STUDIES ON THE CERCARIA OF THE RAT TREMATODE, *PLAGIORCHIS MURIS* (TANABE)

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(With 29 Tables, 10 Text-figures and 2 Plates)

Contents

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I. Introduction.

Plagiorchis muris, a trematode parasite, was first found from the small intestine of the rat by TANABE (1921), and later the sparrow, bat and dog were added to the final hosts by HIRASAWA and ASADA (1929) and MIYATA (1940) respectively. MCMULLEN (1937) reported that the human body is also infected with this parasite experimentally. The first intermediate hosts of this parasite are fresh water snails, *Limnaea pervia* and *Limnaea japonica*, and the second intermediate hosts comprise thirteen species of fresh water animals. Though the general outline of the life history of this parasite has already been made clear by the efforts of such eminent scholars as above mentioned, fundamental studies on the behavior of the cercaria have remained almost untouched.

Up to the present, concerning the behavior of the larva of the trematode parasite,only the feature of escape from the snail, attack on the intermediate hosts, and the encystation of the cercaria in the intermediate hosts have been observed. The present study has been directed chiefly to the problem of the artificial encystation of the cercaria in vitro. First, a morphological study of the cercaria has been made. Then the seasonal fluctuation of the infetion rate of the snail and the behavior of the larva in different media has been observed. The encysting of cercariae was observed in vitro as affected by the blood sera of animals. From this the writer

has obtained some new facts which are to be noted.

II. Material and method.

In the vicinity of Sapporo the large-sized Limnaea, *Limnaea japonica*, is very common. The snail is found to be severely infected with the larvae of *Plagiorchis muris*. The snails were brought into the laboratory and kept separately each in a petri dish after washing carefully with tap water. The dishes were covered with wire net in order to prevent escape, and placed in the dark room. When the room temperature fell below 22°C, they were transferred into the thermostat (22–24°C) as the cercaria may leave the snail very slowly at low temperature. In this envirommenT, however, the cercaria generally emerges from the snail within one or two hours.

The blood used for the experiment of encystation was collected from the heart in case of small animals, in the sterilized test tube with the injection-syringe without a bacillus, but in the large animals it was taken from the jugular vein. After five to twenty hours the serum came out of the blood naturally.

Details of the methods employed in each experiment respectively will be explained later.

III. Morphological description of the cercaria.

The morphological study of *Plagiorchis muris* was made chiefly with living specimens, by means of vital staining with neutral red and nile blue, and in addition cercariae killed in formalin or alcohol and stained with DELAFIELD's haematoxylin were also used. Special attention was paid to the parts of the body of cercaria which are closely related to the encystation behavior.

The cercaria changes body shape variously as it moves. The oral sucker is subterminal in position, spherical to oval in shape and feebly muscled. The mouth opening is directed towards the ventral surface. The ventral sucker is rudimentary and somewhat muscular at this stage, situating just behind the posterior third of the body. The digestive system is very simple in structure. On the dorsal wall of the oral sucker there is a large stylet with a sharp tip and a thickening at the distal two-thirds of the length.

It is flattened ventrally and 0.024 mm. to 0.03 mm. long, the width at the base being about one-fifth or one-sixth of the length.

Two kinds of glands, viz. penetration gland and cystogenous gland, are found in the cercaria. The former consists of four or five large globlet-shaped cells, which are found on each side of the body close to the ventral sucker, the duct starting from the outer margin of each cell to open separately at the base of the cephalic spine or stylet. Therefore, on each side of the stylet there are four or five openings of this gland. The mass of cells is easily stained deeply with 0.02% neutral red solution, while 0.02% nile blue solution stains very slowly. However, the duct of the cell is stained with 0.02% nile blue more rapidly than with 0.02% neutral red. The other gland is situated behind the ventral sucker. It consists of a large number of scattered cells.

TABLE 1. The size of body, tail and stylet in ten cercariae emerged from the snail host.

Body (mm.)	Tail (mm.)	Stylet (mm.)
0.275 × 0.094	0.135 × 0.024	0.024 × 0.0055
0.248 × 0.094	0.135 × 0.022	0.024 × 0.0055
0.248 × 0.090	0.139 × 0.022	0.026 × 0.0043
0.263 × 0.105	0.143 × 0.026	0.026 × 0.0043
0.263 × 0.098	0.143 × 0.024	0.026 × 0.0055
0.248 × 0.090	0.135 × 0.023	0.024 × 0.0055
0.275 × 0.094	0.143 × 0.023	0.029 × 0.0055
0.248 × 0.094	0.135 × 0.022	0.024 × 0.0050
0.275 × 0.094	0.143 × 0.023	0.030 × 0.0055
0.263 × 0.090	0.135 × 0.022	0.024 × 0.0055

The tail of the cercaria is about a half length of the body when extended, but it becomes extremely shorter in contraction, being sometimes 0.0375 mm. long with a width of 0.03 mm. The tail is connected with the end of the body, and attaches in an indentation which is found on the ventral side of the posterior body end. The writer by vital staining has found for the first time some wrinkles or club-shaped projections on the inner side of the above body indentation. The function of these projections is not clear. This indentation shows a different colour from the other parts of

the body by the vital staining using nile blue solution, becoming violet while the other parts are blue. The excretory vessel in the tail of the cercaria is a narrow tube running down almost to the tip. This vessel becomes very narrow at the tail root, but it widens immediately to open in a wide excretory bladder which is of Y-shape in the fixed specimen. However, the shape of the bladder varies according to the movement of the body in living specimen as shown in Fig. 1.

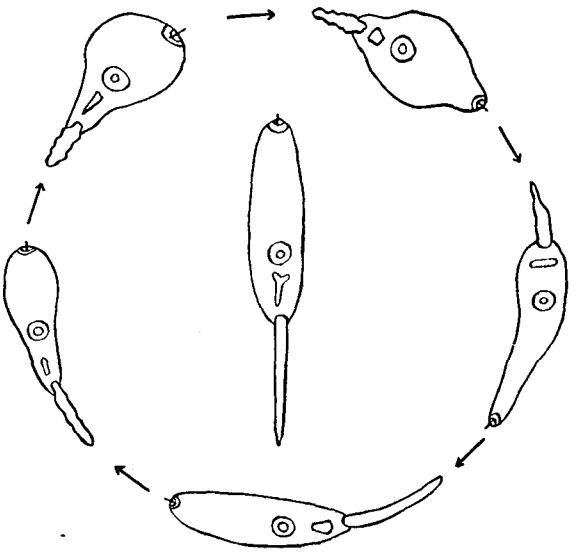

Fig. 1. Change of the shape of the excretory bladder with the creeping movement of the cercaria.

