PARASITOLOGICAL AND HISTOPATHOLOGICAL STUDIES ON RHESUS MONKEYS INFECTED WITH CHINESE MAINLAND STRAIN OF *SCHISTOSOMA JAPONICUM*

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Abstract. Fifteen rhesus monkeys were infected by cutaneous exposure each with 200 or 300 cercariae of *Schistosoma japonicum*. The dynamic distribution of schistosomula in the skin showed that 77-90% of them were found in the connective tissue, while 10-23% migrated in the hair follicles and sebaceous glands at different time intervals after cercarial penetration. Dead schistosomula recovered from the skin varied from 8.7% to 28.7%. The average rate of adult worm recovery was 74.4% and 61.3% in the 6th and 15th weeks of infection, thereafter the rate decreased to 32.3% and 9.0% in the 19th and 42nd weeks, respectively. The mean length of mature pair-worms was 13.2 ± 2.3 mm in male and 18.0 ± 1.9 mm in female 6 weeks of worm age. Afterwards the body length of females and their sexual gland diminished markedly. The mean prepatent period was 35.0 ± 0.6 days. The average size of mature eggs in the feces was $86.6 \pm 5.4 \times 64.3 \pm 3.6$ μm, and the peak of eggs passage in the feces occurred between 7th and 15th weeks after infection, later on the number of eggs markedly decreased. Skin reaction to the primary infection was slight. The pathological changes observed in liver were chiefly cellular infiltration of portal spaces and the lesions produced by egg granulomas. The mean volume of single-egg granulomas of the productive stage in liver was 22.7 ± 10.5 mm$^3 \times 10^{-3}$. The most intensive damages in the gastro-intestinal tract were observed in the large intestine.

The results showed that rhesus monkey is a permissive host for the Chinese mainland strain of *S. japonicum* and spontaneous recovery occurred due to gradual disappearance of the worms at later stages.

INTRODUCTION

In the field of bio-medical science research, the rhesus monkey *(Macaca maulatta)* is a common and important laboratory non-human primate. Although rhesus monkeys infected with *Schistosoma japonicum* of Japanese and Taiwan Province strains have been studied extensively, little information is available about the Chinese mainland strain (Hsü and Hsü, 1956, 1960; Hsü *et al,* 1962, 1969; Davis *et al,* 1963; Cheever *et al,* 1974).

The present study was undertaken to obtain fundamental data on the rhesus monkey in the course of infection with the Chinese mainland strain of *S. japonicum* under laboratory conditions.

MATERIALS AND METHODS

Infection and autopsy of animals

The rhesus monkeys were bought from Guangxi Zhuang Autonomous Region, China. Repeated fecal examinations prior to exposure did not reveal schistosome infections in any of the animals used. Nine normal monkeys were artificially infected by cutaneous exposure for each animal to 200 *S. japonicum* cercariae obtained from pools of artificially infected *Oncomelania hupensis* from Anhui Province, at the lower reaches of the Yangtze River. The animals were sacrificied 6, 15, 19, 32 and 42 weeks post-infection and portal perfusion of infected animals was performed by the conventional method in our Institute. Worms were collected and counted. In addition, 6 monkeys were used for study of the dynamic distribution of schistosomula in skin. A small area of abdominal shaved skin infected with 300 cercariae was taken at different time intervals of 0.5, 2, 6, 24, 48, 72 and 96 hours post-infection from each animal. Recovery of schistosomula from skin followed the method described by Clegg and Smithers (1968) and the schistosomula were stained by 0.05% neutral red-potassium hydroxide to determine

their viability (He *et al,* 1985).

Observations on morphology

The collected worms were fixed in 5% neutral formalin and the body length of pair-worms was measured. Subsequently, they were stained with acid carmin and mounted *in toto.* Under the light microscope, male worms were observed for the number of testes and female worms for the size of ovary. As the ovary is oval shaped, ovary index is defined as the length × width (mm) × 1000. The number of eggs in the uteri of females was examined.

