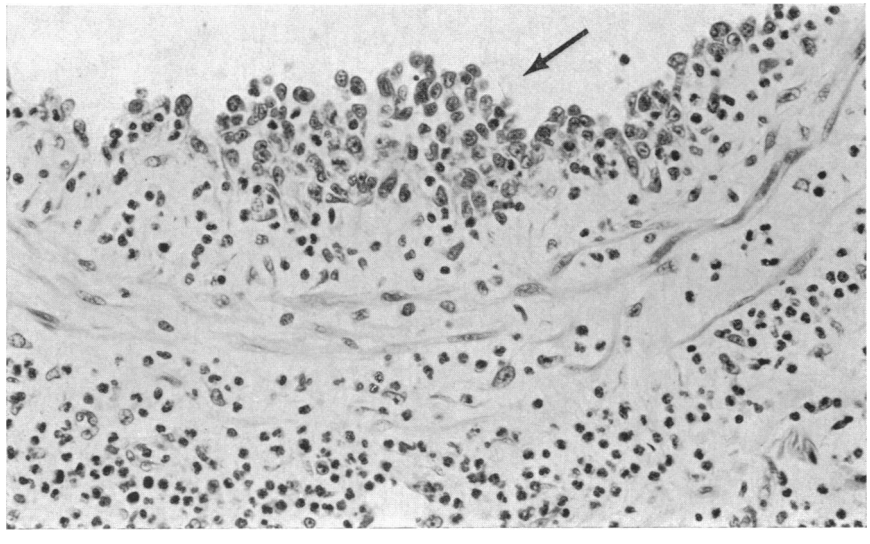

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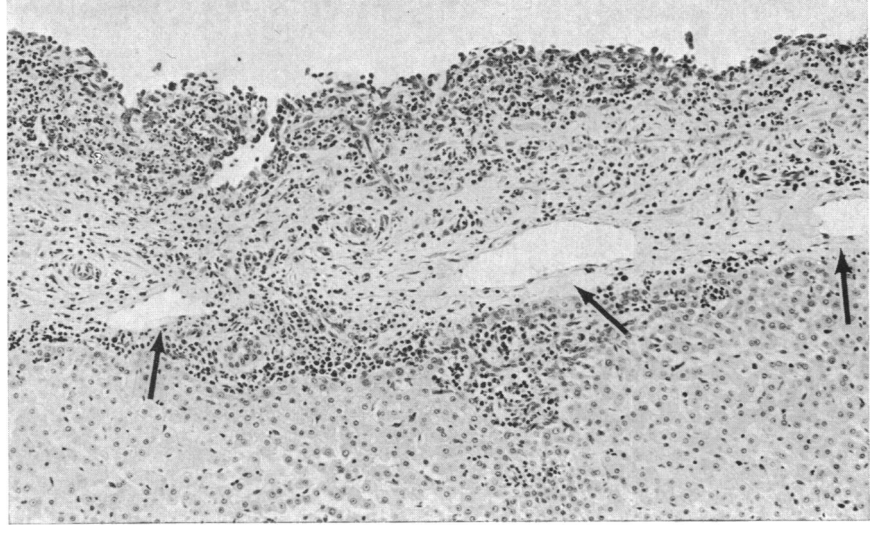

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Table 3. Results of challenge of rhesus monkeys with 600 *S. mansoni* cercariae 12 weeks after an initial exposure to 100 cercariae

No. of weeks between initial exposure and sacrifice	No. of weeks between challenge exposure and sacrifice	Worm recovery (%)	Percentage egg distribution			No. of worm pairs	Eggs per worm pair in the tissues (thousands)		Eggs per worm pair per day in the faeces	No. of monkeys	Monkey no.
			Liver	Small intestine	Colon		Total	Viable			
27	0	54	12	29	56	17	3.95	3.28	240	4	11 b, e
27	14	35	4	23	73	127	5.15	3.69	140	1	11 a
27	14	32	62	26	12	107	1.09	0.46	22	1	11 f
27	14	49	20	44	36	128	5.00	4.11	137	1	11 g
27	14	8	26	62	12	25	2.25	1.44	180	1	11 i
0	14	44	15	52	33	82	3.30	2.22	159	2	12 a, b

frequency and intensity from that seen in uninfected animals.