As mentioned above nile blue yields good result for the vital staining of the duct of penetration glands, while neutra red stains easily the mass of cells of this gland. So the writer recommends double staining using both stains for the demonstration of the penetration glands of the cercaria.

IV. Experiments on the behavior of the cercaria.

A. The influence of light on the emergence of the cercaria from the host snail.

When the infected snail is kept in an aquarium, the cercariae

emerge from the host in great numbers within a short time, and swim actively in the water.

The writer observed the influence of light on the emergence of the cercaria from the snail, using 16 snails which were proved to be infected with many cercariae. The snails were divided into three groups. The first group was placed in the thermostat kept at 27°C, and the second group was placed in a light room at 20 or 22°C. The second group was illuminated by the 10 watt electric lamp at the distance of 38 cm. during the night, sometimes 100 watt electric lamp having been used at the distance of 43 c m. for two hours. The third group was placed in a dark room at a temperature of 19 or 20°C. Each snail was transferred into a new petri

TABLE 2. Influence of light to the emergence of cercariae from the snails.

Group No.	Temperature	Light or dark	Snail No.	Number of cercariae emerged from the snails at 2 hour interuals excepting 5.5 hours in IX.											
				I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
I	27°C	Dark	1	6480	3440	3010	1404	256	258	2700	648	4380	5940	301 0	to Light 540
			2	3780	3010	1720	1624	1020	1032	2160	432	3440	2700	946	108
			3	7560	4300	3440	3780	1720	to 10 W. 840	to dark 3240	1620	6020	3240	3010	540
			4	4320	60	172	1624	1624	516	1080	432	3440	432	3010	432
II	20~21°C	Light	1	11	0	1	0	0	to Dark 3010	1720	40	344	540	688	648
			2	85	7	1	0	0	1290	3440	to 100 W. 324	to 10 W. 688	432	688	756
			3	170	35	16	7	to 10 W. 3440	1140	2160	30	30	1080	1778	1080
			4	79	18	3	2	7	258	220	7	22	to Dark 10800	1720	1080
			5	5	1	0	0	688	4300	2560	860	780	2160	1080	2160
			6	12	5	1	0	0	0	10	1	86	5400	1720	1648
III	19~20°C	Dark	1	540	1200	430	60	35	to 10 W. 4	0	2	0	to Dark 3780	3010	to Light 324
			2	3780	3010	3440	2160	3010	2150	216	45	25	216	860	5
			3	1296	344	946	540	258	50	7	4	2	to Light 324	1032	to Dark 648
			4	3180	688	258	80	70	80	1080	432	1080	216	3	3240
			5	324	3440	2580	324	35	4	7	80	7740	324	Died	
			6	3248	3010	1032	432	40	12	30	300	2580	216	172	5400

I-VIII is from 11.30 am. to 3.30 am. of next day, IX is from 3.30 am. to 9 am., and X-XII is from 9 am. to 3 pm.

dish after every two hours treatment as shown in the following table (IX in Table 2). The number of cercariae emerged from the snails was counted after weakening of the movement of the cercariae. The results are shown in Table 2.

From the table it is clear that the number of the cercariae escaped from the snail placed in the light room is far less than from those in the dark room. It follows that the emergence of the cercaria is influenced by the intensity of the light. When the snail was transferred from the dark room to the light room, the emergence of the cercariae was much reduced. If the intensity of the light is as low as 10 watt lamp, the number of the cercariae escaped from the snail is only a little more than that of the dark room, and it decreases suddenly by transferring into such low illumination. The cercariae escape more frequently from the snail placed in the dark room at 27°C than from the snail in the dark room at 19–20°C. Thus it is understood that the water temperature and the light intensity affect the emergence of the cercariae, and naturally the night or the dark part of the pond even in the daytime in summer will offer the most favourable condition for the escape of the cercariae from the snail in natural environment.

B. The behavior of the cercaria in the water.

(*a*) *Swinmming and creeping movement.*

The cercaria emerged from the snail moves actively, swimming

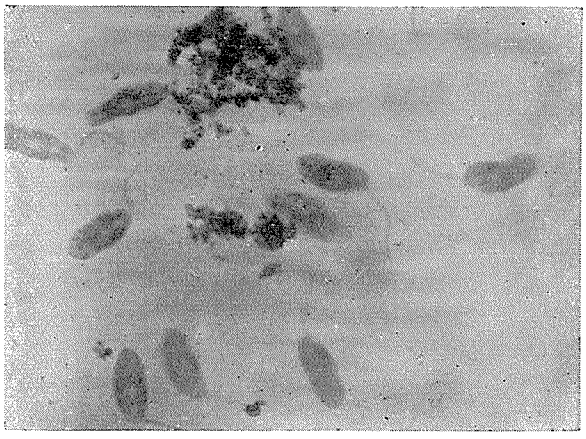

Fig. 2. The cercariae in the water 12 hrs. after emergence.

in the free water or sometimes creeping on a substratum in the water. When swimming it bends the ventral side up, contracting the body. The tail extends greatly and lashes rapidly making a spiral movement. The cercaria is unable to move definitely in one

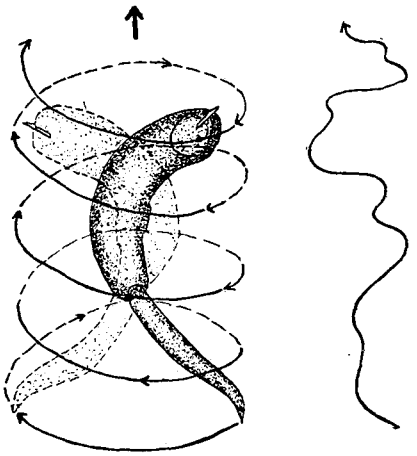

Fig. 3. The spiral movement of swimming cercaria, and a swimming course.

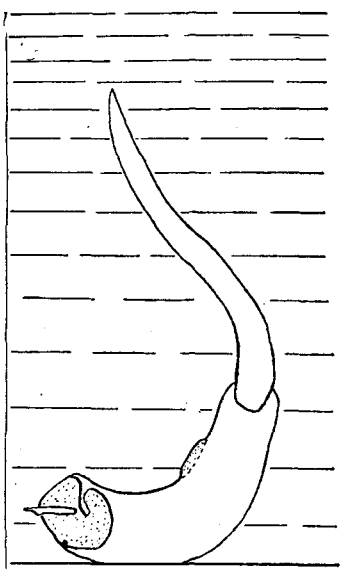

Fig. 4. Resting condition of the cercaria at the water bottom.

direction for any length of time, and so the locomotion looks very erratic. Whenever the swimming cercaria comes in contact with a water surface, the tail ceases the lashing movement and the body begins to stretch, swinging around until the oral sucker can obtain a hold. Then the cercaria comes in contact with a surface, extending its body, the tail resumes the lashing and swimming is started again with the extended body. Swimming and resting of the cercaria appear alternately in the water. Sometimes the resting cercaria changes position without body movement just moving the tail irregularly and lies down at the bottom of the water as shown in Fig. 4.

