EXPERIMENTAL SCHISTOSOMIASIS JAPONICA IN THE RHESUS MONKEY
A COMPARISON OF THE ILAN* (TAIWAN) AND JAPANESE STRAINS

JOHN W. MOOSE, JAMES E. WILLIAMS AND KENTARO YOSHIMURA

Department of Medical Zoology, 406th Medical Laboratory U. S. Army Medical Command, Japan, APO San Francisco 96343

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The prepatent period of the Ilan strain of S. japonicum in three rhesus monkeys varied from 42 to 78 days (avg. 56.3); in an equal number of monkeys, the Japanese strain, from 37 to 40 days (avg. 39.3). Only 28 schistosomes were recovered from the three monkeys infected with the Ilan strain compared to 145 from the three monkeys infected with the Japanese strain. The body length of worms showed that males and females differed significantly between strains at the 5 % and 1 % levels. Miracidia were hatched from eggs obtained from livers of all the monkeys except one monkey which was infected with the Ilan strain. Comparison of the histopathological changes induced by the two strains shows similar patterns of inflammatory response, but the Japanese strain is more severe. Evidence presented shows that the rhesus monkey is susceptible to the Ilan strain of S. japonicum. Differences between the presently studied Ilan strain and the previously known Formosan zoophilic strain (the Changhua** strain) are discussed.

INTRODUCTION

Evidence that the Formosan strain of Schistosoma japonicum is a zoophilic strain was introduced by Hsü and Hsu (1956). Human volunteers did not pass eggs in their stools after being infected. Hsü and Hsu (1960 a ; 1962) later reported that the rhesus monkey (Macaca mulatta) is generally not susceptible to infection with the Formosan strain of S. japonicum. Monkeys infected with this strain did not pass eggs in their stools throughout 70 days of their experiment. However, monkeys infected with the Japanese, Philippine and Chinese strains passed eggs starting on the 33rd, 35th and 39th days, respectively.

Oncomelania formosana was found to be the intermediate host of the Formosan strain of S. japonicum long before the zoophilic characteristics of this strain were established (Yokogawa, 1915). Subsequent experimental studies conducted by Hunter et al. (1952), Dewitt (1954) and Hsü and Hsü (1960 b) indicate that this oncomelanian species will not serve as an intermediate host of the human strains of S. japonicum from the Chinese mainland, Japan, and the Philippines. Thus, O. formosana is susceptible to infection exclusively with the zoophilic strain of S. japonicum in Taiwan.

It is important to note that conclusions drawn from the aforementioned infectivity studies (human, monkey and snail) resulted from experiments using the snail O. formosana,

* 宜蘭
** 彰化
吉村 堅太郎 (米軍 406 医学研究所)
and the *S. japonicum* cercariae obtained from this snail species which was collected from the endemic area of Changhua in Central Taiwan.

Kuntz (1962) reported that a new distribution area of *O. formosana* had been found in Ilan in the northeastern part of Taiwan. *O. formosana* infected with carcariae of *S. japonicum* were also found in this new location.

Moose and Williams (1963, 1964) performed infectivity studies using *O. formosana* originating from Ilan and reported that these snails are exceedingly resistant to infection with the Formosana zoophilic strain from Changhua but are susceptible to infection with the human strains of *S. japonicum* from Japan and the Philippines. Since *O. formosana* snails harboring cercariae of *S. japonicum* have been found in the Ilan endemic area, the question was raised: to what category (human or zoophilic) does the Ilan strain belong (Moose and Williams, loc. cit.)?

Inasmuch as rhesus monkeys are readily available for use as experimental animals, it was decided to compare the infectivity of these animals to the Ilan and Japanese strains of *S. japonicum*.

**MATERIALS AND METHODS**

The principles of laboratory animal care as promulgated by the National Society for Medical Research were observed.

Large field collections of *O. formosana* from Ilan, Taiwan, were sent to this laboratory early in 1965. From these shipments, nine snails shed *S. japonicum* cercariae. After passage of the life cycle through albino mice (SM strain), additional uninfected *O. formosana* from Ilan were exposed to miracidia hatched from eggs obtained from livers of the infected mice. Seventy six snails became infected.

