

## Experimental Transplantation of *Paragonimus miyazakii* Metacercariae into the Pleural Cavity of Albino Rats

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### Introduction

In the oral infection of albino rats with *P. miyazakii* and *P. ohirai*, it is well known that most of the worms migrate into the liver of the hosts before they reach the lungs. But it has been obscure why the worm migrates into the liver, and whether or not the worm can mature without migration into the liver. Hashiguchi and Takei (1969) reported that when the metacercariae of *P. ohirai* were transplanted into the pleural cavity of rats, the larvae easily excysted in the cavity and most of them developed to maturity without migration into the liver of hosts. From these results, it was suggested that the migration of the excysted larvae of *P. ohirai* into the liver of hosts by oral infection was not necessary for the maturation of the worm. The present experiment was designed to know whether *P. miyazakii* can mature or not without migration into the liver of rats, and also to obtain further informations on the affinity of growing worms to the liver or the lungs of the hosts.

### Materials and Methods

Female albino rats (S.D. strain) weighing 90 to 210 g were supplied from Experimental Animal Institute of Kyushu University. They were fed with a commercially prepared diet and water was provided *ad libitum*. Metacercariae of *P. miyazakii* used in the experiment were obtained from a crab, *Potamon (Geothelphusa) dehaani*, collected at Rokuroshi,

Iwakuni City, Yamaguchi Prefecture, Japan. They were transplanted into the pleural cavity of rats under ether narcosis by an injection syringe with a slender vinyl tube through a surgically operated opening in the thorax of rats. Excystation of the metacercariae in the pleural cavity of rats was examined during 1 to 144 hours after the transplantation. Examinations for migrating worms were made during the period from 1 to 67 days after the transplantation. 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 3 to 6 rats were killed with ether, the abdominal cavity was opened and the surface of the liver and abdominal wall was inspected for the hemorrhages stained with Evans-blue. The abdominal surface was flushed with Ringer's solution, and the washings were collected for microscopical examination. Then the thorax was opened and the pleural surface was thoroughly flushed with Ringer's solution; the washings were examined for free worms under the dissecting microscope. All of the abdominal and pleural organs were removed separately, and washed with Ringer's solution several times for collecting the free worms. The liver and lungs were removed from the other organs and examined for hemorrhages and worm cysts, and then minced with scissors in large Petri dishes. The minced liver and lungs were pressed between two glass plates, and examined for penetrating worms under the microscope. The worms recovered were fixed in 70 % alcohol, stained with carmine and mounted

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in balsam. Morphological observations were made on these stained and mounted worms.

### Results

#### *Excystation of the metacercariae*

In order to secure the excystation of the larvae in the pleural cavity of rats, the examination for the cysts before and/or after excystation was conducted. In the empty cysts a small pore probably made by the larvae was found in a part of the inner cyst. Therefore, in the present observations the number of empty cysts found in the pleural cavity was regarded as that of excysted larvae.

As shown in Table 1, the empty cysts were first observed in 2 of the 30 examined 6 hours after the transplantation. The excystation rate of the larvae to total number of cysts recovered was 0 to 6.7% from 1 to 9 hours after the transplantation, then reached 18.0% at 12 hours and 33.3 to 56.0% during 24 to 144 hours. Among encysted larvae recovered, dead ones were first found in 6 out of 10 at 96 hours later and all cysts recognized to be dead 144 hours after the transplantation.

#### *Migration of the worms*

The rats transplanted with metacercariae were all positive for *P. miyazakii*, harbouring 1 to 10 worms per host. As shown in Table

2, the recovery rate of the worms obtained during 1 to 67 days after the transplantation was 31.9% in average, ranging from 13.3 to 90.0% in each examination. In the examination after one day, 2 out of 7 excysted worms were found in the abdominal cavity. After 6 days, 12 (84.7%) of the worms recovered were recognized in the abdominal cavity penetrating into the liver or muscle of abdominal wall of the hosts. During 6 to 25 days, most of the worms migrated into the abdominal cavity, showing the recovery rate of 66.7 to 100.0%. Of the 53 worms recovered in the abdominal cavity during this period, 38 (71.1%) were detected from the liver of the rats. After 30 days, only 1 of the 7 worms recovered was recognized in the liver and the remainder was found in the pleural cavity freely and/or in the worm cyst in the lungs. All but a few worms were recognized in the worm cyst of the lungs from 47 to 67 days after the transplantation. The number of worm cysts in the lungs was 25 in total, 16 (64.0%) of which were found in the right lung.