Moreover, the writer observed the speed of cercaria swimming up and down in the water using a test tube 10 cm. in length and 1 cm. in diameter, graduated every one centimeter. The tube was held vertically, keeping the water at 20°C. The results are given in Tables 3 and 4.

TABLE 3. Time required by cercariae for swimming upward 1 cm. in water.

Cercaria No.	Time (Sec.)	Cercaria No.	Time (Sec.)
1	28.0	11	28.5
2	45.0	12	34.8
3	43.5	13	37.0
4	44.4	14	42.2
5	24.0	15	35.0
6	53.0	16	39.4
7	50.7	17	46.7
8	33.4	18	46.8
9	30.0	19	26.5
10	23.8	20	29.5

TABLE 4. Time required by cercariae for swimming downward 1 cm. in water.

Cercaria No.	Time (Sec.)	Cercaria No.	Time (Sec.)
1	22.0	6	39.0
2	33.4	7	56.5
3	45.0	8	39.0
4	45.0	9	37.4
5	44.0	10	38.0

The creeping movement of the cercaria is caused by the altermate movement of extension and contraction of the body, adhering with the oral and ventral suckers alternately. The writer also observed the speed of creeping of cercaria for the distance of 1 mm. on the slide glass by the aid of the microscope, and obtained results as shown in Table 5.

TABLE 5. Creeping speed of the cercariae for the distance of 1 mm.

Cercaria No.	Time (Sec.)	Cercaria No.	Time (Sec.)
1	11.0	6	10.5
2	11.2	7	12.0
3	11.0	8	10.5
4	10.8	9	11.0
5	12.0	10	10.2

(*b*) *Phototaxis.*

The reaction of the cercariae to light was observed in a dark room. The source of light was a 100 watt electric lamp. A horizontal beam of light from an aperture of 3 cm. diameter, was transmitted directly to a microscope stage on which an observation aquarium was held. As the aquarium a amall petri dish was employed covered with black paper which has an aperture of 2 mm. diameter. The cercariae used within one hour after escaping from the host snail proved most active. In this case the cercariae escape from the light showing negative phototaxis. Naturally, sunlight affects the animal in the same way.

C. Relation between longevity and water temperature.

Observations were made on the longevity of the cercaria under laboratory conditions. It was found that the life of the cercariae after emergence from the snail is comparatively short. At a temperature of 22°C the cercariae become extremely inactive until at last they move slowly by creeping on the bottom of the petri dish after twenty-four hours. After forty-eight hours almost all of them die. At about 27°C the behavior is almost similar to that at 22°C, while at about 17°C they live longer than at 22 or 27°C. However, the direct sun light shortens the longevity of the cercariae at any temperature killing them within a few hours.

D. Relation between the distribution of the cercariae and the depth of water.

For this observation a glass tube was used 5 mm. in diameter

and 20 cm. in length, with a rubber stopper at the end, and centimeter graduation. This tube was set vertically in a glass pot filled with hot water, in order to keep the temperature at 20°C in the tube. The cercariae used were very active iust after the emergence from the host snail. The number of cercariae was counted every half hour at the various water depths in the tube. It was found that after 4 hours the cercariae almost disappear near the surface of the water, and after 6.5 hours no cercaria appears between the water surface and 6 cm. depth (Table 6). After 20 hours a few cercariae are creeping along the glass wall near 12 cm. depth, and after 25 hours none is found except on the bottom.

TABLE 6. Relation between the distribution of cercaria in various depths of water and time after emergence.

Water depth	Numbers of cercaria in various depth of water in various time after emergence.													
	1 hr.	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6	6.5	20	25hrs.
1	32	28	21	21	8	6	2	2	2	0	0	0	0	0
2	5	3	8	1	1	0	0	0	0	0	0	0	0	0
3	4	5	2	0	0	1	1	0	0	1	0	0	0	0
4	7	3	1	1	1	0	1	0	0	0	1	0	0	0
5	5	6	4	0	0	1	0	0	0	0	0	0	0	0
6	13	9	2	3	1	4	1	1	0	0	0	0	0	0
7	4	8	2	2	3	1	2	4	1	1	1	2	0	0
8	5	4	4	5	5	4	5	6	3	3	2	2	0	0
9	3	4	4	3	5	1	1	2	0	0	0	0	0	0
10	13	11	9	8	15	4	5	2	1	2	2	2	0	0
11	11	15	16	12	19	14	12	13	10	6	4	4	0	0
12	9	10	11	18	11	18	19	16	14	15	13	13	2	0
13	6	8	10	8	8	10	6	11	11	9	8	7	2	0
14	4	5	9	7	10	7	7	2	3	3	5	2	4	0
15	26	28	44	58	60	76	85	88	102	107	111	115	139	147

E. Behavior of the cercaria in different media.

The writer observed the behavior of the cercariae in several kinds of media, viz. salt solution, hydrochloric acid, acetic acid, sodium bicarbonate sol., grape sugar sol., ethyl alcohol, pepton sol., pepsin sol., liver extract, egg albumin, egg york, cow milk, goat

milk, agar agar and blood serum. Watch glasses, small petri dishes or hollow slide glasses at the room temperature (20–22°C) and only active cercariae were used.

In the injurious media the cercariae generally cease swimming or creeping movement, lieing down on their backs at the bottom, but for a little while only they exhibit extension and contraction of the body, moving the tail slightly. The media used in these observations were injurious to the cercariae, excepting 0.1–0.5% sodium bicarbonate solution which exerted normal influence on the swimming movement in every respect, as compared with the tap water. Among the media acetic acid and hydrochloric acid proved most injurious; the body of the cercariae without any movement slightly bent towards ventrad in the former medium and greatly contracted into a globular form in the latter. The cercariae which ceased movement in a moment in the various media floated on the surface with body and tail extended. In the dense salt solution of sodium bicarbonate the body was extremely bent towards ventrad, and the tail was contracted exceedingly as also in the ethyl alcohol.

TABLE 7. Time required for the cessation of movement.