Six female rhesus monkeys weighing between 1.5 to 2.5 kg were used for this experiment. During a brief period of isolation and conditioning, their stools were found negative for schistosome eggs and *Salmonella*. The animals were also negative for tuberculin reactivity. The monkeys were divided into two groups of three animals each. Monkeys in Group I (nos. 1, 3 and 30) were each exposed to 400 cercariae of the Ilan strain of *S. japonicum*. Monkeys in Group II (nos. 34, 35 and 469) were each exposed to 400 cercariae of the Japanese strain. The cercariae were obtained by crushing 76 infected *O. formosana* from Ilan and 76 infected *O. nosophora*, which harbors the human strain of *S. japonicum*, from Kofu, Japan. The cercariae of both strains were pooled separately, counted on a glass slide and applied to the shaven abdomen of each animal. The monkeys had previously been anesthetized with Sernyl*. In order to ascertain whether or not an equal distribution of the sexes of the parasites had been achieved, albino mice (SM strain) were exposed to the cercarial pools. Ten mice each were infected cutaneously with 50 cercariae of the Ilan strain and ten mice were likewise infected with the Japanese strain. The mice were killed 42 days after exposure. The worms recovered from each animal were sexed and totaled by group.

To determine the presence of *S. japonicum* eggs in feces, monkey stool examinations employing the AMS III concentration technique (Hunter et al., 1948) were performed 30 days after infection and were continued six days a week through the 88th day. The monkeys were killed on the 90th day by an injection of Toxital**. The viscera of each

* Parke, Davis and Company, Detroit, Michigan, USA.
** Jensen-Salsbery Laboratories, Inc., Kansas City, Missouri, USA.
monkey were first perfused and then manually dissected. The location, number and size of schistosomes recovered was recorded. For histopathological study, portions of the liver, lungs, mesenteric lymph-nodes, kidneys, heart, spleen, and small and large intestines were fixed in 10% formalin, embedded in paraffin, sectioned at six microns thickness and stained with hematoxylin and eosin. The miracidial hatching test was performed with unfixed liver tissue from each monkey.

RESULTS

A. Parasitological Findings

The number of male and female worms recovered from both groups of infected mice showed that a relatively equal sex ratio was attained from each cercarial pool. The worm yield of the Ilan strain was 132 (26.4%) males and 162 (32.4%) females; the Japanese strain, 152 (33.7%) males and 141 (31.3%) females. One mouse in the latter group died nine days after infection; therefore, final tabulation was done with worms harvested from nine animals.

The prepatent period of the monkeys in Group I infected with the Ilan strain was 42 days for Monkey No. 3; 49 days for Monkey No. 1; and 78 days for Monkey No. 30. In Group II infected with the Japanese strain, the prepatent period was 37 days for Monkey No. 35, and 40 days for Monkeys Nos. 34 and 469. All stools remained positive throughout the experiment, with the exception of Monkey No. 3 (Group I) which passed eggs on days 43 and 47 but was negative until the 66th day. However, in this monkey eggs were found continuously thereafter.

Worm recovery data, results of the miracidial hatching test and measurements of undamaged worms are shown in Tables 1 and 2. Briefly, altogether 28 adult worms were recovered from the three monkeys infected with the Ilan strain compared to 145 from the three monkeys infected with the Japanese strain. The body length of worms recovered from the former group of monkeys was shorter than that of the worms recovered from the latter group. By the F test, the difference between the mean body length for the two strains was statistically significant at the 1% level for females and the 5% level for the males. Miracidia were hatched from eggs obtained from livers of all the monkeys except one monkey which was infected with the Ilan strain.