Fecal examination

For the determination of the prepatent period, the animals were separated individually from the 32nd day after exposure onward. Feces passed by each animal were collected daily and hatching method for miracidia of *S. japonicum* was carried out. The day prior to the hatching of miracidia was regarded as the last day of the prepatent period. Examinations of eggs per gram of feces (EPG) were made at the intervals of 7, 11, 15, 19, 23, 27, 31, 35 and 39 weeks after infection according to the method described by Ho (1963). Two hundred mature eggs from the feces on 41 days after infection were measured.

Histological examination

Histological sections were prepared from skin biopsies and various internal organs of animals taken or sacrified at different time intervals. They were fixed in 10% formalin, serial paraffin sections were made and stained with hematoxylin-eosin. The developmental stages of egg granulomas in liver were divided as previously described by Hsü *et al* (1972). The productive stage of egg granulomas containing a single mature egg were measured. The volume of each granuloma was calculated by the formula $V = \pi AB^2/6$, in which A and B stand for the two perpendicular axes.

RESULTS

Dynamic distribution and recovery rate of schistosomula in skin

The numbers of schistosomula recovered from the skin at different time intervals after infection are shown in Table 1. In general, the recovery rate of schistosomula in skin of the rhesus monkey following 0.5-24 hours post-infection was higher than that from 48-72 hours. However, 96 hours after infection, schistosomula can no longer be detected in their skin. Our results indicated that some schistosomula just transformed from cercariae die during peretration into the skin of the rhesus monkey; the average percentage of dead schistosomula per animal varied from 8.7% to 28.7% according to the different time intervals after infection.

The dynamic distribution of schistosomula in skin at different time intervals after cercarial penetration in rhesus monkey is shown in Table 2. Approximately 77-90% of the schistosomula were found in the connective tissue, while 10-23% migrated in the sebaceous glands and hair follicles during their early migrating in skin at different time intervals after cercarial penetration.

Recovery of adult worms

Worm recovery rate of rhesus monkeys infected with *S. japonicum* at different time intervals is shown in Fig 1. 74.4% of cercariae applied were recovered as adult worms 6 weeks after infection. By the 19th week the mean percentage of adult worms decreased greatly to 32.3%. By the 32-42nd week after infection, worm recovery averaged only 7.0-9.0%.

Size of worms and development of sexual glands

The mean length of mature pair-worms of both sexes was 13.2 ± 2.3 mm in male and 18.0 ± 1.9 mm in female for the 6th week of infection. The average ovary index in females was 100.0 ± 17.4 and the average number of ova present in their uteri was 158.2 ± 77.5. However, on the 19th week, the body length of females diminished markedly and their average ovary index and number of uterine ova also reduced greatly (Table 3).

Size and shape of eggs

The size of mature eggs of *S. japonicum* in the feces of rhesus monkeys on day 41 post-infection was 86.6 ± 5.4 × 64.3 ± 3.6 μm, and the index of egg which defined as the ratio of egg width/length × 100 was 74.6 ± 5.9.

Table 1

Recovery of *S. japonicum* schistosomula from skin of rhesus monkey at different time intervals after cercarial infection.

Time post-infection (hours)	Total no. cercariae infected	Total no. schistosomula recovered	%	Average number of schistosomula recovered per monkey			
				live		dead	
				$\bar{X} \pm SD$	%	$\bar{X} \pm SD$	%
0.5	1800	496	27.6	70.7 ± 25.9	85.6	11.9 ± 9.4	14.4
2	1200	238	19.8	53.9 ± 33.1	90.6	5.6 ± 2.1	9.4
6	1200	240	20.0	53.8 ± 11.9	89.7	6.2 ± 3.4	10.3
24	1800	278	15.4	42.2 ± 15.7	91.3	4.0 ± 1.9	8.7
48	1200	115	9.6	20.6 ± 10.5	71.3	8.3 ± 6.3	28.7
72	300	2	0.7	0.4 ± 0.6		0	
96	300	0	0	0		0	

Table 2

Dynamic distribution of *S. japonicum* schistosomula in skin of rhesus monkey after cercarial infection.