The granulomas surrounding eggs containing mature miracidia were measured in all animals. The average diameter of such granulomas in the liver increased from 320 μm to 405 μm between the sixth and seventh weeks after infection and decreased thereafter to 255 μm at 27 weeks. A similar pattern was noted in granulomas in the intestinal submucosa. The diameters of these granulomas averaged about 30 μm less than those of hepatic granulomas. The granulomas in monkeys exposed to 100 cercariae were similar in size to those in animals exposed to 600 cercariae. The Hoeppli phenomenon (Hoeppli, 1932) was frequently seen surrounding eggs in the colonic submucosa 6–7 weeks after infection and was less frequently noted 11–14 weeks after exposure. The Hoeppli phenomenon was not seen in monkeys exposed to 100 cercariae or in monkeys killed more than 14 weeks after exposure.

Dead worms were seen in the lungs of one monkey infected for 27 weeks. The lungs of the remaining animals were unremarkable except for the presence of scattered granulomas in a few animals, and frequent lessions associated with lung mites. No schistosomal pulmonary arteritis was present.

Lesions unrelated to the schistosome infection included frequent submucosal nodules in the colon, which contained nematode larvae, presumably *Oesophagostomum* sp. A spirurid larva was seen in the oesophageal submucosa of one monkey and cysticerci were present in two. Protozoa resembling *Sarcocystis* sp. were noted twice in skeletal muscle, which was examined only sporadically.

Immunity to challenge infection

When monkeys initially exposed to 100 cercariae were challenged with 600 cercariae 12 weeks later, nearly every conceivable response was observed. In two monkeys (11a and 11g, Table 3) most worms of the challenge infection matured, and the number of eggs in the faeces and tissues was as expected for the

Fig. 5. A second occlusive pylephlebitic lesion in the same monkey. No dead worm is evident, but the "U" shaped group of giant cells (arrows) apparently outlines the space formerly occupied by a dead male schistosome. Except for extension of the periportal inflammatory infiltrate into the parenchyma, the hepatic cells are not affected (× 100).

Fig. 6. Early mural endophlebitis in a portal branch 500 μm in diameter. There is marked oedema. Most of the cells on the luminal surface of the lesion appear to be plump endothelial cells (arrow). Occasional eosinophils are also present (× 250).

Fig. 7. Recanalizing channels (arrows) are seen in the thickened endothelium of a portal branch 1000 μm in diameter. Active endophlebitis also persists (× 100).

number of worms present. In a third monkey (11f), a moderate number of worms from the challenge infection were present, but the number of eggs in the faeces (22 eggs per worm pair per day) and in the tissues (1 090 eggs per worm pair) was greatly reduced. The female worms recovered from this monkey were only half as long as those recovered from the other animals. The fourth monkey (11i) was apparently completely resistant to challenge and the number of worms recovered was that expected from the first infection alone.

DISCUSSION

The behaviour of *S. mansoni* infections in rhesus monkeys was similar during the first 12 weeks after exposure to either 100 or 600 cercariae, i.e., the development of the worms in the monkeys, the passage of eggs in the faeces, and the accumulation of eggs in the tissues were in proportion to the intensity of infection. Between 12 and 27 weeks after infection, about two-thirds of the worms died in monkeys exposed to 600 cercariae, while the number of worms did not change in monkeys exposed to 100 cercariae. The number of eggs per worm pair passed in the faeces of the heavily infected monkeys decreased, while a statistically significant increase in the number of eggs per worm pair in the faeces was seen in the lightly infected monkeys. The increase in egg passage after the twelfth week of infection was entirely unexpected and remains unexplained, but in each of two experiments using 4 monkeys, a similar increase was observed. Under the conditions of these experiments, the worm burden could be predicted only within fairly broad limits by the counting of eggs in the faeces, as the number of eggs per worm pair passed in the faeces was influenced considerably by the difference in host response to light and heavy infections. Ritchie et al. (1966) have previously noted this phenomenon. Resistance to reinfection (Smithers & Terry, 1965), is apparently less critically dependent on the intensity of infection than is the course of the primary infection.

