

AD

**REPORT No.** FE-368-6 (Annual Report)

**GRANT No.** DA-CRD-AFE-S92-544-70-G158

**STUDIES ON HOST-PARASITE RELATIONSHIPS OF  
SCHISTOSOMA JAPONICUM IN TAIWAN**

by

Jui-Kuang Chiu, Dr. Med. Sc.  
Professor of Parasitology  
College of Medicine  
National Taiwan University  
Taipei, Taiwan, Republic of China

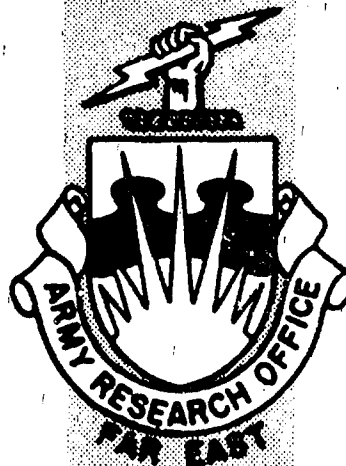
D D C  
RECEIVED  
DEC 28 1971  
C

AD734437

September 1971

Reproduced by  
**NATIONAL TECHNICAL  
INFORMATION SERVICE**  
Springfield, Va. 22151

**U. S. ARMY RESEARCH AND DEVELOPMENT GROUP  
FAR EAST  
APO San Francisco 96343**



38

Unclassified

Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and including annotations must be retained when the original report is classified)

1. ORIGINATING AGENCY (Corporate author)

National Taiwan University  
College of Medicine  
Taipei, Taiwan

2a. REPORT SECURITY CLASSIFICATION

Unclassified

2b. GROUP

3. REPORT TITLE

Studies on Host-Parasite Relationships of Schistosoma Japonicum in Taiwan (U)

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)

Annual Report No. 6, 1 March 1970 - 28 February 1971

5. AUTHOR(S) (Print name, middle initial, last name)

Jui-Kuang Chiu

6. REPORT DATE

6 December 1971

7a. TOTAL NO. OF PAGES

23

7b. NO. OF FIGS

12

8a. CONTRACT OR GRANT NO.

DA-CRD-AFE-S92-544-70-G158

8b. ORIGINATOR'S REPORT NUMBER(S)

FE-368-6

9. PROJECT NO.

3A061102B71Q

c.

Task

4 00 110FE

9. OTHER REPORT NUMBERS (Any other numbers that may be assigned to this report)

10. DISTRIBUTION STATEMENT

Approved for public release; distribution unlimited.

11. SUPPLEMENTARY NOTES

12. SPONSORING MILITARY ACTIVITY

U. S. Army R&D Group (Far East)  
APO San Francisco 96343

13. ABSTRACT

For the past 3 years, studies were designed to investigate: 1. the distribution of oncomelanid snails in Taiwan, 2. the susceptibility of oncomelanid snails from various areas to infection with human and zoophilic strains of Schistosoma japonicum 3. the nature of definitive host for Ilan strain of S. japonicum, and 4. adaptation of S. japonicum in Oncomelania hupensis chiui. In addition, gel-diffusion studies on sera from animals infected with S. japonicum were also made.

Best Available Copy

Unclassified



Unclassified

~~SECRET~~

16. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Schistosomes Strains Human Japan Philippines Laboratory Animals Distribution Identification Gel-diffusion Infectivity Snails Taiwan						

Unclassified

REPORT No. FE-368-6 (Annual Report)

GRANT No. DA-CRD-AFE-S92-544-70-G158

DA Project/Task Area/Work Unit No. 3A061102B71Q 00 11CFE

STUDIES ON HOST-PARASITE RELATIONSHIPS OF  
SCHISTOSOMA JAPONICUM IN TAIWAN

by

Jui-Kuang Chiu, Dr. Med. Sc.  
Professor of Parasitology  
College of Medicine  
National Taiwan University  
Taipei, Taiwan, Republic of China

September 1971

U. S. ARMY RESEARCH AND DEVELOPMENT GROUP  
FAR EAST  
APO San Francisco 96343

Approved for public release; distribution unlimited.

## ABSTRACT

Studies were designed to investigate: 1. the distribution of oncomelaniid snails in Taiwan, 2. the susceptibility of oncomelaniid snails from various areas to infection with human and zoophilic strains of Schistosoma japonicum, 3. the nature of definitive host for Ilan strain of S. japonicum, and 4. adaptation of S. japonicum in Oncomelania hupensis chiui in the past 3 years. During the last one year, efforts have been made to complete the studies. In addition, gel-diffusion studies on sera from animals infected with S. japonicum were also made. Results obtained are as following.

1. Parasitological studies at Jui-Ping area, a new habitat for O. h. chiui, demonstrated that none of the 2,682 snails examined was found to be infected with S. japonicum. Susceptibility studies, however, showed that similarly to the snail from Alilao, the snail from the new habitat is also highly susceptible to all geographic strains of S. japonicum. The snail is found on a hill side along the coast, at least 4 km long in distance from Jui-Ping to the west.

Intradermal tests for S. japonicum were performed among 736 people, and 19 (2.6%) showed positive reactions. Significantly higher rate was found in males (3.3%) than in females (1.8%). Stool examinations of 605 people including 19 positive skin test reactors were all negative for S. japonicum eggs. The infection rates for intestinal helminths were: Ascaris lumbricoides 36.5%, hookworm 53.6%, and Trichuris trichiura 14.4%. Of the 499 school children examined by single Scotch-tape technique, 278 (55.7%) were found to be infected with Enterobius vermicularis.