Medium			Time creeping ceased (Min.)	Time movement of body and tail ceased (Min.)
Kinds	%	pH		
NaCl	1	6.8	150	240
	2	6.6	2.5	5.5
	4	6.4	1	3.5
	10	5.4	1	0.2
HCl	0.005	3.6	25	40
	0.01	3.1	14	18
	0.02	3.0	15	20
	0.05	2.8	5	13
	0.1	2.6	In a moment	9
	0.2		"	8
	0.3		"	7
	0.5		"	3
	1.0		"	1
	2.0		"	In a moment
CH_3COOH	0.01	5.0	20	30
	0.02	4.5	8	12
	0.05	4.2	2	2.5

Medium			Time creeping ceased (Min.)	Time movement of boby and tail ceaced (Min.)
kinds	%	pH		
	0.1	3.6	In a moment	2
	0.2	3.4	"	1.5
	0.5	3.2	"	1
	1.0	3.0	"	In a moment
$NaHCO_3$	0.1	8.8	Swimming and creeping even after 6 hrs.	
	0.2	8.9		
	0.3	8.9		
	0.5	9.0		
	1.0	9.1	180	300
	2.0		10	30
	4.0		3	5
	5.0		In a moment	2
Gape sugar sol.	0.5		270	360
	1.0		"	"
	2.0		90	150
	5.0		20	35
	10.0		8	13
Ethyl alcohol	10		In a moment	In a moment
	20		"	"
	40		"	"
Pepton sol.	2		120	180
	4		90	120
Pepsin sol.	2		180	300
	4		120	180
Extract of pig liver	1		30	60
Egg albumin	100		120	180
	50		200	240
Egg yolk	100		120	180
Cow milk	100		110	150
	50		120	180
Goat milk	100		110	150
Agar agar	1		30	90

From these observations it becomes clear that the movement of the cercaria ceased quickly when pH is less than 6.8 in NaCl sol., 5.0 in CH_3COOH or 3.6 in HCl respectively. In case of $NaHCO_3$ sol., 8.8 to 9 in pH value, very active movement of the cercariae is induced. However the solution, having pH 9 is injurious to the cercaria. Consequently it may be said that the cercaria is resistant in the basic solution being very active in the slightly basic solution, while it is weak in the acid. In the animal blood serum the cercaria shows characteristic behavior which will be described in Chapter VI.

F. Recovery of the movement of the cercaria.

Observations were made on the recovery of movement of the cercariae transferred into the water after having been affected by chemicals. The chemicals used were 0.05% CH_3COOH and 5% $NaHCO_3$ sol. The cercariae subjected to these solutions had ceased their movement within two minutes. Soon after the solutions had caused that sessation they were thrown away and replaced with tap water which was changed thrice within about one minute. Then the behavior of the cercariae was observed with the aid of the binocular microscope. The cercariae recovered activity moving the stylet and tail and then creeping. The results of these observations are shown in Tables 8 and 9.

TABLE 8. Recovery of movement of the cercariae subjected to 0.05% and 0.1% acetic acid.

% of sol.	Affected time (Min.)	Time tailmovement began in water (Min.)	Time bodymovement began in water (Min.)	Time creeping movement began (Min.)
0.05	2	2/3	1	2
	3	1. 1/3	2	3
	4	1. 1/3	2	3. 1/6
	5	2	2. 1/6	3. 1/3
	7	2	2. 2/3	3. 1/2
	10	3	4	7
0.1	5	2. 1/2	3	7
	10	3. 1/2	4	12

TABLE 9. Recovery of movement of the cercariae subjected to 5% sodium bicarbonate.

Affeted time (Min.)	Time tailmovement began in water (Min.)	Time bodymovement began in water (Min.)	Time creeping movement began (Min.)
2	1/6	1/4	2
6	2	3	2
7	2	3	6
10	2	3	6–7
12	3	4	6–7
13.5	15	19	60

When the cercariae subjected to 5% $NaHCO_3$ for 15 minutes found themselves in the water, the most of them floated on the water surface and usually the stylet fell out of the body. However, when 0.6% salt sol. was used instead of tap water, the cercaria moved the tail slightly after 40 minutes and even after 2.5 hours the tail was seen moving while the body remained still. Perhaps recovery of normal activity does not occur.

V. Investigations and experiments on the infection of the larva to the intermediate and final hosts.

A. Seasonal fluctuation of the infection rate of the first intermediate host, *Limnaea japonica*.

It was found that 586 among 1144 individuals of the snail collected in nature, viz. 49.65%, were infected with the larvae of *Plagiorchis muris*. The infection rate increases gradually from May to August showing 92.72% in August, and decreases gradually towards the winter as shown in Table 10.

TABLE 10. Seasonal fluctuation of the infection of cercaria of *P. muris*.

Date	Month	V	VI	VII			VIIII		IX	
	Day	29	22	11	18	30	8	26	9	26
Nos. of snail examined		203	177	80	150	116	110	100	110	88
Nos. of snail infected		25	80	37	77	89	102	88	49	21
Infection rate		12.3%	45.19	46.25	51.33	76.72	92.73	88.0	55.45	23.86%

B. Two new second intermediate hosts.

As the second intermediate hosts of this parasite, ten species of animals have been already reported by TANABE (1920), HIRASAWA and ASADA (1929) and McMULLEN (1937). In the present experiment the writer tried to infect the beach-flea, *Anisogammarus annandalei*, midge larva, *Chironomus dorsalis*, Cyclops and tadpole of the frog with this parasite. These animals were collected from the ditch and brook in the campus of the university. They were left in the tap water for two or three days, and were examined for natural infection of this parasite. Only the material which was free from the parasite was used. Five or ten animals were kept in each small petri dish and the tap water added with one water drop inclucing many cercariae. They were examined after half an hour or one hour. From these experiments the writer found that *Chironomus dorsalis* and *Anisogammarus annandalei* are second intermediate hosts of this parasite, while cyclops and the tadople are not infected.

According to Prof. K. OKABE of the Institute of Parasitology, Kurume Medical College, *Neocaridina denticulata* is also a second intermediate host of this parasite (unpublished).

It is possible to enumerate, at present, thirteen species of animals as the second intermediate hosts as shown in Table 11.

TABLE 11. The second intermediate hosts of *P. muris*.