Time post-infection (hours)	Total no. schistosomula found	No. schistosomula in connective tissue	%	No. schistosomula in sebaceous gland	%	No. schistosomula in hair follicle	%
0.5	173	156	90	3	2	14	8
2	162	125	77	15	9	22	14
6	137	115	84	9	7	13	9
24	90	79	88	3	3	8	9
48	41	36	88	1	2	4	10
72	0	0	0	0	0	0	0
96	0	0	0	0	0	0	0

Table 3

Body length and sexual glands in pair-worms of *S. japonicum* from rhesus monkey.

Age of worms (weeks)	No. measured (pairs)	Male		Female		
		Length (mm) $\bar{X} \pm SD$	With 7 testes (%)	Length (mm) $\bar{X} \pm SD$	Ovary index* $\bar{X} \pm SD$	No. egg in uterus $\bar{X} \pm SD$
6	112	13.2 ± 2.3	77.2	18.0 ± 1.9	100.0 ± 17.4	158.2 ± 77.5
19	10	13.0 ± 1.7	90.0	13.0 ± 2.0	50.2 ± 7.9	35.1 ± 47.8

* Ovary index is defined as the length × width (mm) × 1000

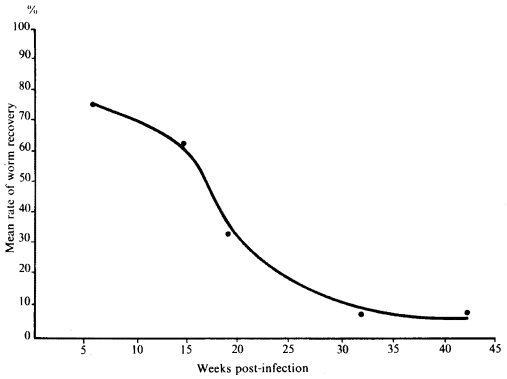

Fig 1—Worm recovery rate of rhesus monkeys at different time intervals after *S. japonicum* infection.

Prepatent period and duration of egg passage in the feces

The mean prepatent period of *S. japonicum* in rhesus monkey was 35.0 ± 0.6 days. The results of the passage of eggs in the feces of rhesus monkeys during various intervals after infection are presented in Fig 2. It was shown that during the first 7-15 weeks after infection numerous eggs were deposited in their feces and averaged about 100-200 EPG. Thereafter, the number of eggs passed in the feces markedly decreased to about 30 EPG on 19 weeks after infection and remained about 10 EPG level until termination of the experiment 39 weeks after exposure.

Histopathological changes

Skin: Penetrating schistosomula were more numerous in the epidermis than the dermis until 48 hours post-infection. In general, the dermal response produced in rhesus monkey by the invading schistosomula in primary infection was slight. 0.5-6 hours post-infection, the majority of schistosomula were found within the tunnel in the stratum Malpighii (Fig 3). No cellular infiltration could be seen in the surrounding of schistosomula in the epidermis, however a slight hyperemia and exudation were encountered in the dermis. 24-48 hours post-infection, edema became prevalent and some neutrophil infiltration surrounded the vessels of the dermis. After 72 hours the perivascular infiltration subsided gradually. At 96 hours the skin was essentially normal.

Liver: The liver was moderately enlarged and greyish-brown. Numerous granulomas were conspicuous on the external and cut surfaces in monkeys killed 6 weeks after exposure. Microscopic examination of the liver showed chiefly cellular infiltration of portal spaces and the lesions produced by egg granulomas. Inflammatory lesions of the intrahepatic portal branches (pylephlebitis) were seen in all of the animals (Fig 4). The productive stage of granuloma containing single egg (Fig 5) were measured. The average volume of such granulomas in the liver was $22.7 \pm 10.5\ mm^3 \times 10^{-3}$. The Hoeppli phenomenon was frequently seen surrounding eggs in the liver. At 19 weeks after exposure, many egg lesions were still in their productive stage but some were in the involutional stage. Most of the egg lesions had well-formed giant cells (Fig 6). In certain cases, giant cells were seen in the process of invading the egg. Some calcified eggs were also seen. At 32 weeks, most egg lesions were in the involutional stage and many were calcified. There was a total reduction in the number and size of remnant egg granulomas.