<table>
<thead>
<tr>
<th>Group (strain)</th>
<th>Animal no.</th>
<th>No. of cercariae</th>
<th>No. of worms recovered</th>
<th>Total no. (%)*</th>
<th>Miracidial hatching test (liver)</th>
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<td>Liver-portal veins</td>
<td>Mesenteric veins</td>
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<td>I (Ilan)</td>
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<td>12</td>
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<td>30</td>
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<td>4</td>
<td>1</td>
<td>0</td>
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<tr>
<td>II (Japanese)</td>
<td>34</td>
<td>400</td>
<td>11</td>
<td>8</td>
<td>2</td>
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B. Histopathological Findings

1. Liver: The nodules induced by eggs of the Ilan strain had sharp margins and were mainly in interlobular areas (Fig. 1). In the center of the nodules, three to six eggs were usually surrounded by multinucleated giant cells. The eggs showed various developmental stages. Those which were necrotic and disintegrated were infiltrated by many eosinophils, lymphocytes and a few histiocytes and plasma cells. Eosinophil infiltration was especially prominent. In general, the outer layer was composed of lymphocytes and eosinophils, but eosinophils were also found diffusely through the lesion. Epithelioid cells and fibroblasts were frequently seen. Some hepatic parenchymal cells were degenerating and had pyknotic nuclei. Lymphocytes and eosinophils infiltrated around interlobular bile ducts and venules.

The structure of nodules induced by eggs of the Japanese strain was essentially the same as with the Ilan strain (Fig. 2). They were composed of some centrally placed eggs, multinucleated giant cells, eosinophils, lymphocytes, histiocytes and plasma cells. Eosinophil infiltration was prominent in some cases, but in others, lymphocytes predominated. Around the wall of interlobular venules, a cellular infiltration composed predominantly of lymphocytes with smaller numbers of plasma cells and comparatively few eosinophils and histiocytes was found.

2. Lungs: Neither nodules induced by eggs nor lesions produced by worms were recognized. However, the findings as described below are based on the assumption that they may be related to schistosomiasis infection. Localized eosinophil infiltration was especially prominent around blood vessels, and diffuse eosinophil infiltration was observed in the alveoli together with prominence of the alveolar epithelium. Comparatively few giant cells and histiocytes were present. Eosinophils infiltrated around bronchioles and mucosal epithelia. Pulmonary pathological changes were essentially the same in monkeys in both groups.

3. Intestines: In general, the pathological changes in the small intestine were less prominent than those in the large intestine. With the Ilan strain (Fig. 3), scattered eggs and hemorrhages were generally recognized in the tunica propia mucosa together with a cellular infiltration composed of mononuclear cells and eosinophils. In the submucosa, many nodules were seen whose structure corresponded to those in the liver. The eggs were surrounded by giant cells and ringed by lymphocytes, eosinophils, and histiocytes. In addition, the nodules usually had some fibroblasts and epithelioid cells. The eosinophil and lymphocyte infiltration was more prominent than that of other cellular components. Proliferative changes in connective tissue were recognized around the
nodules. It was frequently found that eggs not surrounded by giant cells were directly infiltrated by a great many eosinophils and disintegrating polymorphonuclear leucocytes. In these foci, epithelioid cells, fibroblasts and macrophages were recognized. Proliferating connective tissue was present around the inflammatory foci. This type of lesion, with prominent cellular infiltration, was found in the mucosa as well as in the submucosa.

With the Japanese strain (Fig. 4), the pathological changes were comparable to those of the Ilan strain. Catarrhal inflammation was observed in the mucosal membrane, and disintegration or exfoliation of the mucosa was occasionally found. So many large nodules were recognized in the submucosa that the granulation tissue induced by adjacent eggs seemed to be continuous. The structure of the nodules was the same as with the Ilan strain.

4. Other organs: No specific pathological changes were observed in lymphatic nodules, heart, kidney, or spleen in monkeys infected with either strain.

DISCUSSION

Data obtained from the parasitological results of this study in rhesus monkeys show the following significant differences between the Ilan and Japanese strains of *S. japonicum*; (1) The prepatent period of the Ilan strain varied in each monkey from 42 to 78 days (average 56.3); the Japanese strain, from 37 to 40 days (average 39.3); (2) Only a total of 28 schistosomes were recovered from the three monkeys infected with the Ilan strain compared to 145 from the group infected with the Japanese strain; (3) The body length of both male and female worms recovered from the monkeys infected with the Ilan strain was significantly shorter than that of the Japanese strain; and (4) Miracidia were not hatched from the liver of one monkey infected with the Ilan strain although they were hatched from the livers of the other two monkeys infected with this strain and from the livers of three other monkeys infected with the Japanese strain.

