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FOLLOW-UP STUDIES OF *CEBUS APELLA* EXPOSED TO HEAVY INFECTIONS WITH *SCHISTOSOMA MANSONI*

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SUMMARY

Studies conducted in intact and splenoctomized Capuchin monkeys (*Cebus apella*) showed that they are a suitable model to study experimental schistosomiasis. All of them passed viable eggs after and throughout the infection. Egg production was higher between the third and the fifth month after the infection. Thereafter it decreased slightly but remained in high levels throughout the experiment.

The increase in egg production after repeated challenges was not as impressive as that seen after the first one. This pattern is different from that observed in Rhesus monkeys in which it was reported a progressive diminution of egg production.

Liver fibrosis in *Cebus apella* was observed only in monkeys submitted to repeated challenges from the beginning. However, the typical pattern of pipe-stem fibrosis and the picture of portal hypertension were not obtained so far.

INTRODUCTION

Several species of subhuman primates have been submitted to infection with *Schistosoma mansoni*. While all of them could be experimentally infected, their susceptibility and course of infection varied considerably [11].

This report deals with the course of primary and challenge infections in capuchin monkeys, and has the following main purposes: 1) Obtention of a experimental model which might reproduce the schistosomotic fibrosis found in man; 2) to observe the morphological and functional changes of the portal system and 3) to evaluate the influence of previous infections on the animal resistance to challenges as judged by the egg excretion, liver lesions and mortality.

MATERIALS AND METHODS

The data presented are from 10 adult monkeys as shown in Table I. Splenectomy was performed on 4 of them for use in an experiment on the susceptibility of *Cebus* monkeys to malaria though only the first two were artificially inoculated with *Plasmodium simiun*. These animals were used for the schistosomiasis experiment 6 to 8 months after the termination of the malaria studies and were healthy and active when selected for the present work. According to DEANE [5] these animals have not shown to be susceptible to malaria.

None of the monkeys studied was found to pass *S. mansoni* eggs prior to experimental infection.

Each animal was exposed to infection by placing a predetermined number of cercaria (Table I) on a shaven abdominal area for 45 minutes. Albino mice exposed to 100 to 150 cercariae each were used as infectivity controls.

As the first monkey died after a 700 cercariae infection the subsequente animals were infected with 500 to 600 cercariae.

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TABLE I

Number of *Schistosoma mansoni* cercariae and time schedule for infection, challenges and laparotomy in *Cebus* monkeys

Monkey number	Number of cercariae									Laparotomy (months after infection)
	Primary infection	Challenges (time in months from primary infection)								
		1 mth.	2 mths.	3 mths.	4 mths.	5 mths.	8 mths.	12 mths.	more than 1 year	
1	700	—	—	Died *						—
2 ***	600	—	—	—	Died **					—
3 ***	600	—	—	—	—	—	—	500	550(x2) died *	12, 15, 23
4	500	—	—	—	—	550	—	650	—	4, 10, 17
5	600	—	—	—	—	—	Died **		—	5
6	550	—	—	—	—	—	—	650		5, 10
7	550	—	—	—	—	—	—	600(x4)****		5, 10
8	550	—	—	—	—	—	Died **			8
9 ***	200	200	Died *							—
10 ***	200	200	200	200	200	—	Died *			—

* Death probably related to infection

** Death unrelated to infection (after laparotomy in monkeys no. 2 and 8 and after fight in monkey no. 5)

*** Splenectomized monkeys

**** Four challenges were performed in monthly intervals

Monkeys no. 9 and 10 were submited to different schedules of infection in order to study the effects of repeated infections in monthly intervals in a period when a strong immunity could possibly not be established [13].

Monthly fecal examinations began in the fifth week after exposure. Egg counts were performed on two or three samples obtained from a 24 hours specimen of feces, according to the technique described by FERREIRA [6]. Briefly, it consists in a 2% water dilution (for example, 10 g of feces in 500 ml of water) of a 24 hours well mixed sample of feces. The diluted material is not strained. Two or three egg counts are performed in a counting chamber (0.5 ml of volume or 25 × 20 × 1 mm). The whole area is counted and the total number of eggs is calculated as follows: eggs per gram = number of eggs × dilution (50) × 2. With the total weight in grams of the 24 hours specimen, the daily egg excretion will be easily obtained.

Egg counts in monkey no. 3 were started in the 16th month after the primary infection (Fig. 1).