#### *Development of the worms*

As shown in Table 3, average size of the worms increased from 1.0 by 0.5 mm to 4.6 by 2.3 mm 65 days after the transplantation. After 30 days, when worm cysts were first found in the lungs of the hosts, the size of

Table 1 Excystation of *P. miyazakii* metacercariae in the pleural cavity of albino rats

Hrs. after transplantation	No. of rats used	No. of metac. transplanted	No. of empty cysts*	No. of encysted metac.	Excystation rate per total recovered (%)
1	3	30	0	20	0
3	3	30	0	23	0
6	3	30	2	28	6.7
9	3	30	0	22	0
12	2	20	3	13	18.8
24	3	30	14	11	56.0
48	3	30	5	5	50.0
96	3	30	5	10**	33.3
144	3	30	14	14***	50.0

\* Number of empty cysts means that of excysted larvae.

\*\* 6 were dead.

\*\*\* All dead.

Table 2 Results of experimental transplantation of *P. miyazakii* metacercariae into the pleural cavity of albino rats

Days after transplantation	No. of rats used	No. of metac. used	No. of worms recovered	Recovery rate (%)	No. of worms found in					Recovery rate of worms from abdominal region (%)	No. of cyst in lungs
					Abdominal cavity	Muscle of abdominal wall	Liver	Pleural cavity	Lungs		
1	3	30	7	23.3	2			5		28.6	
2	3	30	5	16.7				5		0	
4	3	30	5	16.7				4	1	0	
6	3	30	14	46.7	2	2	8	2		84.7	
9	3	30	9	30.0	2		6	1		88.9	
11	3	30	9	30.0	1	1	4	3		66.7	
13	3	30	5	16.7			4	1		80.0	
16	3	30	8	26.7	2	1	5			100.0	
18	3	30	5	16.7	1		4			100.0	
20	3	30	4	13.3			2	2		50.0	
25	3	30	9	30.0	4		4	1		88.9	
30	3	30	7	23.3			1	4		14.3	1
47	3	30	7	23.3						0	3
55	3	30	13	43.3				1		0	6
65	2	20	18	90.0				2		0	7
67	3	30	19	63.3	1			1		5.3	8

Table 3 Development of *P. miyazakii* in albino rats by pleural transplantation

Days after transplantation	No. of worms measured	Average and range (in mm)		No. of worms with uterine eggs per total examined (%)
		Length	Width	
6	12	1.0 (0.8-1.3)	0.5 (0.4-0.6)	0/12
9	9	0.8 (0.3-1.4)	0.5 (0.4-0.7)	0/9
11	9	1.1 (0.9-1.9)	0.5 (0.4-0.7)	0/9
13	5	1.7 (1.0-2.1)	0.8 (0.5-0.9)	0/5
16	8	1.8 (1.0-2.0)	0.7 (0.4-0.9)	0/8
18	5	1.6 (1.1-2.5)	0.8 (0.5-1.3)	0/5
20	4	2.0 (1.3-2.7)	0.9 (0.6-1.2)	0/4
25	6	2.1 (1.7-2.4)	0.9 (0.6-1.1)	0/6
30	6	2.0 (1.5-2.3)	1.3 (0.8-1.7)	0/6
47	6	3.9 (2.4-5.6)	2.3 (1.8-2.9)	0/6
55	9	3.9 (3.3-4.9)	2.4 (1.6-3.5)	1/9 (11.1)
65	13	4.6 (3.1-6.0)	2.3 (1.7-2.7)	4/13 (30.8)

worms was 2.0 by 1.3 mm, but no uterine eggs were found. The eggs in the uterus were not recognized until 47 days, but they were found in 1 (11.1%) of the 9 worms after 55 days and in 4 (30.8%) of the 13 worms

after 65 days.