2. Studies of the egg distribution of S. japonicum in various organs of mice and hamsters indicated that there are three types of egg distribution in mice. The Ilan and Changhua strains of S. japonicum belong to the first type in which the eggs deposited in the liver are greatest in number and proportion at 40 days of infection, and then decrease while the infection is longer. In contrary, the proportion of eggs deposited in the small intestine is going up from 40 days to 70 days after infection. The Chinese parasite shows the second type of egg distribution. Similar egg distribution is observed in the liver and small intestine in the first and second types. But significantly higher proportion of egg deposition in the large intestine is found. The Japanese and Philippine parasites are the third type in which the egg deposited in the small intestine are the greatest in number and proportion usually through the period from 40 to 60 days after infection.

The Ilan and Changhua S. japonicum demonstrated similar pattern of egg distribution. More eggs were quantitated in the liver at the early stage than the later stage of infection. In the large intestine, however, more eggs were quantitated in the later stage than in the early stage of infection. The characteristics of egg distribution in the Japanese S. japonicum was the lowest proportion of eggs deposited in the liver, and exceedingly high proportion of egg distribution in the small intestine at the early stage of infection. On the other hand, the characteristic egg distribution of

the Chinese parasite was observed in the large intestine in which the highest number of eggs were always quantitated and significantly less involvement in the small intestine.

3. Gel-diffusion studies on sera from animals such as rabbits, dogs, monkeys and rats which were infected with S. japonicum were made. Results obtained indicated that no distinct relationship between the precipitating antibody production and the degree of susceptibility of animal to the infection with S. japonicum was observed. The best antibody production was demonstrated in the rabbit, and the lines demonstrated in the tests were also more distinct in rabbits than in other species of animals.

It seems that, in general, the Ilan strain of S. japonicum possesses higher precipitating antibody production than the Changhua strain of S. japonicum.

Table of Contents

I. General Introduction	Page 1
II. Parasitological Studies at Jui-Ping Area: a New Habitat for <u>Oncomelania hupensis chiui</u> in Taiwan	3
1. Introduction	3
2. Materials and Methods	3
3. Results and Discussion	3
III. Egg Distribution of Human Strains of <u>Schistosoma japonicum</u> in Various Organs of Mice and Hamsters	5
1. Introduction	5
2. Material and Methods	5
3. Results and Discussion	6
IV. Gel-Diffusion Studies on Sera from Animals Infected with <u>Schistosoma japonicum</u>	8
1. Introduction	8
2. Materials and Methods	8
3. Results and Discussion	8
V. Literature Cited	10
Tables	11-27
List of Publication and Graduate Students	28
Distribution list	29
DD Form 1473	30



List of Tables

Table 1.	Results of examinations on Jui-Ping <u>Oncomelania hupensis chiui</u> exposed to geographic strains of <u>Schistosoma japonicum</u> miracidia.	Page 11
2.	Results of intradermal test for <u>Schistosoma japonicum</u> among inhabitants of Jui-Ping area, by age and sex.	12
3.	Prevalence of intestinal helminthic infections among inhabitants of Jui-Ping, by sex.	13
4.	Prevalence of pinworm infection among school children of Jui-Ping area, by school and sex.	14
5.	Egg distribution of Japanese strain of <u>Schistosoma japonicum</u> in various organs of mice, according to days of infection.	15
6.	Egg distribution of Philippine strain of <u>Schistosoma japonicum</u> in various organs of mice, according to days of infection.	16
7.	Egg distribution of Chinese strain of <u>Schistosoma japonicum</u> in various organs of mice, according to days of infection.	17
8.	Egg distribution of Japanese strain of <u>Schistosoma japonicum</u> in various organs of hamsters, according to days of infection.	18
9.	Egg distribution of Chinese strain of <u>Schistosoma japonicum</u> in various organs of hamsters, according to days of infection.	19
10.	Precipitin lines found in sera of rabbits infected with Changhua strain of <u>Schistosoma japonicum</u> along with the worm recovery rate.	20
11.	Precipitin lines found in sera of rabbits infected with Ilan strain of <u>Schistosoma japonicum</u> along with the worm recovery rate.	21
12.	Precipitin lines found in sera of dogs infected with Changhua strain of <u>Schistosoma japonicum</u> along with the worm recovery rate.	22

13. Precipitin lines found in sera of dogs infected with Ilan strain of <u>Schistosoma japonicum</u> along with the worm recovery rate.	Page 23
14. Negative results of gel-diffusion tests on sera of monkeys infected with Changhua strain of <u>Schistosoma japonicum</u> along with the worm recovery rate.	24
15. Precipitin lines found in sera of monkeys infected with Ilan strain of <u>Schistosoma japonicum</u> along with the worm recovery rate.	25
16. Precipitin lines found in sera of rats infected with Changhua strain of <u>Schistosoma japonicum</u> along with the worm recovery rate.	26
17. Precipitin lines found in sera of rats infected with Ilan strain of <u>Schistosoma japonicum</u> along with the worm recovery rate.	27

