Migration and Growth of *Paragonimus ohirai* and *P. miyazakii* Metacercariae Artificially Introduced into Rats

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The migration and growth of lung flukes, *Paragonimus*, in a final host varies according to species and/or definitive host. In oral infections of albino rats with *P. ohirai* and *P. miyazakii*, most of the worms of both species migrate into the liver of their hosts before reaching the lungs. *P. miyazakii*, however, has a tendency to stay in the liver for a longer period than *P. ohirai* in the case of oral infections of rats (Yokogawa *et al.*, 1964a; Tada, 1969). The reasons why these worms migrate into the liver, or for the duration of time they stay in this organ, remain uncertain.

The present study was undertaken to compare the migration and growth of *P. ohirai* and *P. miyazakii* the metacercariae of which had been introduced into the pleural cavity of rats. These investigations were also carried out to investigate if both species introduced could mature without migration into the liver of the rats.

MATERIALS AND METHODS

Ninty-three albino rats weighing 90 to 252 g used in this study were supplied from Experimental Animal Institute of Kyushu University, Fukuoka, Japan. They were fed with a commercially prepared diet and water was provided *ad lib*. Metacercariae of *P. ohirai* and *P. miyazakii* used in the experiment were dissected from the crabs, *Sesarma* (*Holometopus*) *dehaani* collected from Maruyama River, Hyogo Prefecture, and *Potamon* (*Geothelphusa*) *dehaani* from a small stream near Iwakuni City, Yamaguchi Prefecture, Japan respectively. They were introduced into the pleural cavity of rats under ether narcosis by hypodermic syringe attached to a slender vinyl tube through a surgically produced opening in the thorax. Examinations for migrating worms were made from 2 to 40 days after introduction of *P. ohirai*, and from 1 to 67 days of *P. miyazakii*. To facilitate examination each of the rats received an intravenous injection of 10 to 15 ml of 0.3 % Evans-blue solution per kg body weight 15 minutes before autopsy (Yokogawa *et al.*, 1962).

In each examination 2 to 4 rats in the case of *P. ohirai* and 3 to 6 rats in *P. miyazakii* were killed with ether, the peritoneal cavity was opened, and the surface of the liver and peritoneal wall was inspected for the hemorrhages stained with Evans-blue. The peritoneal surface of rats was flushed with Ringer's solution; the washings were examined for free worms under a dissecting microscope. All of the internal organs of rats were removed separately, then washed with Ringer's solution several times for recovery of free worms. The liver and lungs were removed from the other organs and examined for hemorrhages and lung capsules, and then minced with scissors in petri dishes. The minced liver and lungs were examined for penetrating worms, as thin strips pressed between two glass slides. The worms recovered were preserved in 70 % alcohol, stained with carmine and mounted

in balsam. Morphological observations and measurements of the worms were made on these stained and mounted worms. Unless otherwise specified, all measurements are in millimeter.

RESULTS

The worms recovered from the liver, peritoneal cavity and muscles underlying the peritoneal lining of the body cavity of rats are show in Figure 1 as total percentage recovery of worms in each examination. The size of the worms recovered are given in Figure 2 as the mean length obtained from the measurements of 17 to 43 mounted specimens of *P. ohirai* in each examination, and of 4 to 13 specimens of *P. miyazakii*, respectively.

All rats exposed to *P. ohirai* by introduction of the metacercariae into the pleural cavity were positive for the species. The average recovery of *P. ohirai* was 76.5 (40.0 to 100.0) %. In the case of *P. miyazakii*, an average recovery of 31.9 (13.3 to 90.0) % occurred.