Discoverer	Published year	Second intermediate host of *P. muris*	Developmental stage of host
HIRASAWA & ASADA	1929	*Anax parthenope*	Larva
		Orthetrum albistylum	"
		Caloptoryx atrata	"
		Ephemera strigata	"
		Cybister japonicus	Adult
		Chironomus plumosus	Larva, nymph, adult
		Culex pipiens	Larva, nymph
		Asellus aquaticus	Adult
		Turbellaria sp.	"
McMULLEN	1937	*Stagnicola emarginata angulata*	Larva
OKABE (unpublished)	(1950)	*Neocaridina denticulata*	Adult
YAMASHITA	1950	*Chironomus dorsalis*	Larva, nymph, adult
		Anisogammarus annandalei	Adult

C. Observation on the infection of the midge, *Chironomus dorsalis*.

(a) *Penetration into the midge body.*

Observations were made by using the watch glass or hollow slide glass as the container under the microscope. When the cercariae are put into the water with the midge larvae, *Chironomus dorsalis*, they attach themselves rapidly to the caudal tuft of setae of antenna, anterior or posterior pseudopod, especially often to the former two. The cercariae after attaching to the host's body creep on the body surface for a while, and finally penetrate into the host's body from the soft part between the body segments of the host, inserting the anterior end of the body at right angles to the host's body and moving the stylet to and fro rapidly. Further penetration into the host's body is effected by the movement of stylet and body aided by the secreta of the penetration glands. When about one-third of the body of the cercaria penetrates into the host, the tail is very easily detached from the body. Whenever the wriggling detached tail comes in contact with some substratum, it moves actively extending and contracting alternately for some minutes, as if it were still on a living cercaria.

From the penetrated part of the host's body the body fluid of the host runs out, inculuding shining green granules which were secreted from the penetration glands of the cercaria. These granules dissolved into the water soon. The cercaria continues the movement, taking sometimes Dharma form and sometimes pyriform, until finally the whole body enters into the host. It takes only 5 or 6 minutes from the first attachment to the finish of the penetration.

The cercaria having penetrated into the host creeps for two to 10 minutes, seeking for a suitable postition in the body. The creeping movement gradually becomes slow. Then the cercaria begins the rotation movement as soon as the creeping has ceased, and the encystation takes place. It takes 2 or 3 minutes to form the cyst following the beginning of the rotation movement. Within twenty minutes after encystation the white colour of the outer layer of the cyst changes to yellow, and gradually to brown. At the inner side of this layer there is a thin membranous layer called "true cyst". After three days great change occurs in the body of

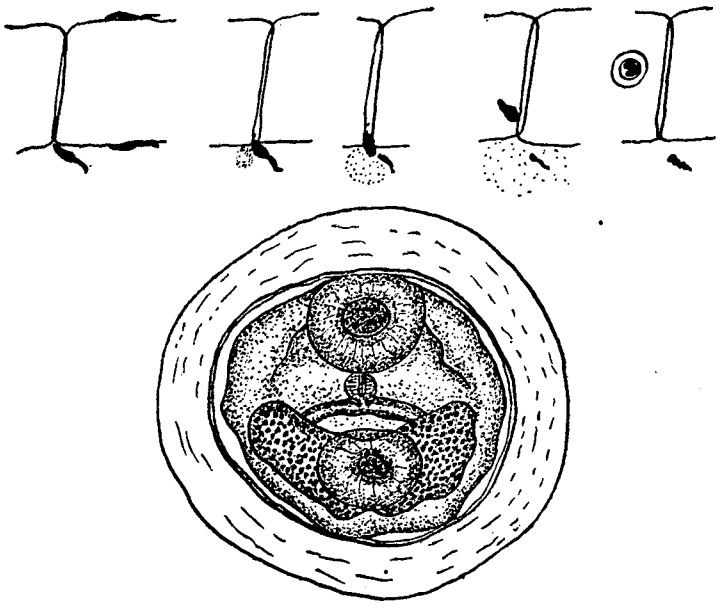

Fig. 5. Various stages of penetration and encysted larva (metacercaria).

the cercaria as will now be described.

The stylet is removed from the body of the encysted cercaria, and remains in the cyst. The penetration gland disappears. In the excretory bladder black granules appear which in number, and fill the bladder after five days. The cercaria develops into the metacercaria which has the ability of infection within five or six days after infection. Therefore the cercaria continues active rotation movement for about three or four days within the cyst, and after that the rotation gradually becomes slow, and almost ceases after five or six days.

The size of the "true cyst" of this parasite is 0.085 mm. to 0.12 mm. in long diameter and 0.08 to 0.115 mm. in short diameter, and the whole cyst including the outer layer is 0.215 mm. to 0.23 mm. in long diameter and 0.118 mm. to 0.22 mm. in short diameter. The thickness of the outer layer is very different with the individual, while that of the "true cyst" is almost the same, namely 0.0037 mm. From this fact for the measurement of the cyst size it is thought better to use the "true cyst" rather than the whole cyst including the outer layer.

(*b*) *Favorite parasitic part in midge larva.*

Two groups including each fifty midge larvae were used for this observation of which the first showed light infection, while the second had fairly high infection. At first all midge larvae of these groups were examined by flattening under a cover slip, and the position of the parasites and numbers of cysts were recorded. The results are shown in Tables 12 and 13.

TABLE 12. Number of encysted cercariae in various parts of body of midge larva in light infection group.

Host No.	Part of host body and numer of cysts														
	Body segment												Post. pseudopod	Blood gill	Anal gill	Total
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII				
1										1	1					2
2											4					4
3								1					1			2
4						1	1			1		1				4
5											2	1				3
6								1	1							2
7		1									1					2
8									1		1					2
9											1	1				2
10		1						3	1	1	1		1			8
11						1			1		1					3
12	1	1		1	1						1	2				7
13							1	1	1	1		1	2			7
14							1				1	1				3
15					1							1	2			4
16	1						1	1	1	1		3				8
17		1		2		1					1	1				6
18				2							1	3				6
19											1		1			2
20			1				1		1				1			4
21											1		1			2
22				2		1	1						4			8
23						1	2	1		1						5

Host No.	Part of host body and number of cysts															
	Body segment												Post. pseudopod	Blood gill	Anal gill	Toal
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII				
24												1	3			4
25						1					3			1		5
26					1						2	1	1			5
27		1					2				3					6
28					1		1					1				3
29	1	2									1	2	1			7
30		1	1	1	1											4
31		1					1		1							3
32									1	1	1	1				4
33											1	2				3
34					1					1		1				3
35							1									1
36												1				1
37			1		1						3	1				6
38				1		1					1					3
39				1		1		1	1	1	4					9
40			1				1				1	1	1			5
41						1							1			2
42											3	2	2			7
43												2				2
44											1	1				2
45		1	1									3	2			7
46	1							1			3					5
47		2	1					1	2		2					8
48		3	1								1	3				8
49				3						1	2	1	1			8
50	1			1					1		1	1	1			6
Total	5	15	7	14	7	9	14	11	13	10	51	40	26	1	0	223
%	2.2	6.7	3.1	6.3	3.1	4.0	6.3	4.9	5.8	4.5	22.9	17.9	11.7	0.5	0	100

TABLE 13. Number of encysted cercariae in various parts of body of migde larva in fairly high infection group.