Comparison of the histopathological changes induced by the two strains showed that they are essentially similar. However, relatively minor differences were observed in the histopathological lesions induced by eggs of the two strains. Greater eosinophil infiltration was noted in the inflammatory nodules in livers of monkeys infected with the Ilan strain. Whether this difference can be attributed to a greater degree of hypersensitivity reaction is conjectural. Another minor difference is that in the monkeys infected with the Japanese strain the lesions in the intestines were more severe than those produced by the Ilan strain. However, we have to take into consideration that although both groups of monkeys were sacrificed at the same post-infection interval of 90 days, the stages of the egg lesions were not necessarily the same. If there were differences between Ilan strain and Japanese strain infections in rates of migration and maturation and the number of eggs deposited in tissue, differences in the host response (in predominant reactive cells and number of lesions) would be reflected in the inflammatory reaction to the parasite.

It is obvious from the foregoing discussion that the rhesus monkey is a more susceptible host for the Japanese strain of *S. japonicum* than for the Ilan strain. However, the striking finding obtained from this study is that the Ilan strain developed to maturity in each of the three monkeys used. Not only did the infected animals pass eggs in their feces, but when autopsied 90 days after infection, they all showed classic lesions of schistosomiasis japonica.

In their early paper, Hsü and Hsü (1960 a) reported that rhesus monkeys experimentally infected with the Changhua strain of *S. japonicum* do not pass eggs in their feces.
Based on this observation, it was then concluded that this primate species is not susceptible to the Changhua strain. Hsi and Hsi (1962) later reported positive stool findings in 3 of 103 rhesus monkeys infected with this strain. However, two of these monkeys had been debilitated by Salmonella infections. This is in contrast to the results obtained in the present study. Eggs were found in fecal examinations of all three monkeys infected with the Ilan strain and all were found to be free of Salmonella enteritis.

Hsi, Davis, and Hsi (1962) reported that from a rhesus monkey autopsied 90 days after infection with the Changhua strain, only immature worms measuring 0.2 to 2.1 mm were recovered. Histopathologically, this animal did not show any residual evidence of parasitism; no eggs were found in the liver or in the gastrointestinal tract.

It has been well documented that neither man nor rhesus monkeys are susceptible to infection with the Changhua strain; yet, based on the parasitological and histopathological findings of the authors' experiment, it is evident that the rhesus monkey is susceptible to the Ilan strain of S. japonicum.

The information presented here plus that already reported by Moose and Williams concerning the snail host-parasite relationship existing in the Ilan endemic area, show that the Ilan strain of S. japonicum differs from the strain found in Changhua and so with their snail hosts in these two localities. Apparently, the Ilan strain of parasite and its intermediate host have not reached the same extent of evolutionary changes as described by Hsi and Hsi (1962) in regard to the zoophilic strain in Changhua. The Ilan strain of S. japonicum still has the capability of reaching maturity in the rhesus monkey and the O. formosana snails in Ilan are susceptible to infection with human strains of the parasite.

We are grateful to Dr. H. F. Hsi and Dr. S. Y. Li Hsi, Research Associate Professor, Department of Preventive Medicine and Environmental Health, College of Medicine, University of Iowa, for their kind interest and help in supplying us with large field collections of naturally infected O. formosana from Ilan, Taiwan.

REFERENCES


Hunter, G. W., Hodges, E. P., Jahnnes, W. G., Diamond, L. S., and Ingalls, J. W., Jr. (1948): Studies on schistosomiasis. II. Summary of further studies of methods of reco-


EXPLANATION OF FIGURES

Fig. 1: Monkey liver, 90 days after infection with the Ilan strain of *S. japonicum*, showing granuloma with schistosome eggs. Hematoxylin and eosin stain, × 180.

Fig. 2: Monkey liver, 90 days after infection with the Japanese strain of *S. japonicum*, showing granuloma with schistosome eggs. Hematoxylin and eosin stain, × 180.

Fig. 3: Monkey intestine, 90 days after infection with the Ilan strain of *S. japonicum*, showing schistosome eggs. Hematoxylin and eosin stain, × 180.

Fig. 4: Monkey intestine, 90 days after infection with the Japanese strain of *S. japonicum*, showing schistosome eggs. Hematoxylin and eosin stain, × 180.