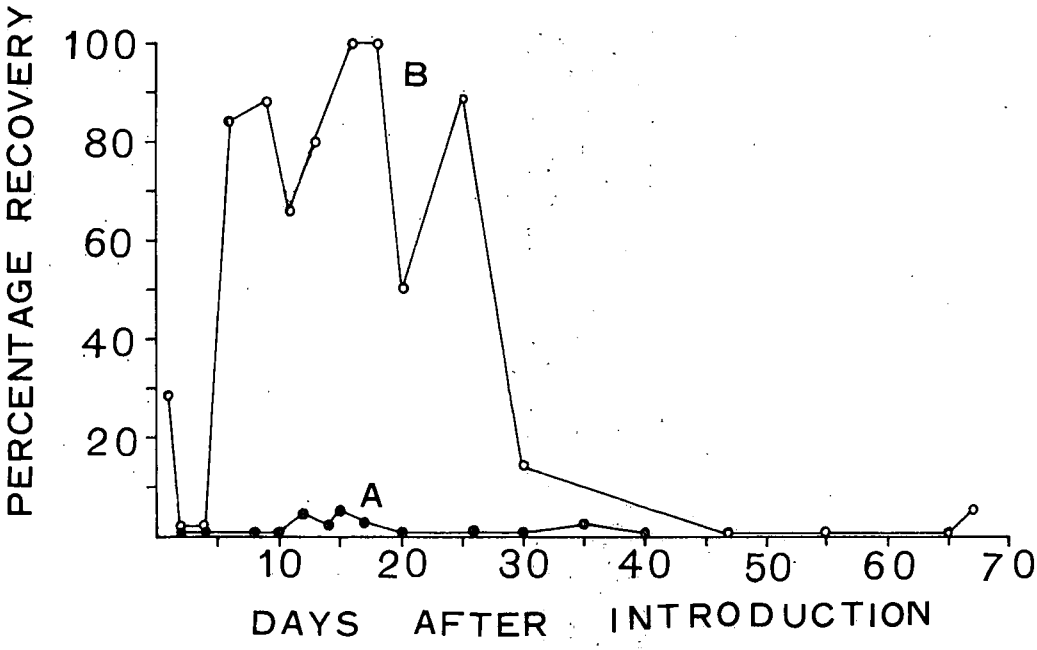

FIGURE 1. Percentage recovery of *P. ohirai* (A) and *P. miyazakii* (B) from the liver, peritoneal cavity and muscles of rats, to total worms recovered in each examination.

Migration and growth of P. ohirai

All of the worms recovered 2 to 10 days after exposure were free in the pleural cavity. Two days after introduction 49 worms were recovered from the pleural cavity of 4 rats. Twenty of these worms measured 0.6 by 0.2 in average, ranging 0.4 to 0.7 long by 0.1 to 0.2 wide. The worms recovered 6 and 10 days later were of approximately equal length, each averaging a little over 1.0 long by 0.4 wide.

In 14 animals which had been exposed to 280 metacercariae 12 to 17 days previously, 218 worms were recovered. Of these worms 3 were found in the liver and 4 were free in the

peritoneal cavity. After 17 da a lung capsule containing 2 worms was observed in left lung of a host.

During the 20th and 40th days of introduction all but one worm, which was found 35 da later free in the peritoneal cavity, were found in the pleural cavity, lungs and lung capsules. Most of the worms recovered after 30 da were detected in lung capsules, mostly in pairs. The largest worm recovered after 20 da was 2.8 long and its reproductive system was similar to that of the adult worms, but without eggs in the uterus. The eggs in the uterus were first observed in 14 (45.2 %) of the 31 worms at da 25, then in 42 (95.5%) of the 44 worms at 30 da, and 29 (96.7%) of the 30 worms at 40 days. At da 40 the worms averaged 6.0 long by 3.7 wide, ranging from 3.0 to 8.1 long by 2.2 to 4.9 wide.