## Section I

### General Introduction

Schistosoma japonicum was first discovered in Taiwan by Takegami (1914)<sup>1</sup> in Changhua County, in the central part of the western coastal plain of the island. This parasite is defined as a zoophilic or non-human strain of S. japonicum. Experimentally the snail host, Oncomelania hupensis formosana, in Changhua, although susceptible to the local strain of S. japonicum, was found to be refractory to any human strains of the parasite from Japan, the Philippines and mainland China (DeWitt, 1954<sup>2</sup>; Hsu and Hsu, 1960<sup>3</sup>). Therefore, it was stated that no oncomelamid snails on the island was susceptible to the infection with the human strain of S. japonicum, and because of this no indigenous human schistosomiasis has been established in Taiwan. In 1962, Kuntz<sup>4</sup> found a new endemic area for S. japonicum in Ilan County, in the northeastern part of Taiwan. Subsequently a considerable amount of effort has been made to determine whether the parasite prevalent in Ilan is the human strain or not. So far, no human case has been discovered, and three rodents were the only natural hosts that have been found in this area. Therefore, it is considered that the Ilan S. japonicum may also be a non-human strain. Susceptibility studies on O. h. formosana from this area to infection with S. japonicum, however, revealed that they were slightly susceptible to the human strain of S. japonicum from Japan and the Philippines (Moose and Williams, 1964)<sup>5</sup>. In the meantime, in the course of a study on Paragonimus in the northern tip of Taiwan, a new species of snail, O. h. chiui, was incriminated as the snail host for P. iloktsuenensis at Alilao village, Taipei County (Chiu, 1961)<sup>6</sup>. Subsequent studies on the susceptibility of this species of snail to infection with the geographic strains of S. japonicum demonstrated that O. h. chiui is highly susceptible not only to the two zoophilic strains from Taiwan, but also to the three human strains of S. japonicum from Japan, the Philippines and mainland China (Chiu, 1965<sup>7</sup>, 1966<sup>8</sup>, 1967<sup>9</sup>, 1968<sup>10</sup>). As a result of these studies, it is essential that further investigations be carried out to further establish the possibility of human schistosomiasis becoming established in Taiwan. Therefore, studies on host-parasite relationships of S. japonicum and its host have been initiated and designed to investigate:

1. The distribution of oncomelamid snails in Taiwan, as well as searching for new foci of Oncomelania hupensis chiui.
2. The susceptibility of oncomelamid snails from known sites or from new focus to infection with both the human and non-human strains of S. japonicum.
3. The nature of the definitive host for the Ilan strain of S. japonicum.
4. Adaptation of S. japonicum in O. h. chiui snails.

Main findings of the first two years of studies were as follows:

1. A new habitat for O. h. chiui was found at Jui-Ping village, on the north coast of Taiwan, approximately 10 km west of the mouth of the Tamsui River.

2. O. h. formosana from , villages in Ilan have been demonstrated to be susceptible only to the Ilan strain of S. japonicum and resistant to the Changhua, Japanese, Philippine and Chinese strains of the parasite.

O. h. formosana from 17 villages in Changhua were tested with two Formosan strains of S. japonicum. The infection rates varied from 21.3 to 73.8% for Changhua parasite, and 0-11.4% for the Ilan parasite. O. h. formosana from Kaohsiung, on the other hand, have been demonstrated to be resistant to Ilan, Changhua and Japanese parasite.

3. Fourteen species of animals have been exposed to the Ilan strain of S. japonicum to study the nature of the definitive host of this strain of parasite. Some species of animals have been exposed to the Changhua, Japanese, Philippine and Chinese strains of S. japonicum. Results obtained from the studies on the prepatent period, relative amount of egg passage in stools, duration of egg passage in the feces, worm recovery rate, and egg distribution in various organs of mice and hamsters indicate that the Ilan strain is more closely related to the Changhua strain than to the other strains. The dog, goat and squirrel have been found to be excellent host for the Ilan S. japonicum.

4. The Ilan strain of S. japonicum has been passed in O. h. chiui for 5 generations using the mouse as the definitive host. The infectivity of the parasite was tested in O. h. formosana and mice. A few differences were observed between the parasite maintained in O. h. chiui and O. h. formosana.

5. Intradermal tests among inhabitant of the Ilan endemic area indicated that 28 (2.6%) of 1,088 people examined showed positive reactions. However, the stool examinations of 727 people including 28 positive reactors were all negative for the ova of S. japonicum.

Continuous efforts have been made in the last one year to complete the present studies. In addition, gel-diffusion studies on sera from animals infected with S. japonicum have been made to utilize the blood of infected animals used in the study and the new immunology laboratory which has just been set up in our department. Results obtained are reported as followings:

## Section II

### Parasitological Studies at Jui-Ping Area: a New Habitat for Oncomelania hupensis chiui in Taiwan

#### 1. Introduction

Intensive surveys for oncomelaniid snails revealed a new habitat for O. h. chiui at Jui-Ping village, Linkou District, Taipei County, in the second year of the present project. Subsequently, parasitological studies were carried out to determine the distribution of O. h. chiui at this area, the susceptibility of the snail to the infection with geographic strains of S. japonicum, and the presence of S. japonicum at this area.

#### 2. Materials and Methods

O. h. chiui was searched around the area to determine the distribution of the snail. The snails were collected from each place where O. h. chiui was found, and were examined for natural infection with S. japonicum by crush preparations.

The susceptibility of O. h. chiui from this area to infection with geographic strains of S. japonicum including the Changhua, Ilan, Japanese, Philippine, and Chinese S. japonicum was studied. Each strain of the parasite was tested on 50 snails along with a control. Each snail was exposed individually to 5-7 miracidia. Ninety-eight to 157 days after infection, the surviving snails were examined by means of crush preparations.

A total of 736, 399 males and 337 females, received the intradermal test for S. japonicum. The antigen was obtained from the U. S. Army 406th Medical Laboratory in Japan. Fecal containers were given out to people who received skin testings, and they were asked to submit stool specimens for examination in 2 days. As a result, 605 people, 337 males and 268 females, submitted stools. Stools were examined by direct smear and the AMS III concentration technique.

The Scotch-tape technique was performed among primary school children in the morning to determine the pinworm infection rate in this area. A total of 499 school children (264 males and 235 females), consisting of 152 from Jui-Ping and 347 from Chia-Pao primary schools, were examined.