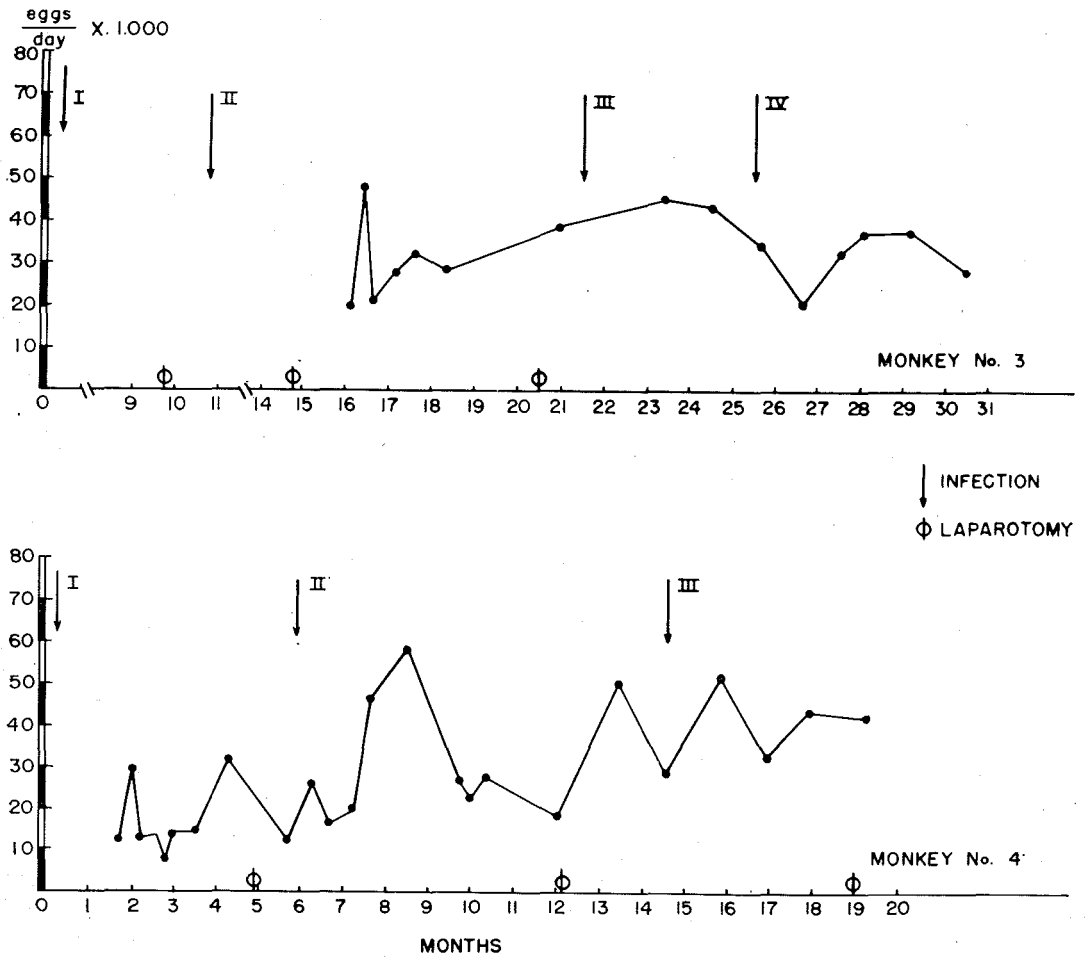

Fig. 1 — Egg counts in monkeys no. 3 and 4 in different periods after infections. The increase of egg production was much less impressive after the third challenge.

The viability of eggs from the feces was determined by the hatching technique and the infectivity of miracidia by exposing susceptible snails (*B. glabrata*) individually to 6 to 8 miracidia per snail.

Before exposure to the primary infection each animal was submitted to a laparotomy. The spleen was exposed for the determination of intrasplenic pressure and the performance of a splenoportogram. In splenectomized animals portal pressure and portography were performed after dissection of a branch of the superior mesenteric vein.

Liver and lymph node biopsies which were used as controls for each monkey were fixed in Helly's fluid, embedded in paraffin and stained by hematoxilin-eosin, elastic and reticulin stains. When necessary Masson's thrichrome stain was also used.

The same techniques were used after infections during the laparotomy or necropsy.

Peripheral venous blood obtained from the anesthetized animal before surgery was used for hemagglutination tests, according to KAGAN [8] as modified by HOSHINO [7].