The number of eggs in the tissues reached a level of 4 500 per worm pair 7 weeks after exposure and did not change thereafter. The short period required for the development of this steady state reflects the very rapid rate of egg destruction in monkey tissues (Cheever & Powers, 1971). With the great differences in the number of eggs per worm pair passed in the faeces, it is surprising that similar differences in the number of eggs per worm pair in the tissues are not seen. Variations in the rate of egg destruction in the tissues or differences in the proportion of eggs passed in the faeces may be responsible.

Pathological changes were related to the intensity of infection but were of a similar nature in heavily and lightly infected monkeys. Inflammatory lesions of the colonic submucosa and serosa were the most conspicuous lesions, grossly and microscopically, and in our experience the extent of colonic involvement has been the most important factor in the production of clinical illness in the monkeys. Phlebitic lesions of the intrahepatic portal branches were seen frequently. The venules occluded by leucocytes resembled lesions produced by dead worms in monkeys (Hsü et al., 1970; Striebel, 1969; Cheever & Powers, 1971). Remnants of dead worms were frequently seen in the livers of animals with this type of phlebitic lesion. However, these occlusive phlebitic lesions were often more frequent than could be accounted for by the number of worms that could possibly have died in the animals in question. Eggs were rarely found in either occlusive or mural pylephlebitic lesions. Thus the pathogenesis of most pylephlebitic lesions is not clear, although some are definitely related to the death and destruction of worms. The mural lesions may represent a healing stage of the occlusive lesion, or the occurrence of such a lesion in a vein too large to be occluded by the process. Neither type of lesion was associated with thrombosis and the lesions apparently resolved without residual damage. The pylephlebitic lesions were similar in many ways to those described by Koppisch (1937) in rabbits infected with large numbers of *S. mansoni*, and phlebitic lesions have been described in association with dead worms in many other hosts infected with *S. mansoni* or other schistosome species (McCully & Kruger, 1969). However, we would emphasize that most of the lesions in our monkeys cannot be interpreted as the remnants of lesions about dead worms.

Inflammatory lesions, both in the colon and in the liver, were much more conspicuous during the sixth and seventh weeks of infection than they were later. The size of the granulomas surrounding mature eggs also reached a maximum at 7 weeks and decreased thereafter. In this regard the sequence of events was similar to that described in mice (Andrade & Warren, 1964; Cheever, 1965; Domingo & Warren, 1968; Anderson & Cheever, 1972). The hyperreactivity of monkeys in the first weeks after the beginning of oviposition may be related to the acute toxaemic phase of schistosomiasis in man (Bogliolo, 1964).

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RÉSUMÉ

INFECTION À *SCHISTOSOMA MANSONI* CHEZ LE SINGE RHÉSUS: ÉVOLUTION COMPARÉE CHEZ DES ANIMAUX FORTEMENT OU FAIBLEMENT PARASITÉS

On a étudié quantitativement les rapports entre l'intensité d'une infection à *Schistosoma mansoni* et la réponse de l'hôte parasité. A cet effet, des singes rhésus ont été exposés à 600 cercaires de *S. mansoni* et sacrifiés après 6, 7, 11-14 et 27 semaines; d'autres, exposés à 100 cercaires, ont subi le même sort après 12 et 27 semaines.