Host No.	Part of host body and number of cyst															
	Body segment												Post. pseudopod	Blood gill	Anal gill	Total
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII				
1		6		2	1				1				2			10
2				2		1		3	1	4	5	1	2			19
3				3	2	3		3	3	2	3	5				24
4				4	1	1		1	2	4	3	1	1			18
5		1		1	2	1		1	1	3	5	1	1	1		18
6		1		1				1	2	1	1	3	1			11
7	1	2	1					1		3	1	2	1			12
8					1	1	1	1	1			4	2			11
9		1	1						1	3	2	3	2			13
10								1	4	2		3	1			11
11		1	5				2	1	3	2						14
12		1	1		2	1	2	1	1	2	2	1				14
13				3						4	2	1	1			11
14	3	3	2								2	2	1			13
15	3	2	4	2						2	1	1				15
16	2	2								1	3	3				11
17	5	4	4					1			2	1				17
18		2	2	3								8	1			16
19	2	1	4		2			2		1	1	3				16
20	2	2	3			1	1			1	3	3	3		1	20
21	5	2	1					1		1	3	1				14
22	1	4	2					2		2	1	4	1			17
23	4	5	6							1		2				18
24	3	1	2					2	1			1				10
25	1	4	7					1	1			1				15
26	4	1	3		1		1			5	1	4				21
27	5	4	1		2		1	2	1		1	2	1			20
28	7	5	3				1					4	2			22
29	6	2		3	2		1				1	2				17
30	1	2	2	2	2		2	1		3	1	2	1			19
31	1	2	2	1			2			1		6				15

Host No.	Part of host body and number of cyst															
	Bdoy segment												Post. pseudopod	Blood gill	Anal gill	Total
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII				
32	2	1	1							1	3	3				11
33	5	2	1				1				2					11
34	1	3	1	1			1	1			6	6				20
35	2	2	3								1		6			14
36	1	6	3								1	2				13
37	4	4	6	1						1	2	1				19
38	6			1						2	2	5				16
39	3	1	1						1		2	5				13
40	2	7	3	1		1			1	3	2	2				22
41		4	1							1	4	3				13
42	2	4	1							1	2	2				12
43		4	2				2	1				2	1			12
44	3	3	2					1	1			1	1			12
45	4	5	.			1				2		5				17
46	1	13				2			1	2		2				21
47	3	5	3	1					1	1	2	3				19
48			1		1		1	1		2	4	1				11
49	4	1	6	1			1			1	2	1	1			18
50	1	3	1	1							1	4	1			12
Total	100	129	92	34	19	13	20	31	28	65	80	123	32	1	1	768
%	13.0	16.8	11.9	4.4	2.4	1.7	2.6	4.0	3.6	8.5	10.5	16.0	4.1	0.2	0.2	100

The result shows that the number of parasite cysts is far greater in the hind part than in the other ports of the host's body in the light infection, while it shows almost the some number in the fore and hind parts of the body in the fairly high infection group. In severe infection the cercariae are rater more numerous in the fore body than in the hind part as shown in Table 15 in the next section. In the blood gills and anal gills as well the cysts are very few or none.

The frequency of the cysts in each part of the host's body is as shown in Table 14. According to the finding in the light infection group the cysts are more often in the hind body than in

the fore body of the host, but in the high infection group they are very frequent, showing the same rate either in the fore or hind parts of host's boby. The occurrence of the cyst in the central part of the host's body is not frequent in any case.

TABLE 14. Infection frequency of the cyst in various parts of body of midge larva.

Grade of infection	Part of host boby and frequency of cyst														
	Body segment												Post. pseudopod	Blood gill	Anal gill
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII			
Light	5	11	7	9	7	9	12	9	12	10	31	27	17	1	0
Fairly high	34	42	36	19	12	10	15	23	19	32	36	46	21	1	1

As above mentioned the number and frequency of the cyst parasited to the host go parallel. Probably the favorite parasitic position of the cercaria of this parasite is the hind part of the host's body including the 11th and 12th segments, and the next is the fore part of the body including the 2nd and 3rd segments.

(c) *Number of parasitic cysts and life span of the infected midge larva.*

The midge larva which is infected artificially with this parasite either to a light or fairly high degree develops into the nymph and adult stages, retaining the metacercaria of the parasite within the body. Observations on the longevity of the midge larva heavily infected with this parasite were made. The death of the host is easily judged by the change of the body colour, from red to green or blue, and also by the ending of movement of the inner organs, viz. air bladder, intestine, etc. The results of the observations are as given in Table 15.

The life of the host becomes shorter in proportion to the increase of the number of parasites. The host infected with about eighty parasites does not die within very short time, but it can not develop into a nymph. The death of the host may be due to the oppression upon the inner organs especially by the air bladder made by the existence of the cyst.

TABLE 15. Relation between numbers of encysted cercariae and the longevity of midge larvae.

Longevity of host (hrs.)	Various parts of host body and number of cysts																
	Body segments												Post. pseudpod	Blood gill	Anal gill	Total	Average
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII					
1.5	17	35	29	17	31	41	23	34	37	43	26	20	17	1		371	371
2	20	22	13	3	11	25	20	16	11	16	23	18	13	1	2	214	
	16	20	27	11	9	6	4	14	10	45	16	25	16		4	223	226.5
	18	31	22	10	5	12	7	19	30	11	14	24	8	4	1	216	
2.5	22	26	13	5	2	1	11	8	6	23	30	27	12	2		188	
	26	35	18	10	10	16	3	6	4	7	25	32	3			195	189.3
	18	25	15	2	8	13	5	4	5	13	23	35	18	1		185	
3	19	11	4	10	1	4	3	1	6	6	15	14	10			104	
	18	12	13	7	7	2	5	4	3	14	16	22	14			137	
	28	25	15	5	5	6	10	6	6	6	13	19	7	1		152	
	12	17	9	8	9	14	12	8	20	25	11	7	9			161	
	30	25	29	18	20	7	7	2	10	10	12	11	10			196	155.1
	17	31	25	10	5	2	5	3	10	10	18	20	5	1	1	163	
	15	20	15	8	5	9	11	6	10	15	15	19	6			154	
	25	24	28	8	13	6	8	1	3	14	11	7	11	1	2	162	
	18	20	12	10	12	8	18	10	17	20	15	6	1			167	
3.5	12	21	16	18	3	3	6	9	7	18	13	8	1		1	136	136
4	17	19	16	4	3	1	9	6	2	13	12	8	4			114	114
4.5	12	19	15	13	2	4	7	2	4	8	9	7	5			107	107
Over 6	11	18	13	5	1	1	1	0	1	7	13	7	3			81	81
Total	371	455	347	182	162	181	175	159	202	324	330	341	173	12	11	3426	171.3

D. Infection of beach-flea, *Anisogammarus annandalei*.

The cercaria penetrates the soft parts between not only the body segments, but also the leg segments of the beach-flea, *Anisogammarus annandalei*. In this host the feature of the cyst differs from that of the other hosts except that of the gill, the cyst appearing as a black point which is covered with a soot like black

layer on the forth or fifth day after infection. The larva in a black cyst also developed into the mature metacercaria which becomes the adult when given to the mouse experimentally. When many cercariae are put into the water together with *Anisogammarus*, the number of cercariae penetrated into the host are not so much even after two hours. This may ascribe to the fact that the part between body segments of leg segments of *Anisogammarus* is harder than that of the midge larva.