RESULTS

1) *Parasitological data* — All the capuchin monkeys passed schistosome eggs after and throughout the experimental infection. The prepatent period varied between 6 and 7 weeks (43 and 50 days) both in heavily and slightly infected animals.

The eggs were viable and the miracidia were infective for snails. In all animals eggs appeared in the feces in greatest numbers between the third and the fifth month after

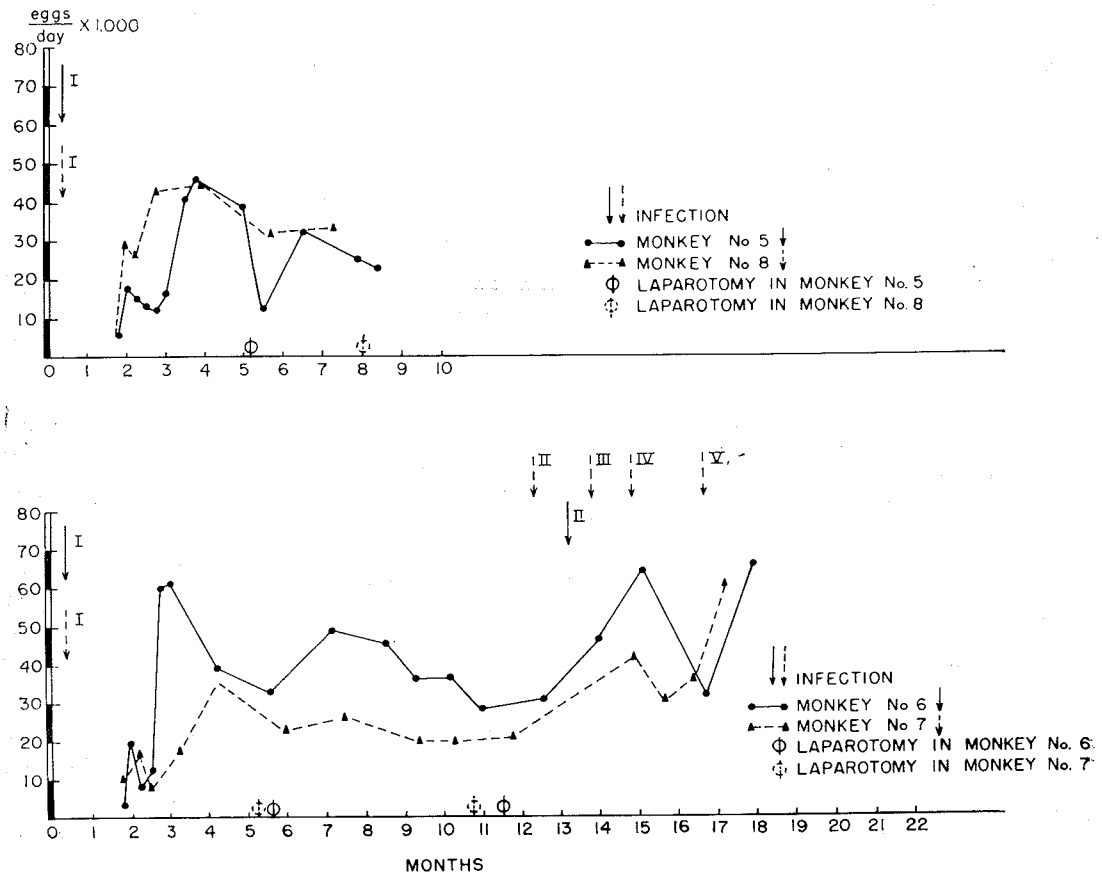

Fig. 2 — Egg counts in monkeys no. 5, 6, 7 and 8. Egg production remained in high levels throughout the experiment and increased after challenge.

exposure. Thereafter eggs production decreased slightly but remained in high levels throughout the experiment.

The relatively short intervals of observation in monkeys no. 9 and 10 did not allow us to compare the number of eggs in feces to the number of cercariae used for infection.

An increase in the egg production was observed in all monkeys submitted to challenges (Figs. 1 and 2). Such increase seems to be less impressive after repeated challenges.

2) *Splenoportography and intrasplenic pressure* — Were determined during surgery in monkeys 4, 5, 6, 7, before and in different periods after infection. No significant increase of portal pressure neither an evident collateral circulation was observed (Fig. 3). Monkeys no. 9 and 10 were not submitted to laparotomy after infection.