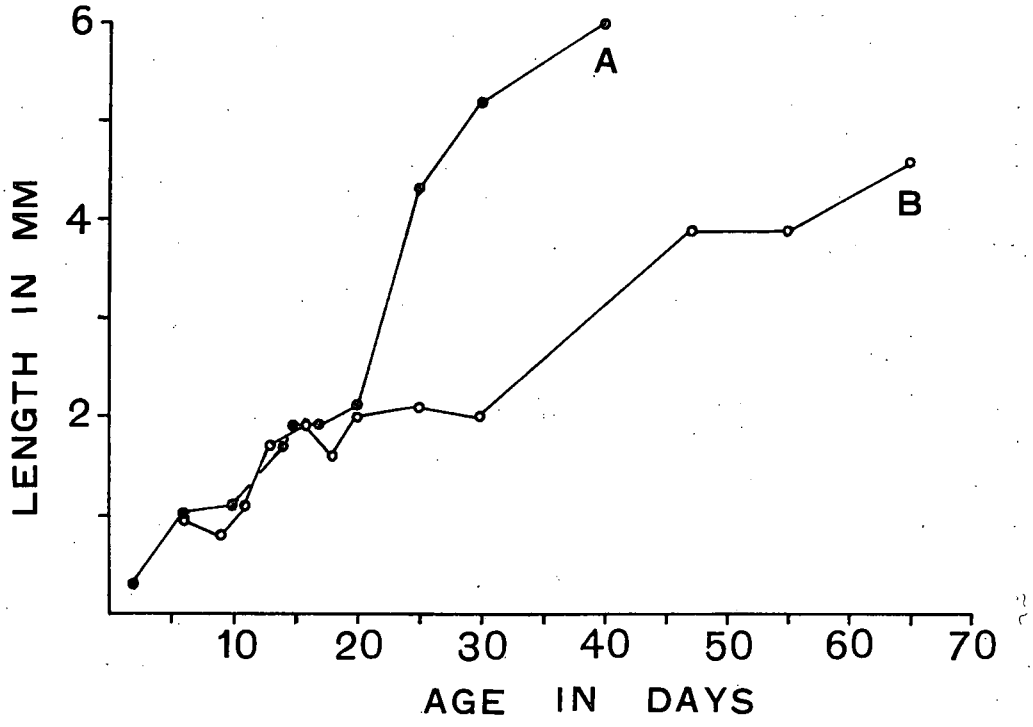

FIGURE 2. Growth, expressed as increase in body length of *P. ohirai* (A) and *P. miyazakii* (B) in rats infected by introduction of metacercariae into the pleural cavity.

Migration and growth of P. miyazakii

Two of 7 worms were found free in the peritoneal cavity as early as 1 da after introduction into the pleural cavity of the host. After 2 to 4 da the worms were not found in the cavity. Upon leaving the pleural cavity during 6 to 25 da, the worms followed normal migratory pattern. Most often they were found in the liver, but were also seen in the muscle underlying the peritoneal lining and free in the peritoneal cavity. Six days later, 12 of the 14 worms recovered were found in the peritoneal cavity, liver and muscles underlyng the peritoneal lining. The worms averaged 1.0 long by 0.5 wide, the largest worm attaining a length of 1.3. In a period 16 to 18 days after introduction, all of the worms recovered were

found in the liver, peritoneal cavity and muscles; no worms were observed in the pleural cavity or in the lungs. The worms recovered 16 and 18 da later, averaged 1.8 long by 0.7 wide and 1.6 by 0.8 wide, respectively. Two worms recovered at 20 da and 8 worms at 25 da from the liver and peritoneal cavity of rats, showed no great difference in size.

After emigrating from the pleural cavity to the liver, peritoneal cavity and muscles, the worms immigrated into the pleural cavity. They then induced lung capsules to be produced in the host. After 30 da, when a lung capsule was first found in hots, the worms were 2.0 long by 1.3 wide but the eggs in the uterus were not observed. All but a few worms were found in the lung capsules of rats from 47 to 67 da after introduction of metacercariae into the pleural cavities. Uterine eggs were first found in 1 of the 9 worms at da 55, and in 4 of the 13 worms at da 65. The largest worm recovered throughout the experiment was 6.0 long by 2.7 wide at da 65. This worm contained numerous uterine eggs.

DISCUSSION

Infections of albino rats with *P. ohirai* yielded recovery rates of 2.5 to 68.8 % (Miyazaki, 1940) and 63.0 to 69.5 % (Ohkura, 1963a), while infections with *P. miyazakii* yielded recoveries of 30.7 % (Hashiguchi *et al.*, 1968), 29.3 % (Tada, 1969) and 23.9 to 32.2 % (Yoshida, 1970). In this study, the recovery rate averaged 76.5 (range 40.0 to 100.0) % in *P. ohirai* and 31.9 (range 13.3 to 90.0) % in *P. miyazakii*. Comparisons of recovery rate of the worms, suggest that the metacercariae of both species introduced into the pleural cavities of rats are able to infect rats as metacercariae by normal (*per os*) infections. The low values obtained for *P. miyazakii* are probably due to the low natural infectivity of this species to rats.

Ohkura (1963a) found that in rats fed metacercariae of *P. ohirai* peak numbers of worms from the liver were recovered at da 14; the proportion of the worms from this organ to those recovered was 73.6 %. In contrast to Ohkura's (1963a) studies, all but a few specimens *P. ohirai* metacercariae introduced into the pleural cavities of rats in the present study were found free in the pleural cavity and then produced lung capsules in the lungs of hosts.