The size of the cyst is not different from that of the midge.

E. Infection of white mouse as a final host.

The larvae of the midge, beach-fleas or dragonfly-larvae infected with this parasite were given to the white mice which were confirmed to be free from any helminth parasite by the examination of faeces for ten days before used. Many adults of the parasite were obtained from the small intestine of the final host. On the fifth day of the experiment in which the metacercariae of these intermediate hosts were found in the faeces of the final host a few eggs of Plagiorchis trematode were detached after which the eggs increased in number gradually day by day. The mice were killed on the 12th day, and many adult worms were found right below the duodenum. The body size of the adult worm is 0.8 mm. to 2.1 mm. in length and 0.23 mm. to 0.82 mm. in breadth, taking a flattened long elliptical shape. The colour is light or orange yellow. They were identified as *Plagiorchis muris* (TANABE).

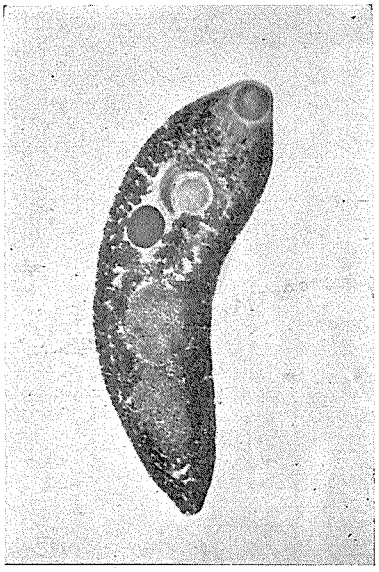

Fig. 6. Adult worm of *P. muris* obtained from the white mouse by experimental infection.

F. Reduction of infectivity of cercaria under influence of certain factors.

A series of experiments was carried out to learn about the

degree of retention of infectivity by the cercaria which had been subjected to chemical treatment and recovered movement after treatment, as described before. The recovered cercariae were put into the tap water together with the midge larvae, and the penetration behavior was observed for two hours with the aid of the binocular microscope. The chemicals used were 0.05% acetic acid and 5% sodium bicarbonate.

As shown in Table 16, many cercariae which bad been subjected to 0.05% acetic acid for 5 minutes penetrate the host body and encyst, while only a few cercaria treated for 7 minutes pene-

TABLE 16. Infection to midge larvae of cercariae affected by 0.05% acetic acid for various durations of time.

Affected time (Min.)	No. of host	Various parts of host and number of cysts															Total
		Body segment												Post. pseudopod	Blood gill	Anal gill	
		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII				
2	1		3	1										2			4
	2												2				2
	3	1															3
	4	1			1	1							2	1			6
	5	1		1							1		1	1			5
3	1						1						2	2			5
	2		1								2		1	3			7
	3												4	1			5
	4		2	1		1							1				5
	5		1	1						2				2			6
4	1			1									2	1			4
	2			1							1		1	1			4
	3			2	1					1				1			5
	4				2								2	2			6
	5			1							1		1	1			4
5	1									1		1					2
	2							1									1
	3											1	1				2
	4										1		2				3
	5			1						1			1	1			4
7	1												1				1
	2												1				1
	3											1					1
	4												1				1
	5												1				1
10	1																0
	2																0
	3																0
	4																0
	5																0

trate and encyst, but the cercaria affected for 10 minutes did not penetrate. So it is clear that the cercaria loses infectivity or penetration power upon subjection to the action of 0.05% acetic acid for longer than ten minutes.

Many cercariae affected by 5% sodium bicarbonate for 7 minutes retain the infectivity; only a few cercaria affected for 12 minutes still have infecting power; those affected for 13.5 minutes lose their infectivity. It is noted that the infecting activity is exceedingly different between the 7 minutes and 10 minutes

TABLE 17. Infection to midge larvae of cercaria affected by 5% sodium bicarbonate for various durations of time.

Affected time (Min.)	No. of host	Various parts of host anst and number of cysts														
		Body segment											Post. pseudopod	Blood gill	Anal gill	Total
		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII				
2	1			3	1	1							1				6
	2		1						1					1			3
	3	1		1					1		1						4
	4					1						1	1		1		4
	5		1	1								1	1				4
6	1			1	1						1		1				4
	2			1								1	2	1			5
	3												1	3			4
	4		2														2
	5		1	3				1			1	1	1	3			11
7	1	3	2							1							6
	2	1	2														3
	3											2	1	1			4
	4							1		1	1		2				5
	5			1									1				2
10	1																0
	2																0
	3														1		1
	4		1														1
	5		1					1									2
12	1																0
	2			1									1				2
	3												2				2
	4												1				1
	5																0
13.5	1																0
	2																0
	3																0
	4																0
	5																0

treatment of two groups of the cercariae treated with 5% sodium bicarbonate, though the recovery of movement of these two groups was accomplished within the same time.

The relation between the infectivity of cercaria and temperature was observed. The cercariae were put into slender test tubes with water, and the tubes placed in a hot water tank. Thus the temperature and the affecting time were regulated. After recovering movement in the tap water the midge larvae were placed in the test tubes.

TABLE 18. Infection to midge larvae of cercariae affected by various temperatures.