Intestines: The most intensive damages in the gastro-intestinal tract were produced in the large intestine. The histopathological changes of the intestines seemed to be due mainly to deposited eggs. Microscopic examination of the intestines showed variable numbers of eggs in the mucosa and eggs and granulomas in the submucosa and muscular layers (Fig 7). There was moderate edema and minimal fibrosis of the submucosa in

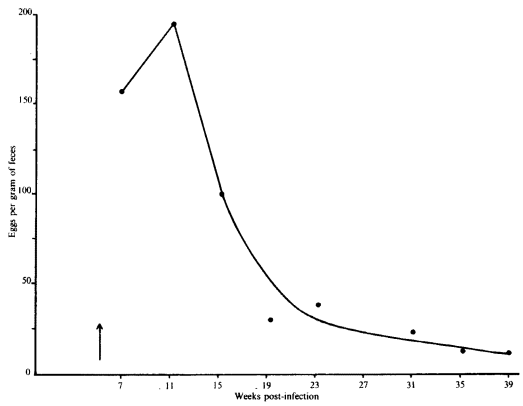

Fig 2—Passage of eggs in the feces of rhesus monkeys after *S. japonicum* infection. Arrow indicates the day of beginning to pass eggs.

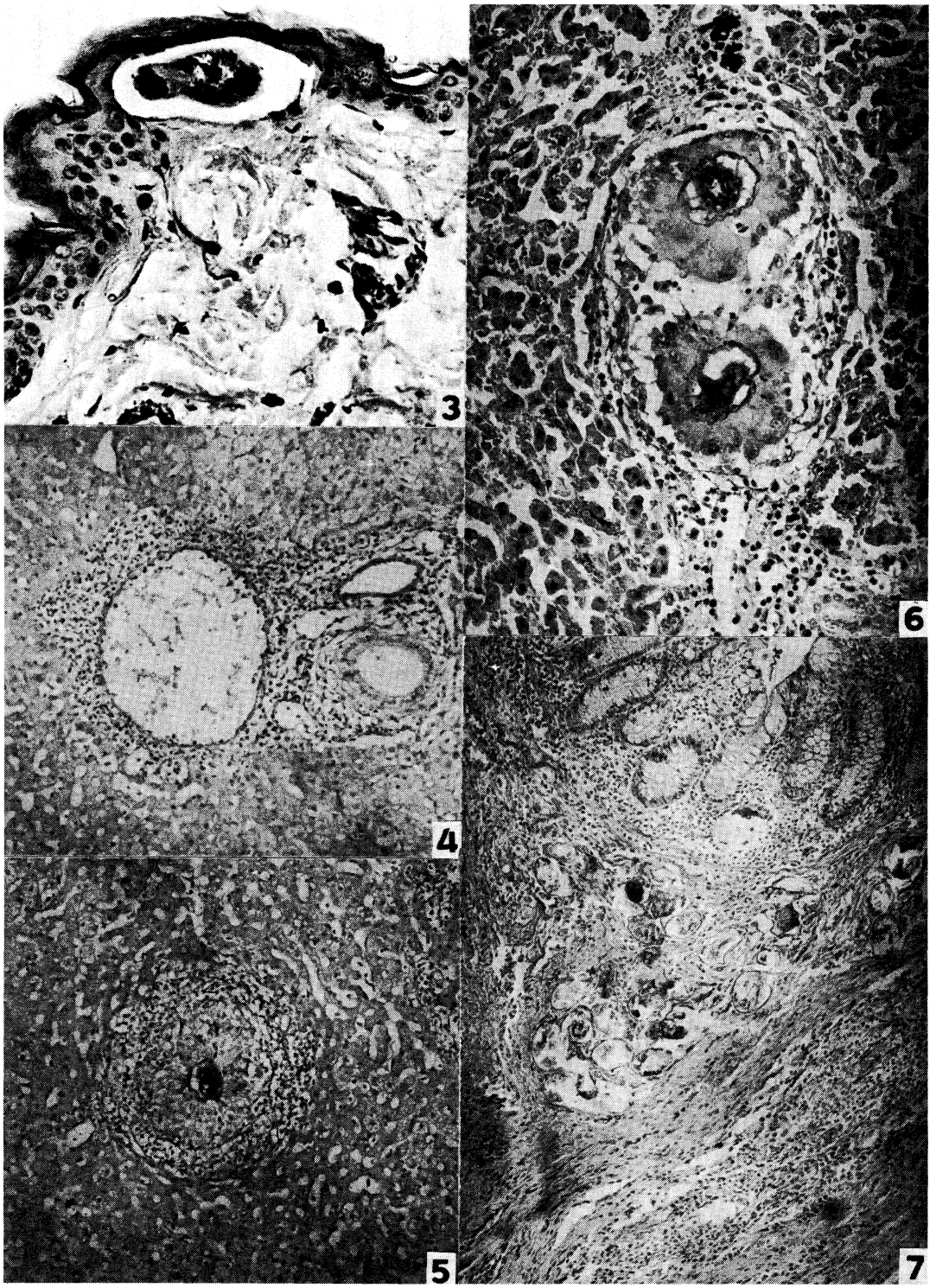

Fig 3—Skin, 2 hours post-infection, showing schistosomulum lying within the tunnel in Malpighi layer. × 260
Fig 4—Liver, 6 weeks post-infection, showing intensive cellular infiltration of portal space and pylephlebitis. × 100
Fig 5—Liver, 6 weeks post-infection, showing single-egg granuloma of productive stage. × 100
Fig 6—Liver, 19 weeks post-infection, showing various numbers of giant cells in egg granulomas. × 200
Fig 7—Rectum, 42 weeks post-infection, showing multiple lesions produced by deposition of eggs. × 100

rectum, which was approximately twice as thick as the submucosa of normal monkeys.

DISCUSSION

Hsü and Hsü (1956, 1960) first reported that rhesus monkeys, like Taiwanese monkeys *(Macaca cyclopis)* and humans, are not susceptible to infection with the Taiwan Province strain of *S. japonicum,* and that 4 rhesus monkeys were negative for the schistosome eggs in the stools throughout 70 days of daily examinations after exposure. The results of pathological study indicated that although in rhesus monkeys infection with 2,000 to 15,000 cercariae of the Taiwan Province strain of *S. japonicum* produced moderate lesions in the liver, the lesions were of short duration and were completely reversible with restitution of normal architecture in 60 to 90 days (Hsü *et al,* 1962).