Yokogawa *et al.* (1964a) and Tada (1969) reported liver migration for *P. miyazakii* in rats fed metacercariae. In addition, they demonstrated the tendency for this species to remain in the liver of rats for a longer period than *P. ohirai*. Observations in this study support the results of Yokogawa *et al.* (1964a) and Tada (1962) for normal infections of *P. miyazakii*. In my experiments with this species it was observed that metacercariae excysted in the pleural cavity but most of them would migrate to the liver, penetrating this organ and muscles underlying the peritoneum, then making their way to the pleural cavity where they induced a host lung capsule. Moreover, the migration schedule of *P. miyazakii* in the present study, into the liver, muscles and lungs, mostly agreed with that found for infection *per os* as reported by Hashiguchi *et al.* (1968). Thus, significant differences between migration of *P. ohirai* and *P. miyazakii* in rats infected by introduction of metacercariae into the pleural cavities were found.

Yokogawa *et al.* (1962) observed migrations of *P. westermani* in rats and cats, noting that a liver migration seemed not to be the normal route. They (1964 a, b) also reported on the migration of *P. miyazakii* in rats and cats. They reported that in rats the worms occurred in both the liver and muscles, but in cats they occurred only in the liver before reaching the lungs of the hosts.

On the basis of these observations, the migration of *P. ohirai* and *P. miyazakii* in rats infected by introduction of metacercariae into the pleural cavities may be summarized as follows : In *P. ohirai* the metacercariae will excyst in the pleural cavity and most of them induce lung capsules in the host. They remain in the pleural cavity for periods of 17 to 20 days without migration into the liver and peritoneal cavity of rats. Metacercariae of *P. miyazakii* will also excyst in the pleural cavity, but most of them make their way into the liver, peritoneal cavity and muscles where they undergo a rather prolonged migration, coming to rest in lung capsules of the host.

Adult worms derived from metacercariae introduced into the pleural cavity of rats, then recovered at necropsy up to 65 days did not differ in size when compared with measurements given in the literature for *P. miyazakii* in the same host species for similar periods after infection *per os*. Measurements of all developmental stages of *P. ohirai* similarly introduced showed that the worms tended to be somewhat smaller than that those recovered from infections *per os* reported by Miyazaki (1940) and Ohkura (1963b). The difference found between *P. ohirai* and *P. miyazakii* might be caused by the remaining in the pleural cavity without migration into the liver of the hosts. The liver has been considered to be an important organ for the lung flukes as related to their nutrition (Tomita, 1956; Ohkura, 1963b). Hashiguchi (unpublished data) observed two host capsules in the liver of one albino rat 20 metacercariae of *P. ohirai per os* and obtained two fully mature worms from one capsule, but no worms from the other. In this case, the size of the worms recovered were larger than those from the capsules in the lungs of the same host. Similar results have also been reported by Kawashima *et al.* (1966) for heterotopic parasitism of *P. miyazakii*. They obtained the worms from the lungs, liver, kidney and peritonea of albino rats and reported that the largest worm was found in the capsule in the liver of the hosts. In the case of *per os* infections, their observations suggest that the worms undergo considerable growth and development while remaining in the liver for periods of several days.

SUMMARY

The migration and growth of *P. ohirai* and *P. miyazakii* is reported. Metacercariae were introduced into the pleural cavity of albino rats. The location of the worms and tissue involved were followed by examining exposed hosts at different time intervals. The metacercariae of *P. ohirai* introduced into rats excysted easily in the pleural cavity, and most of them developed to maturity without migration into the liver. *P. miyazakii* metacercariae also excysted in the pleural cavity, but most migrated, in 6 to 25 da, into the liver or muscles underlying the peritoneal lining. These worms then returned to the pleural cavity and induced lung capsule formation in the host. Comparisons of worms recovered from

normal (*per os*) infections and these experiments, show that *P. ohirai* in the present experiment was smaller and *P. miyazakii* was of equivalent size. Results suggest that liver migration is not necessary for *P. ohirai* to attain maturity. *P. miyazakii* had a liver migration even though the metacercariae had been initially introduced into the pleural cavity of the definitive host. After migration to the liver *P. miyazakii* returned to the site of introduction.

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