Durant les 12 premières semaines, le nombre d'œufs éliminés dans les selles, par paire de vers et par jour, a été sensiblement le même chez les animaux des deux groupes, et les nécropsies pratiquées à l'issue de cette période ont montré la présence de quantités similaires d'œufs dans les tissus. Entre la 12e et la 27e semaine, deux tiers environ des vers adultes sont morts chez les animaux fortement parasités; on a noté simultanément une migration des vers survivants des veines du côlon vers les veines de l'intestin grêle et une diminution du nombre d'œufs excrétés qui est passé de 186 à 96 par paire de vers et par jour. Par contre, chez les singes exposés à 100 cercaires et suivis jusqu'à la 27e semaine, on n'a constaté aucun signe de mort des vers et aucune migration; le nombre des œufs excrétés est passé de 200 à 300 par paire de vers et par jour entre la 12e et la 27e semaine. Il n'y avait aucun lien apparent entre les proportions relatives d'œufs présents dans le côlon et dans l'intestin grêle et l'élimination des œufs dans les selles,

Les lésions macroscopiques et microscopiques, bien que plus marquées chez les singes exposés à 600 cercaires, ont été qualitativement similaires dans les deux groupes d'animaux. L'infiltration inflammatoire de la sous-muqueuse du côlon et du hile hépatique était beaucoup plus intense à la 7e qu'à la 11e et à la 27e semaine. La taille des granulomes renfermant des œufs mûrs observés dans le foie et dans la sous-muqueuse intestinale à décru après la 7e semaine. Des lésions inflammatoires des branches intrahépatiques de la veine porte (pyléphlébite) étaient présentes chez la moitié environ des animaux sacrifiés après la 11e semaine. L'atteinte de la paroi des grosses veines était fréquente; chez les singes exposés à 600 cercaires, les petites veines étaient souvent obstruées par des leucocytes. Dans de nombreux cas, on a trouvé des vers morts dans le foie des animaux présentant des lésions veineuses occlusives, mais la pathogénie de ces dernières n'est pas complètement élucidée.

REFERENCES

Anderson, L. A. & Cheever, A. W. (1972) *Bull. Wld Hlth Org.*, **46**, 233-242

Andrade, Z. A. & Warren, K. S. (1964) *Trans. roy. Soc. trop. Med. Hyg.*, **58**, 53-57

Bogliolo, L. (1964) *G.E.N. (Caracas)*, **19**, 157-236

Cheever, A. W. (1965) *Amer. J. trop. Med. Hyg.*, **14**, 211-226

Cheever, A. W. & Powers, K. G. (1969) *Ann. trop. Med. Parasit.*, **63**, 83-93

Cheever, A. W. & Powers, K. G. (1971) *Amer. J. trop. Med. Hyg.*, **20**, 69-76

Domingo, E. O. & Warren, K. S. (1968) *Amer. J. Path.*, **52**, 369-379

Hoeppli, R. (1932) *Chin. med. J.*, **46**, 1179-1186

Hsü, S. Y. Li, Davis, J. R., Hsü, H. F., Mergner, W., & Lust, G. L. (1970) *Amer. J. trop. Med. Hyg.*, **19**, 785-791

Koppisch, E. (1937) *Puerto Rico J. publ. Hlth.*, **13**, 1-54

McCully, R. M., & Kruger, S. P. (1969) *Onderstepoort J. vet. Res.*, **36**, 129-162

McMullen, D. B., Ritchie, L. S., Oliver-Gonzales, J., & Knight, W. B. (1967) *Amer. J. trop. Med. Hyg.*, **16**, 620-627

Powers, K. G. & Cheever, A. W. (1972) *Bull. Wld Hlth Org.*, **46**, 295

Ritchie, L. S., Knight, W. B., McMullen, D. B., & Lichtenberg, F. (1966) *Amer. J. trop. Med. Hyg.*, **15**, 43-49

Smithers, S. R. & Terry, R. J. (1965) *Parasitology*, **55**, 701-710

Striebel, H. P. (1969) *Ann. N.Y. Acad. Sci.*, **160**, 491-518