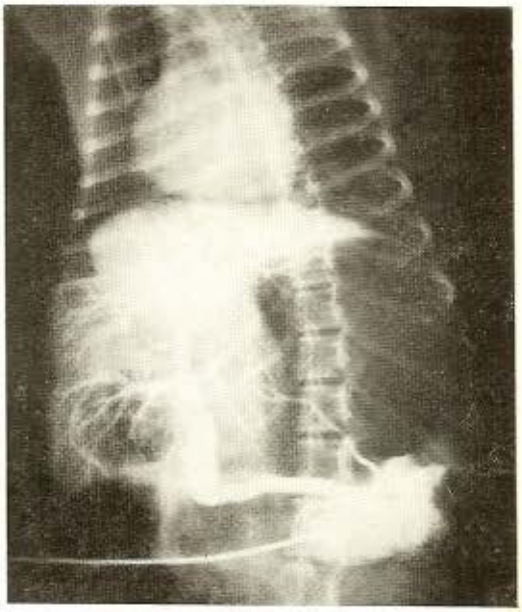

Fig. 3 — Splenoportography of monkey no. 4 after infection. No difference was observed when compared with the control.

3) *Clinical and serologic data* — Death related to infection in monkeys no. 1, 3, 9 and 10 were preceded by anorexia, diarrhea with mucus and blood.

Hemagglutination tests performed in blood samples obtained during the operation in monkeys no. 3, 4, 5, 6 and 7 showed that all of them were positive and that the titers increased progressively after infection (Table II).

TABLE II

Results of hemagglutination tests performed in *Cebus* monkeys exposed to *Schistosoma mansoni* cercariae

Monkey no.	Quantitative serology (reciprocal of titer)									Time of determination
	20	40	80	160	320	640	1280	2560	5120	
3	+	+	+	+	+	+	+	+	+	After the 3rd. challenge
4	—	—	—	—	—	—	—	—	—	Before infection
4	+	+	+	+	—	—	—	—	—	4 months after infection
4	+	+	+	+	+	+	+	—	—	10 months after infection and 5 months after the 1st. challenge
5	—	—	—	—	—	—	—	—	—	Before infection
5	+	+	+	+	+	+	+	—	—	5 months after infection
6	—	—	—	—	—	—	—	—	—	Before infection
6	+	+	—	—	—	—	—	—	—	5 months after infection
6	+	+	+	+	+	+	—	—	—	10 months after infection
7	—	—	—	—	—	—	—	—	—	Before infection
7	+	+	+	—	—	—	—	—	—	5 months after infection
7	+	+	+	+	+	+	—	—	—	10 months after infection

4) *Morbid anatomy* — At operation before infection no pathologic finding was observed except in monkey no. 7 who showed a small nodule at the serosal surface of the small intestine. Liver and lymph node biopsies were normal in all of them.

A progressive splenomegaly was observed after infection but the influence of splenic pressure determinations and of contrast injections on this increase of size cannot be discarded.

In the liver of animals without multiple infections portal fibrosis was not accentuated, but present, often accompanied by a marked inflammatory infiltrate, made up of mononuclear cells, chiefly histiocytes, lymphocytes, plasma cells and eosinophils. There was granulomata formation around viable and non viable eggs. Portal tracts exhibited slight fibrosis and moderate edema. Among the inflammatory infiltrate, small proliferated venules with swollen endothelial lining were occasionally observed.

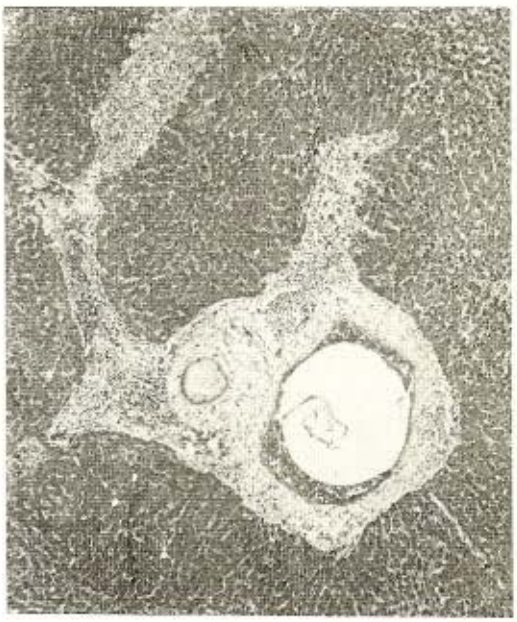

Fig. 5 — Same animal after four challenges and after 6 months of primary infection showing a large portal tract, enlarged by fibrosis and inflammatory infiltrate. Masson's thrichrome. 100 ×