#### 3. Results and Discussion

A total of 2,682 O. h. chiui snails were collected and examined for natural infection with S. japonicum. Not a single snail was found to be infected with schistosome. O. h. chiui is found on a hill side along the coast, at least 4 km long in distance from Jui-Ping to the west. An

average size of the 100 snails from Jui-Ping (4.7 mm long by 2.5 mm wide) is larger than those from the type locality, Alilao (3.8 mm long by 2.0 mm wide). The apex of the shell is eroded in 15 of the 100 snails from Jui-Ping, and 88 of the 100 snails from Alilao.

Susceptibility studies of the snail to the infection with geographic strains of S. japonicum indicated that similarly to the snail from Alilao, the snails from the new habitat have been demonstrated to be highly susceptible to all geographic strains of S. japonicum from Changhua, Ilan, Japan, the Philippines and mainland China (Table 1). The infection rates ranged from 44.7% to 93.8%. Not a single snail was infected in the control group.

Results obtained from the intradermal tests are shown in Table 2 according to age and sex of the examinees. Of the 736 people who received intradermal tests, 19 (2.6%) showed positive reactions with S. japonicum antigen. It was the same positive rate as we obtained in Ilan. In Ilan 28 (2.6%) of the 1,088 people examined showed positive reactions with S. japonicum antigen. When the rates were observed among four age groups, the highest positive rate was observed in the age group of from 41 to 60 years (4.6%). No positive reactor was detected in the age group of from 21 to 40, and it is not known why the rate for the 1-20 years group was as high as 2.4%. On the other hand, significantly higher rate was seen in males (3.3%) than in females (1.8%). Stool examinations of 605 people including 19 positive skin test reactors were all negative for S. japonicum eggs.

Results obtained from both direct smear and AMS III concentration technique of fecal examinations are shown in Table 3 according to the sex of the examinees. Of the 605 people examined, 444 (73.4%) were found to be infected with one or more helminths. Only the common parasites, Ascaris lumbricoides, hookworm and Trichuris trichiura, were found. The highest rate of infection was found for hookworm in which 324 (53.6%) of the 605 people examined were found infected. Most of the examinees were farmers who used nightsoil in the cultivation of vegetables. A little higher rate was observed in males (55.8%) than in females (52.2%). The infection rates for Ascaris lumbricoides and Trichuris trichiura were 36.5% and 14.4%, respectively. The rates between sex for these two species of parasites were close.

Of the 499 school children examined by single Scotch-tape technique, 278 (55.7%) were found to be infected with Enterobius vermicularis (Table 4). The infection rates for the two primary schools were similar. But a significantly higher infection rate was observed in males (60.2%) than in females (50.6%).

### Section III

#### Egg Distribution of Human Strains of Schistosoma japonicum in Various Organs of Mice and Hamsters

##### 1. Introduction

Very little is known about the definitive host for the Ilan strain of S. japonicum. The only report dealing with the finding of reservoir host was made by Kuntz (1965)<sup>4</sup>. He examined 300 wild and domestic animals, and found 2 Rattus rattus and 1 Bandicota indica nemorivaga naturally infected with S. japonicum. In later studies, Khaw and Fan (1966)<sup>11</sup> after examining 1,361 stools, organs and tissues of 356 pigs, 188 buffaloes, 20 dogs, 247 poultry and 550 rodents were unable to find either the adult worms or ova.

In the last two years, the susceptibility of various species of mammals to the infection with the Ilan strain of S. japonicum has been studied to understand the nature of the definitive host for this parasite. Some species of animals have also been exposed to the other geographic strains of S. japonicum for the comparison. The degree of susceptibility of each species of animal is based upon the following information: a) length of the prepatent period, b) quantitation of eggs in stools, c) duration of egg passage in the feces, d) worm recovery rate, e) location of worms in the host, f) distribution of eggs in various organs of mice and hamsters, g) egg hatching ability, and h) growth and development of the parasite.

The first four items have been studied, and the last two items are still under study. Results obtained from the study on egg distribution of the Ilan and Changhua strains of S. japonicum in various organs of mice and hamsters have been reported in the previous annual reports. In order to complete the study, egg distribution of the three human strains of S. japonicum in various organs of mice and hamsters was studied for the comparison.

##### 2. Materials and Methods

The laboratory mouse (Swiss Webster) and hamster (Golden Syrian) have been subjected to quantitative percutaneous infections of cercariae obtained from pools of infected snails. The Japanese strain was obtained from O. h. nosophora, the Philippine strain from O. h. quadrasi and the Chinese strain from O. h. chiui. Animals were sacrificed at intervals of 40, 50, 60 and 70 days after infection. Following the recovery of worms, the liver, small intestine, large intestine, stomach, spleen, mesentery, lungs and brain were removed and assayed for eggs by trypsin digestion. Eggs quantitated were pooled together and tabulated in Tables 5-9 according to the days of infection. Most eggs were found in the liver, small intestine and large intestine. The eggs found in other organs were low in number and were therefore pooled.

### 3. Results and Discussion

In mice infected with the Japanese parasite (Table 5), the greatest number of eggs were quantitated in the small intestine (62.7%) 40 days after infection, followed by the liver (26.8%), large intestine (10.1%) and others (0.4%). Eggs deposited in the small intestine increased in number and proportion (87%) 50 days, but decreased rapidly in number and proportion (17.3%) 70 days after infection. The number in the liver, on the other hand, decreased at 50 days (9%), and increased suddenly at 70 days (79.9%). Decrease of the eggs in proportion was observed in the large intestine through 70 days after infection, and there was no significant change in the other organs throughout the study period. It was significant that most eggs deposited in the small intestine and liver regardless of the days of infection.