### Discussion

The recovery rate of *P. miyazakii* by oral infection to albino rats was reported to be

30.7% (Hashiguchi *et al.*, 1968) and 29.3% (Tada, 1969) in average. Therefore, the recovery rate (31.9%) obtained in the present experiment showed that the fluke could infect albino rats by pleural transplantation as by oral infection.

Most of the worms transplanted into the pleural cavity took their normal route of migration penetrating into the liver or muscle of abdominal wall, then returned to the pleural cavity and produced worm cysts in the lungs. Moreover, the time of migration of the worms into the liver and lungs of hosts mostly agreed with that in the case of oral infection (Hashiguchi *et al.*, 1968).

For the lung flukes, *Paragonimus*, the lungs of hosts are considered to be the final organ on their migration route. In the pleural transplantation of *P. ohirai*, most of the worms were found freely in the pleural cavity without migration into the liver, and thereafter they produced worm cysts in the lungs of rats (Hashiguchi and Takei, 1969). Therefore, *P. ohirai* seems to be able to develop to maturity in the pleural cavity and the lungs of the hosts. On the contrary, *P. miyazakii* appears to need migration into the liver of the hosts for maturity.

Kawashima *et al.* (1966) reported the heterotopic parasitism of *P. miyazakii*, observing typical worm cysts in the liver of the hosts. The same results were ascertained by Hatsushika *et al.* (1968) and Yoshida (1970) in *P. miyazakii*, but in the case of *P. ohirai* such parasitism was found very rarely. Miyazaki (1940) recognized a worm cyst without worms in the abdominal wall of albino rats 35 days after the infection with *P. ohirai*. Besides, Hashiguchi (unpublished data) observed after 52 days two worm cysts in the liver of one albino rat fed orally with 20 metacercariae of *P. ohirai* and obtained two fully mature worms from one cyst but no worms from the other. In the present experiment the heterotopic parasitism was not recognized.

Tada (1969) pointed out that *P. miyazakii* had a tendency to remain in the liver of hosts for a longer time than *P. ohirai*, and

also he emphasized that these difference between the two species might be due to the difference of affinity to albino rats. The results obtained in the present experiment in *P. miyazakii* and in the previous one in *P. ohirai* (Hashiguchi and Takei, 1969) seem to give a interesting suggestion on the host-parasite relationship of both species.

### Summary

The metacercariae of *P. miyazakii*, which were transplanted into the pleural cavity of albino rats, excysted in the cavity and developed to maturity. But, most of the worms migrated into the liver or the muscle of abdominal wall during 6 to 25 days after the transplantation, then they returned to the pleural cavity, and produced worm cysts in the lungs of the hosts. Development of the worms by pleural transplantation was similar to that by oral infection. Thus, it seems to be necessary to migrate into the liver of hosts for maturation of *P. miyazakii* in albino rats. Besides, the affinity of worms to the liver or the lungs of the hosts was apparently different by developmental stages of the worms even in abnormal infection such as pleural transplantation.

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### 宮崎肺吸虫メタセルカリアのダイコクネズミ胸腔内移植

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大平肺吸虫をネズミ胸腔内に移植すると、大部分の虫が、宿主の肝臓へ移行することなく、成熟しえた(橋口・武井, 1969)。今回は、宮崎肺吸虫を用いて同様な実験を試み、肝臓への移行や、虫の発育時期と宿主肝臓ならびに肺臓の関係を検討した。

移植されたメタセルカリアは、ネズミ胸腔内で脱のうし感染しえた。しかし、大部分の虫は、6~25日後に宿主の肝臓や腹壁筋に穿入したのち、ふたたび胸腔に移行

し、肺臓に虫のう腫を形成した。また、虫の発育面でも経口感染による従来成績とほぼ一致した。

これらの事実から、宮崎肺吸虫のネズミ感染では、成熟のために宿主肝臓への移行が必要なものと考えられた。また、胸腔内移植のような異常感染でも、虫の発育時期による宿主肝臓および肺臓に対する親和性には、明らかな差異がみられた。