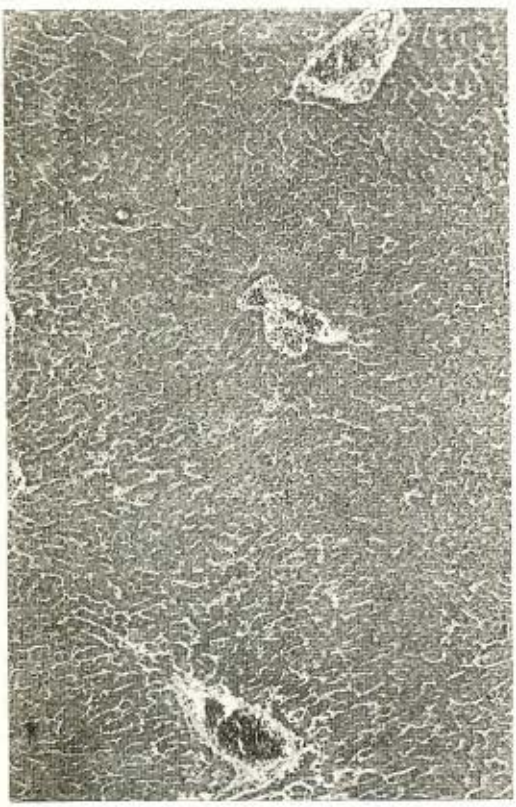

Fig. 4 — Monkey no. 10 showing before infection a normal liver structure. Masson's thrichrome. 80 ×

The lobular limiting plate was frequently disrupted, due to a secondary aggression of the inflammatory infiltrate.

Generally speaking, hepatic cells were preserved.

There was a marked hyperplasia and hypertrophy of the Kupffer cells which exhibited large amounts of black schistosomal pigment in their cytoplasm. No marked differences were observed at laparotomy and at necropsy in biopsy specimens from monkeys submitted to one infection or to challenges performed after long intervals (monkeys no. 1 to 8).

The liver of animals which were subjected to monthly infections from the beginning (monkeys no. 9 and 10) differed from the others in the following aspects: Portal fibrosis and edema were more marked and the inflammatory infiltrate more prominent (Figs. 4, 5 and 6). Dilated small radicles

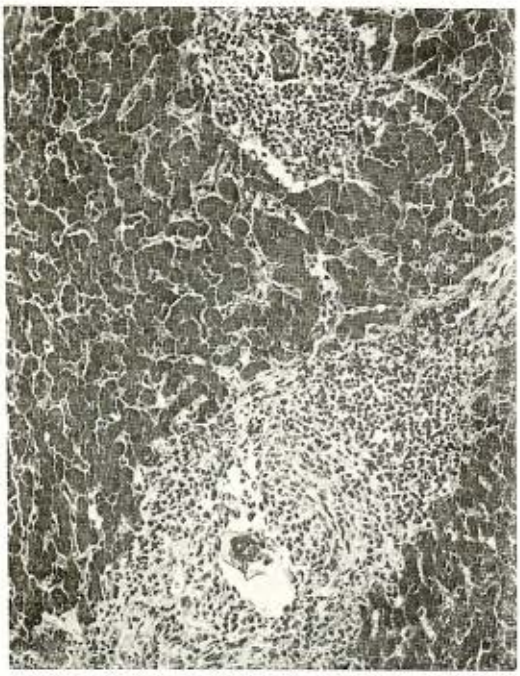

Fig. 6 — Same animal showing a small portal tract with fibrosis, granulomatous formation around viable eggs and focal destruction of the lobular limiting plate. Masson's thrichrome. 300 ×

of the portal vein, engorged with blood, were more frequently seen at the periphery of the portal spaces and the large radicles frequently appeared with dead male schistosomal worms inside. The portal fibrosis involved peripheral and the large, close to the hilum, portal tracts in a fashion slightly resembling human schistosomotic liver scarring. Lymph nodes of the infected animals, when compared to controls, were bigger due to the enlargement of lymph follicles which presented prominent reactive centers. Moreover, in the lymph node there were multiple granulomata around dead and viable eggs. In few cases an eosinophilic thin cap was observed between the granulomata formation and the egg shell (Hoeppli phenomenon) (Fig. 7A)[9]. In others this eosinophilic mass was inside the egg shell, usually in one pole, surrounding partially the miracidium (Fig. 7B). One lymph node also showed small focal hyalin deposits, occasionally affecting the walls of small vessels, which were interpreted as amyloid. There were also present in the lymph node foreign body granulomata, around non viable eggs, circumscribed by scanty peripheral fibrosis.