Similar results were obtained for the Philippine parasite in mice (Table 6). The greatest number and proportion of egg deposition were observed in the small intestine throughout the study from 40 to 60 days after infection. The eggs deposited in the liver (9.7%) was less than that of in the large intestine (16%) at 40 days. But later 50-60 days after infection, eggs quantitated in these two organs were very close. High proportion of egg count was observed in the other organs (10.2%) 60 days after infection.

The egg distribution of Chinese parasite in mice demonstrated different type from other strains of the parasite (Table.7) The egg distribution in the liver and small intestine was very similar to the Ilan and Changhua strains of S. japonicum. The proportion of eggs deposited in the liver decreased while the infection was getting longer. On the other hand, the increase of the proportion of eggs deposited in the small intestine was observed. Involvement of the large intestine was characteristic in the Chinese strain of S. japonicum. Eggs deposited in the large intestine were from 29.1% to 36.1% in proportion through 40-60 days after infection, and it was higher than any other strain of the parasite.

On the whole, there are three types of egg distribution in mice. The Ilan and Changhua strains belong to the first type in which the eggs deposited in the liver are greatest in number and proportion at 40 days of infection, and then decrease while the infection is going longer. In contrary, the proportion of eggs deposited in the small intestine is going up from 40 days to 70 days after infection. The Chinese strain of S. japonicum shows the second type of egg distribution. Similar egg distribution is observed in the liver and small intestine in the first and second types. But significantly higher proportion of egg deposition in the large intestine is found, usually around 30% from 40 to 60 days after infection. The Japanese and Philippine parasites are the third type in which the eggs deposited in the small intestine are the greatest in number and proportion usually through the period from 40 to 60 days after infection.



The egg distribution of the Japanese and Chinese strains of S. japonicum in various organs of hamsters was studied to compare with the Ilan and Changhua strains of the parasite. As seen in Table 8, the proportion of eggs deposited in the liver was very low (3.9-9.9%) in the Japanese S. japonicum regardless of the days of infection as compared with the two Formosan strains of the parasite which are usually more than 20% (Annual Report, 1970). On the other hand, the highest proportion was observed in the small intestine (74.9%) at 40 days. It, however, decreased at 50 days (56.7%) and 60 days (37.4%). The increase of egg distribution was observed in the large intestine from 18.7% (40 days) to 32.5% (50 days) and 58.4% (60 days).

In the Chinese strain of S. japonicum (Table 9), the proportion of eggs deposited in the liver was 28.7% at 40 days, then increased to 36.7% at 50 days and remained approximately the same rate, 37.9% at 60 days. The fewest number of eggs were quantitated in the small intestine through the period of from 40 to 60 days after infection (4.1-12.7%). The eggs deposited in the large intestine were greatest and remained almost the same proportion from 40 to 60 days after infection (57.0-28.9%).

Results obtained indicated that egg distribution of geographic strains of S. japonicum in various organs of hamsters was quite characteristic. The Ilan and Changhua S. japonicum demonstrated similar pattern of egg distribution. More eggs were quantitated in the liver at the early stage than the later stage of infection. In the large intestine, however, more eggs were quantitated in the later stage than in the early stage of infection. The characteristics of egg distribution in the Japanese S. japonicum was the lowest proportion of eggs deposited in the liver, and exceedingly high proportion of egg distribution in the small intestine at the early stage of infection. On the other hand, the characteristic egg distribution of the Chinese S. japonicum was observed in the large intestine in which the highest number of eggs were always quantitated and significantly less involvement in the small intestine.

## Section IV

### Gel-Diffusion Studies on Sera from Animals Infected with Schistosoma japonicum

#### 1. Introduction

Gel-diffusion studies on sera from animals infected with S. japonicum were made to utilize the blood of infected animals used in the present project, and also to utilize the new immunology laboratory which has just been set up in our department. The purposes of this study are 1) to investigate the difference of the precipitating antibody production in suitable and unsuitable hosts, and 2) to compare the precipitating antibody production between the Ilan and the Changhua strains of S. japonicum

#### 2. Materials and Methods

The dog and rabbit were used as suitable host, and the monkey and rat as unsuitable host. Two groups of animals were designed for the Ilan and Changhua strains of S. japonicum. Each group contained 5 each of the dog, rabbit and monkey, and 18 rats. The number of cercariae exposed were: 312-523 for monkey, 326-563 for dog, 228-282 for rabbit and 121-361 for rat. Sera were collected from the monkey, dog and rabbit before the infection, and every 10 days from 20 days of infection till 60 days of infection, and then every 30 days thereafter. Sera of rats were obtained from the heart directly after sacrificing the animal. Three rats were sacrificed each time according to the same time schedule of the other animal species including 3 non-infected rats as control. The preparation of antigen described by Damian (1966)<sup>12</sup> was followed. The gel-diffusion tests performed were of the double diffusion plate technique of Ouchterlony. The agar used was 1% Noble agar (Difco) in 0.02M Phosphate buffered 0.9% NaCl at pH 7.0.

#### 3. Results and Discussion

Results obtained indicated that no distinct relationship between the precipitating antibody production and the degree of susceptibility of animal to the infection with S. japonicum was observed (Tables 10-17). All 10 rabbits began to show one line 40 days after infection regardless of the strain of the parasite. The rabbit numbered C-Rb-1 which was infected with the Changhua parasite demonstrated the second line at 50 days of infection. This line also appeared in two rabbits, I-Rb-23 and I-Rb-24, which were infected with the Ilan parasite 120 and 150 days after infection, respectively. The third line was found in serum of I-Rb-24 40 days after the infection.