In the present study, we have investigated the infection of rhesus monkey with the Chinese mainland strain of *S. japonicum* both by parasitological and histopathological techniques, for purposes of comparison and better understanding of the host-parasite relationships. In contrast to the results of infection with Taiwan Province strain, rhesus monkey infected with the Chinese mainland strain of *S. japonicum* resulted in 74.4-61.3% of worm recovery rate 6-15 weeks after infection. The mature worms recovered in pairs developed well and more than a hundred ova were present in the uterus of females. Eggs were demonstrated in fecal material during 34-36 days after exposure and the peak of egg passage in the feces occurred in the 7 to 15 weeks post-infection. The pathological changes observed in the liver were chiefly cellular infiltration of portal spaces and the lesions produced by egg granulomas. The most intensive damage in the gastro-intestinal tract was produced in the large intestine. These findings during the first 4 months of infection did not differ greatly from those in other hosts, such as mouse, rabbit, dog, cattle, pig, goat and sheep (Ho, 1963). Thereafter, the rhesus monkey showed various sign of resistance to infection. The worm recovery rate reduced greatly and the females and their sexual gland diminished markedly. The number of eggs passed in the feces also decreased dramatically. All of these findings indicated that most worms had died and the fecundity of surviving worms decreased with the rate of oviposition. Based on the results of the present investigation, we may conclude that the rhesus monkey is a permissive host for the Chinese mainland strain of *S. japonicum,* but it differs from the other permissive hosts mentioned above, in that many eggs pass in their feces during the whole period of infection. One of the possible interpretations of the worm elimination in the rhesus monkey is to assume that it is due to an acquired host immune response.

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