The above described reactions, chiefly the foreign body, were also found in the liver.

DISCUSSION

Previous studies [4, 11] on capuchin monkeys (*Cebus apella*) submitted to experimental infection with Schistosomiasis mansoni have led to confliting results. According to SADUN et al. [11] egg excretion seemed to follow a pattern similar to that found in Rhesus monkeys, that is, the number of eggs in the stools reached a peak shortly after the beginning of patency and decreased rapidly afterwards. Many eggs were viable but the percentage of those hatching was considerably lower than in the rhesus. No snails became infected from the resulting miracidia. Some of this aspects were not observed by us [4].

The results of our experiments showed that the egg excretion remained abundant throughout the duration of the experiment (from 2 months to more than 1 year) and that the eggs were infective for snails.

Furthermore, in our follow-up studies, the pattern of egg excretion is similar to that found in chimpanzee by SADUN et al. [12] though their experiments were of shorter duration.

It is worth mentioning that most challenges were followed by an increase of egg excretion. This was not observed in the Rhesus monkey by SMITHERS & TERRY [13] and under certain circumstances by MCMULLEN et al. [10].

These results suggest that resistance in Rhesus monkeys after the primary infection is more evident than that observed in capuchin monkeys. In these monkeys, however, a certain degree of immunity develops, as judged by the resistence to repeated challenges, which otherwise would be fatal. Thus, monkeys no. 9 and 10 died after total challenges of 200 and 800 cercariae respectively whereas monkey no. 7 is alive and well after 4 challenges of 600 cercariae each (Table I).

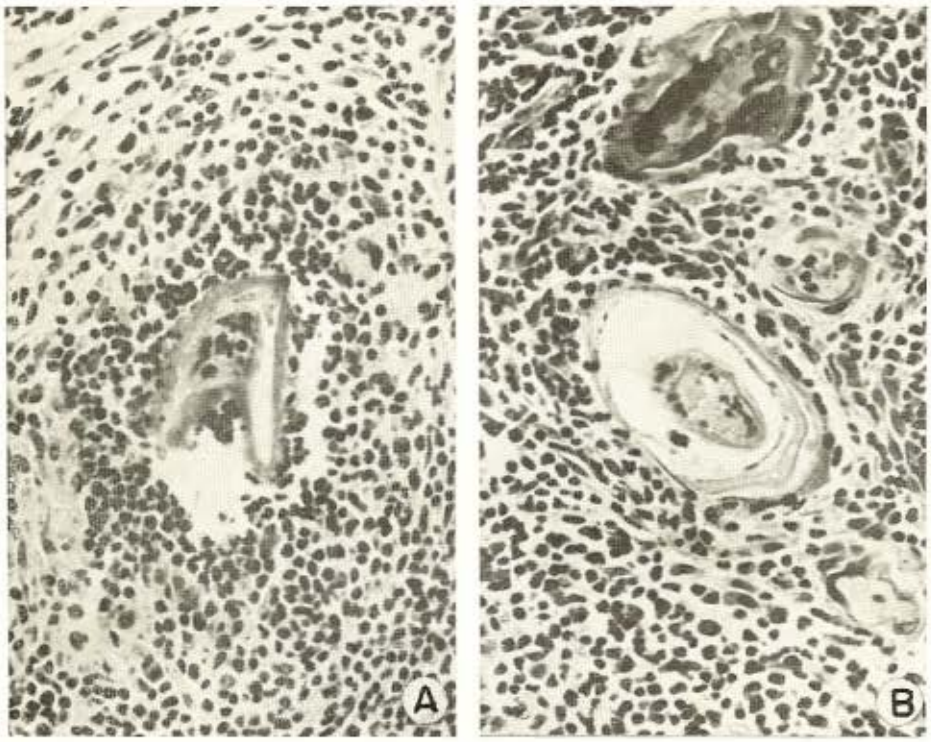

Fig. 7 — A) Lymph node showing granulomatous reaction and Hoeppli phenomenon. H.E., 300 × — B) Eosinophylic thin cap inside an egg shell partially surrounding miracidium in the same lymph node. H.E., 300 ×

The influence of splenectomy on this resistance is not well established. However, if we compare only splenectomized animals, we observe that monkey no. 3 died only after a total challenge of 1,600 cercariae. According to SADUN et al.[12] it is doubtful that splenectomy greatly influences these results.