One of the 5 dogs and 3 of the 5 dogs infected with the Changhua and

Ilan strains of S. japonicum, respectively, showed one line 40 days after infection, and another dog with the Ilan parasite showed the corresponding line 90 days after infection. One dog, C-C-12, infected with the Changhua parasite demonstrated the second line 50 days after infection.

Not a single monkey showed one line after the first infection. Two monkeys infected with the Ilan S. japonicum, however, showed one line 30 days after the challenge infection at the 136th day of the first infection. No line was observed in monkeys infected with the Changhua S. japonicum even after the challenge infection.

Two and three rats infected with the Changhua and Ilan S. japonicum, respectively, gave one line either 50 or 60 days after infection. The rest of rats showed no lines. It was not possible to follow the precipitating antibody in the rat, as the animals were sacrificed in order to collect the sera from the heart.

Results obtained seem to indicate, in general, that the Ilan strain of S. japonicum possesses higher precipitating antibody production than the Changhua strain of S. japonicum. The lines demonstrated in the tests were more distinct in rabbits than in other species of animals.

## Section V

### Literature Cited

1. Takegami, K. 1914. On the "Schistosomadae" in Formosa (Preliminary Report). *Formosan Agr. Review*, 89: 345-350.
2. DeWitt, W. B. 1954. Susceptibility of snail vectors to geographic strains of Schistosoma japonicum. *J. Parasit.* 40: 453-446.
3. Hsu, S. Y. Li and Hsu, H. F. 1960. Infectivity of the Philippine strain of Schistosoma japonicum in Oncomelania hupensis, O. formosana and O. nosophora. *J. Parait.* 46: 793-796.
4. Kuntz, R. E. 1965. Zoophilic schistosomiasis with a report of a new locality on Taiwan. *J. Formosan Med. Assoc.* 64: 649-657.
5. Moose, J. W. and Williams, J. E. 1964. The susceptibility of geographical races of Oncomelania formosana to infection with human strains of Schistosoma japonicum. 406 Med. Lab. Research Report, U. S. Army Med. Command, Japan, Presented at First International Congress of Parasit. in More, Italy, Sept. 1964.
6. Chiu, J. K. 1961. Snail host of Paragonimus iloktsuenensis in Taiwan. *J. Formosan Med. Assoc.* 60: 1173.
7. Chiu, J. K. 1965. Tricula chiui: a new snail host for Formosan strain of Schistosoma japonicum. *J. Parasit.* 51: 206.
8. Chiu, J. K. 1966. Susceptibility of Tricula chiui to infection with geographic strains of Schistosoma japonicum and various species of Paragonimus. *J. Formosan Med. Assoc.* 65: 654-655.
9. Chiu, J. K. 1967. Susceptibility of Oncomelania hupensis chiui to infection with Schistosoma japonicum. *Malacologia*, 6: 145-153.
10. Chiu, J. K. 1968. Cercaria production of geographic strains of Schistosoma japonicum in Oncomelania hupensis chiui. *J. Formosan Med. Assoc.* 67: 259-265.
11. Khaw, O. K. and Fan, P. C. 1966. Schistosomiasis japonica on Taiwan. *Chinese Med. J.* 13: 128-130.
12. Damian, R. T. 1966. An immunodiffusion analysis of some antigens of Schistosoma mansoni adults. *Exp. Parasit.* 18: 255-265.

Table 1. Results of examinations on Jui-Ping Oncomelania hupensis chui exposed to geographic strains of Schistosoma japonicum miracidia.

Strain of parasite	No. snails exposed*	No. snails examined	No. snails infected	Percent infected
Changhua	50	48	45	93.8
Han	50	23	20	87.0
Japanese	50	38	17	44.7
Philippine	50	22	20	90.9
Chinese	50	10	8	80.0
Control	50	39	0	0

\* Each snail was exposed to 5-7 miracidia individually.

Table 2. Results of intradermal test for Schistosoma japonicum among inhabitants of Jui-Ping area, by age and sex.

Age or Sex	Number examined	Number positive	Percent positive
<b>Age</b>			
1 - 20	531	13	2.4
21 - 40	55	0	0
41 - 60	109	5	4.6
61 - 80	41	1	2.4
<b>Sex</b>			
Male	399	13	3.3
Female	337	6	1.8
<b>Total</b>	<b>736</b>	<b>19</b>	<b>2.6</b>

Table 3 Prevalence of intestinal helminthic infections among inhabitants of Jui-Ping, by sex.

Sex	Number examined	No. with helminth		Species of Helminth					
				Ascaris		Hookworm		Trichuris	
		No.	%	No.	%	No.	%	No.	%
Male	337	247	73.3	120	35.6	188	55.8	51	15.1
Female	268	197	73.5	106	39.6	140	52.2	36	13.4
Total	605	444	73.4	226	37.4	328	54.2	87	14.4

Table 4. Prevalence of pinworm infection among school children of Jui-Ping area, by school and sex.

School	Sex	No. examined	No. infected	% infected
Jui-Ping	Male	75	44	58.7
	Female	77	39	50.6
	Total	152	83	54.6
Chia-Pao	Male	189	115	60.8
	Female	158	80	50.6
	Total	347	195	56.2
Grand Total	Male	264	159	60.2
	Female	235	119	50.6
	Total	499	278	55.7



Table 5. Egg distribution of Japanese strain of Schistosoma japonicum in various organs of mice, according to days of infection.

Organ	Days of Infection					
	40		50		70	
	No. eggs	%	No. eggs	%	No. eggs	%
Liver	169,928	26.8	165,710	9.0	190,408	79.9
Small Intestine	398,310	62.7	1,606,250	87.0	41,180	17.3
Large Intestine	64,050	10.1	70,443	3.8	0,050	2.5
Others	2,340	0.4	3,992	0.2	696	0.3
Total	634,628	100	1,846,395	100	238,334	100
No. adults recovered	85 males 77 females		64 males 65 females		12 males 11 females	
No. of mice studied	4		5		2	

Table 6. Egg distribution of Philippine strain of *Schistosoma japonicum* in various organs of mice, according to days of infection.

Organ	Days of Infection					
	40		50		60	
	No. eggs	%	No. eggs	%	No eggs	%
Liver	260,408	9.7	80,976	26.9	35,205	15.6
Small Intestine	1,993,200	74.2	149,365	49.6	135,690	60.0
Large Intestine	430,500	16.0	70,646	23.5	32,250	14.3
Others	3,898	0.1	121	0.04	23,162	10.2
Total	2,688,006	100	301,108	100	226,307	100
No. adults recovered	29 males 28 females		20 males 17 females		7 males 7 females	
No. of mice studied	4		2		2	

Table 7. Egg distribution of Chinese strain of *Schistosoma japonicum* in various organs of mice, according to days of infection.

Organ	Days of Infection					
	40		50		60	
	No. eggs	%	No. eggs	%	No. eggs	%
Liver	27,182	40.1	71,180	37.8	86,223	16.5
Small Intestine	16,110	23.8	62,100	33.0	280,810	50.7
Large Intestine	24,420	36.1	54,800	29.1	185,900	33.6
Others	16	0.02	132	0.1	782	0.1
Total	67,728	100	188,212	100	553,715	100
No. adults recovered	28 males 27 females		35 males 23 females		20 males 13 females	
No. of mice studied	2		2		3	

Table 8. Egg distribution of Japanese strain of *Schistosoma japonicum* in various organs of hamsters, according to days of infection.

Organ	Days of Infection					
	40		50		60	
	No. eggs	%	No. eggs	%	No. eggs	%
Liver	248,467	5.4	790,212	9.9	93,893	3.9
Small Intestine	3,475,200	74.9	4,537,450	56.7	916,702	37.4
Large Intestine	865,410	18.7	2,603,400	32.5	1,430,516	58.4
Others	51,884	1.0	70,055	0.9	7,332	0.3
Total	4,640,961	100	8,001,117	100	2,448,443	100
No. adults recovered	38 males 30 females		51 males 39 females		11 males 10 females	
No. of hamsters studied	5		5		5	

Table 9. Egg distribution of Chinese strain of *Schistosoma japonicum* in various organs of hamsters, according to days of infection.

Organ	Days of Infection					
	40		50		60	
	No. eggs	%	No. eggs	%	No. eggs	%
Liver	44,391	28.7	124,490	36.7	161,523	37.9
Small Intestine	19,668	12.7	14,171	4.1	19,623	4.6
Large Intestine	90,664	58.6	201,461	58.9	242,771	57.0
Others	117	0.1	1,048	0.3	1,828	0.5
Total	154,840	100	342,170	100	425,745	100
No. adults recovered	18 males 17 females		17 males 13 females		18 males 11 females	
No. of hamsters studied	4		5		4	

Table 10. Precipitin lines found in sera of rabbits infected with Changhua strain of Schistosoma japonicum along with the worm recovery rate.

Animal No.	Days of Infection							Cercariae exposed	Worms recovered	Rate (%)
	0*-30	40	50	60	90	120	150			
C-Rb-1	0	1	1, 2	1, 2	1, 2	1, 2	1, 2	235		
C-Rb-2	0	1	1	1				237	176	74.3
C-Rb-3	0	1	1					282	217	77.0
C-Rb-4	0	1	1	1				228	134	58.8
C-Rb-5	0	1						245	172	70.2
Total**								992	699	70.5

\* Control serum collected from rabbits before the infection.

\*\* Excluding C-Rb-1.

Table II. Precipitin lines found in sera of rabbits infected with Han strain of Schistosoma japonicum along with the worm recovery rate.

Animal No.	Days of Infection							Cercariae exposed	Worms recovered	Rate (%)
	0*-30	40	50	60	90	120	150			
I-Rb-23	0	1	1	1	1	1, 2		241	85	35.3
I-Rb-24	0	1, 3	1, 3	1, 3	1, 3	1, 3	1, 2, 3	243		
I-Rb-25	0	1	1	1				259	166	64.1
I-Rb-26	0	1	1	1				266	138	51.9
I-Rb-27	0	1	1	1				241	76	31.5
Total**								1007	465	46.2

\* Control serum collected from rabbits before the infection.

\*\* Excluding I-Rb-24.

Table 12. Precipitin lines found in sera of dogs infected with Changhua strain of Schistosoma japonicum along with the worm recovery rate.

Animal No.	Days of Infection									Cercariae Worms		Rate (%)	
	0*	30	40	50	60	90	102**	122	132	142	exposed		recovered
C-D-10	0	0	0								504	237	47.0
C-D-12	0	1	1, 2	1, 2							521	282	54.1
C-D-13	0	0	0	0	0	0	0	0	0	0	537 (517)#		
C-D-14	0	0	0	0	0	0	0	0	0	0	549 (423)		
C-D-16	0	0	0	0							542	358	66.1
Total##											1567	877	56.0

\* Control serum collected from dogs before the infection.

\*\* The day of challenge infection was made.

# The figures in parentheses are the number of cercariae used for the challenge infection.

## Excluding C-D-13 and C-D-14.



Table 13. Precipitin lines found in sera of dogs infected with Han strain of Schistosoma japonicum along with worm recovery rate.