Finally, prominent degrees of fibrosis were observed only in those animals submitted to repeated infections from the beginning (monkeys no. 9 and 10). This interesting finding reinforces the hypothesis that human schistosomotic fibrosis is most apt to appear in patients exposed to multiple and heavy infections in a period not long enough for the appearence of immunity. In Rhesus monkeys this critical period appears to be of 16 or more weeks after the initial exposure. When challenge was 12 weeks or less after this exposure the results were variable[13].

Splenoportography and intrasplenic pressure performed in monkeys no. 4, 5, 6 and 7 did not show any evidence of portal hypertention or collateral circulation. This is in accordance with the absence or the slight degree of liver fibrosis in these monkeys. Unfortunatelly monkeys no. 9 and 10, which showed the more fibrotic livers were not submitted to those techniques.

Hemagglutination tests disclosed a progressive increase of titers after infection. BRUCE et al.[2] observed that all Rhesus monkeys were positive in the fluorescent antibody test 6 weeks after exposure and that maximal titers were observed at this time.

Hepatic granulomata similar to those observed in man were found by COELHO & MAGALHÃES[3] in *Cebus* monkeys. However, their studies were of short duration. In our liver biopsies both foreign body granulomata around empty egg shell and granulomatous reaction around viable eggs were observed. Hoeppli phenomenon was more frequently seen in lymph node biopsies than in the

liver. A hyalin deposit inside the egg shell, either in one of its pole or substituting the miracidium, was observed by us more frequently than the typical Hoeppli phenomenon.

The lymph nodes showed enormous lymph follicles, with enlarged reactive centers. In one case a hyalin deposit, probably amyloid, was observed. Similar finding was described by ANDRADE & ANDRADE [1] in studying the spleen in the human hepatosplenic form of schistosomiasis.

In the portal tracts dilated proliferated branches of the portal vein were frequently observed, such finding being interpreted as evidence of an early intrahepatic circulatory disturbance. Such proliferated venules were also observed by SADUN et al. [1,2] in the infected chimpanzee.

More severe lesions were observed in monkeys no. 9 and 10. Liver fibrosis was prominent only in these animals though a picture identical to the classical pipe-stem fibrosis was never achieved. BRUCE et al. [2] concluded that the failure to develop severe chronic disease in Rhesus monkey is probably related to the fact that this animal, after a particularly severe reaction during the early stages of the infection, usually recovers by elimination of the worms. On the other hand, a severe fibrosis, resembling human pipe-stem fibrosis was observed by SADUN et al. [1,2] in the chimpanzee after a single exposure to 1,000 cercariae.

It is our feeling that these different species of animals and particularly the Rhesus and Capuchin monkeys should be submitted to repeated heavy exposures in short periods in order to study their ability to develop liver fibrosis after *Schistosoma mansoni* infection.

To sum up, Cebus monkeys are well suited animals for the study of schistosomiasis. They seem to occupy an intermediate position between Rhesus and Chimpanzee monkeys. Egg output was maintained throughout the experiment but liver fibrosis was more difficult to obtain as compared to the chimpanzee.

RESUMO

Estudo evolutivo de macacos Cebus apella *expostos a infestações com cercárias de* Schistosoma mansoni

Estudos feitos em macacos *Cebus apella* demonstraram constituir êsses animais um modêlo útil para o estudo da esquistossomose experimental. Observou-se eliminação de ovos viáveis pelas fezes durante tôda a infecção, sendo mais alta entre o 3.º e 5.º mês após a inoculação com cercárias de *Schistosoma mansoni*. Inoculações repetidas acompanharam-se de aumento da eliminação de ovos embora não tão evidente quanto após a primeira infecção. Tais fenômenos são diferentes daqueles observados no macaco Rhesus, no qual tem sido relatada uma diminuição progressiva da postura.

A fibrose hepática no *Cebus apella* foi observada sòmente em macacos submetidos a infecções repetidas desde o início. Até o momento, contudo, não conseguimos obter o quadro típico da fibrose de Symmers nem hipertensão portal.

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