Animal No.	Days of Infection									Cercariae exposed	Worms recovered	Rate (%)
	0*-30	40	50	60	90	102**	122	132	142			
I-D-20	0	1	1	1						328	155	47.5
I-D-21	0	0	0	0	1	1	1	1	1	563 (515)#		
I-D-22	0	1	1	1						511	188	36.8
I-D-23	0	0	0	0						504		
I-D-24	0	1	1	1						515		
Total ##										837	343	41.0

\* Control serum collected from dogs before the infection.

\*\* The day of challenge infection was made.

# The figure in parenthesis is the number of cercariae used for the challenge infection.

## Including only I-D-20 and I-D-22.

Table 14. Negative results of gel-diffusion tests on sera of monkeys infected with Changhua strain of Schistosoma japonicum along with the worm recovery rate.

Animal No.	Days of Infection								Cercariae exposed	Worms recovered	Rate (%)
	0*-60	90	120	140**	160	170	180	190			
C-My-6	0	0	0	0	0	0	0	0	497(925)#		
C-My-7	0	0	0	0	0	0	0	0	523(1055)		
C-My-8	0								515	210	40.8
C-My-9	0								502	185	34.9
C-My-10	0								317	0	0
<b>Total ##</b>									<b>1334</b>	<b>395</b>	<b>29.6</b>

\* Control serum collected from monkeys before the infection.

\*\* The day of challenge infection was made.

# The figures in parentheses are the number of cercariae used for the challenge infection.

## Excluding C-My-6 and C-My-7.

Table 15. Precipitin lines found in sera of monkeys infected with Ilan strain of Schistosoma japonicum along with the worm recovery rate.

Animal No.	Days of Infection								Cercariae exposed	Worms recovered	Rate (%)
	0*	60	90	120	136**	156	166	176			
I-My-18	0								455	36	7.9
I-My-19	0	0	0	0	0	1	1	1	486 (540)#		
I-My-20	0	0	0	0	0	1	1	1	500 (505)		
I-My-21	0								513	7	1.4
I-My-22	0								494	240	48.6
Total##									1462	283	20.4

\* Control serum collected from monkeys before the infection.

\*\* The day of challenge infection was made.

# The figures in parentheses are the number of cercariae used for the challenge infection.

## Excluding I-My-19 and I-My-20.

Table 16. Precipitin lines found in sera of rats infected with Changhua strain of Schistosoma japonicum along with the worm recovery rate.

Animal No.	Days of Infection	Precipitin line	Cercariae exposed	Worms recovered	Rate (%)
Control	0	0			
C-R-26	20	0	153	48	31.4
C-R-27	20	0	163	36	22.1
C-R-19	20	0	121	22	18.2
C-R-24	30	0	161	45	28.0
C-R-25	30	0	162	57	35.2
C-R-17	30	0	124	11	8.9
C-R-22	40	0	153	56	36.6
C-R-23	40	0	149	65	43.6
C-R-15	40	0	160	32	20.0
C-R-11	50	0	361	50	13.9
C-R-12	50	1	151	18	11.9
C-R-18	50	0	128	47	36.7
C-R-13	60	0	159	20	12.6
C-R-14	60	0	141	5	3.6
C-R-16	60	1	130	52	40.0
C-R-10	90	0	258	8	3.1
C-R-21	90	0	145	15	10.3
C-R-20	90	0	121	17	14.1
Total			2940	604	20.5

Table 17. Precipitin lines found in sera of rats infected with Han strain of Schistosoma japonicum along with the worm recovery rate.

Animal No	Days of Infection	Precipitin line	Cercariae exposed	Worms recovered	Rate (%)
Control	0	0			
I-R-15	20	0	145	19	13.1
I-R-16	20	0	147	28	19.1
I-R-9	20	0	164	32	19.5
I-R-17	30	0	135	3	2.2
I-R-14	30	0	138	7	5.1
I-R-12	30	0	159	11	6.9
I-R-5	40	0	143	67	46.9
I-R-6	40	0	168	34	20.2
I-R-8	40	0	173	14	8.1
I-R-3	50	0	164	43	26.2
I-R-4	50	1	129	37	28.7
I-R-10	50	1	144	13	9.0
I-R-1	60	0	121	35	28.9
I-R-2	60	0	129	27	20.9
I-R-11	60	1	159	22	13.8
I-R-18	90	0	150	5	3.3
I-R-13	90	0	142	4	2.8
Total			2510	401	16.0

## List of Publications and Graduate Students

### I. Publications

1. Cercaria production of geographic strains of Schistosoma japonicum in Oncomelania hupensis chiui. J. Formosan Med. Assoc. 67 (7): 259-265, 1968.
2. Susceptibility of Oncomelania hupensis chiui and related snails to infection with Paragonimus. J. Formosan Med. Assoc. 68 (1): 7-14, 1969.
3. Susceptibility of various species of mammals to infection with Ilan strain of Schistosoma japonicum. Proceedings of the Fourth South-east Asian Seminar on Parasit. & Trop. Med., Schistosomiasis and other Snail-transmitted Helminthiasis. Manila, Feb. 1969, 49-56.
4. Host-Parasite relationships of Schistosoma japonicum in Taiwan. (Abstract of Special Lecture at the 63rd Annual Meeting of the Formosan Med. Assoc.). J. Formosan Med. Assoc. 69 (11): 540-541, 1970.
5. Schistosome skin testing and intestinal parasite survey among inhabitants of Yuan-Shan, Ilan County, Taiwan. Chinese J. Microbiol. 4 (3, 4), 1971. (in press)
6. Proteins and dehydrogenase isozymes of Oncomelania. Chinese J. Microbiol. 4 (3, 4), 1971. (in press)

### II. Graduate Students

WEN, Y. F. M. Sc.  
SU, K. E. M. Sc.  
HWANG, Y. Y. M. Sc.  
CHOW, K. M. Sc.  
CHENG, L. Y. M. Sc.