Affected Temp. (°C)	Time (Min.)	No. of host	Various parts of host and number of cysts: Body segment I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	Post. pseudopod	Blood gill	Anal gill	Total
37	5	1	3	3	3			1					1	3				14
		2	2	2									1	2				7
		3	1		1		2	1					2	1	2			10
		4	3	2	6	1				1			1	4	1			20
		5	3	6	4	1							1	4	1			20
40	10	1		3	2	1	1								4	2		13
		2	1	10	2	1	1			1		2		5	5	2		30
		3	14	8	6	1	1			1			4	1	2	9		47
		4	3	5	3									1	1			13
		5	1	11	1									2	1			16
43	5	1	2	1	2								2	3				10
		2	3	6	1									5			1	16
		3		3	2									2				7
		4	5	2									2	4	1			14
		5		4	2								2	4				12
43	10	1	3	5	2	6						3	2	2	4	1		28
		2	3	9	8	3			1	1	1					1		27
		3	2	7	4	4	1	4	4	2	2	2	2	2	4	2		42
		4	2	5	3	1						1	2	3	1			18
		5		10	12	9		4		1		2	1	2	2			43
45	5	1	3		1								1	2				7
		2		4	1							2	1	3				11
		3	2	1									3	1				7
		4	3			1								3				7
		5		4	1									4				9
45	10	1																0
		2																0
		3													1			1
		4																0
		5																0

Affected Temp. (°C)	Time (Min.)	No. of host	Various parts of host and numbers of cyst															
			Body segment											Post. pseudopod	Blood gill	Anal gill	Total
			I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII				
47	5	1	2			1							2	2	3			10
		2	5		3										1			9
		3	1											3	1			5
		4	1										1					2
		5	5	2	1		1							1				10
50	5	1																0
		2																0
		3																0
		4																0
		5																0

As shown in Table 18 the reduction of the infectivity of the cercariae is not induced by subjection to 43°C temperature for 10 minutes or 47°C for 5 minutes, but it is very clearly influenced by 45°C for 5 minutes. The cercariae lose infectivity absolutely by subjection to 50°C for 5 minutes, creeping around on the body surface of midge larvae even after two hours. Therefore it is clear that the penetration power of the cercaria is affected by these chemicals, though it is not clear whether the encystation power is retained or not. Solution of this question will be discussed later in Chapter VI, Section E.

VI. Experiments on artificial encystation of the cercaria in vitro.

As already described the cercaria shows simple behavior in animal blood serum. When one drop of the water including many cercariae is put on a hollow slide glass or in a watch glass, at first the cercariae are seen swimming actively, but after about one minute every cercaria sinks to the bottom, and begins creeping. At this time the water is removed from the slide glass or watch glass rapidly by a pipette, and replaced with two or three drops of the serum of the serum of body fluid. The behavior of a cercaria is shown in this case to be the same in vitro as in the intermediate host, and it does encyst.

A. General consideration of artificial encystation in vitro.

Four stages in the artificial encystation process of the cercaria

in the blood serum or body fluid can be established as follows.

(*a*) *The first stage.*

This stage extends from the occurrence of the wake-like creeping course of the cercaria in the medium to the detachment of the tail. When the cercaria is affected by the blood serum or body fluid, the creeping movement gradually slows within a very short time, and at the same time a wake-like substance appears behind the body to show the creeping course. It is probable that this substance may be produced by the reaction between the secreta of

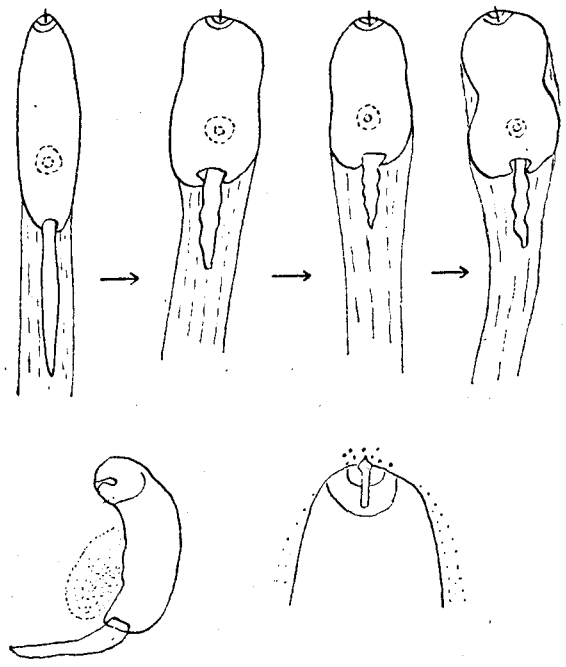

Fig. 7. Development of wake-like substance behind the body of a cercaria and change of body shape with its movement.

the cystogenous gland of the cercaria and the blood serum or body fluid. After one or two minutes the creeping movement of the cercaria almost ceases, leaving only active movement of the tail. Soon after cessation of the creeping movement the cercaria begins the extending and contracting movement of the body very actively, and its body shape becomes Dahma form or pyliform. In this movement of the body, the cercaria actively widens and narrows the

indented parts of the posterior end in which the tail-root attaches. By such movement subsequently the tail is pushed in and out very actively. The movement continues for about one minute ; then the tail is detached, one part of the root leaving the body first and finally the entire part. The excretory tube is likely to be drown out of the body with the detached tail. Just before detaching of the tail, the writer observed some granular substances to be sent out towards the tail in this type. Detaching of the tail usually occurs within six or seven minutes after the affection of the serum, but it occasionally occurs in the early creeping time. The detached tail continues wriggling motion for some time near the body part of the cercaria. Soon after detaching the tail-root swells as shown in Fig. 8, the tail gradually contracts, and finally it ceases all movement.

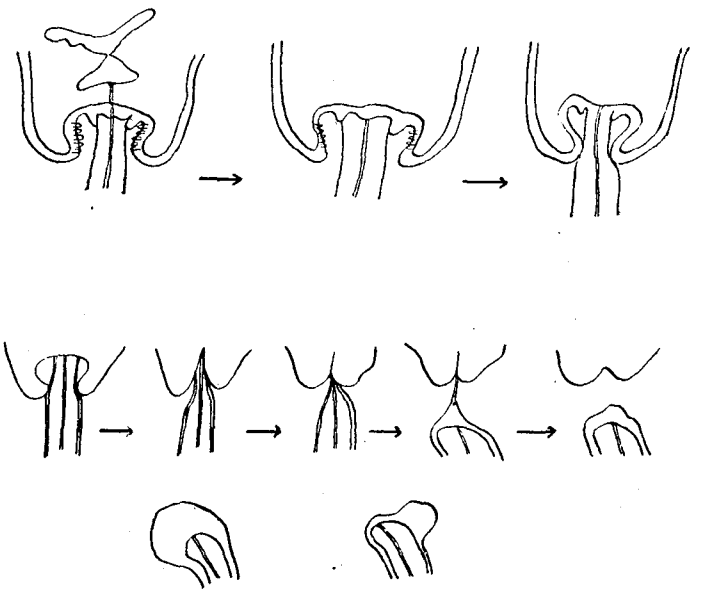

Fig. 8. Stages of detaching of the tail from the body end of cercaria.

(*b*) *The second stage.*

This stage extends from the beginning of the rotation movement to the forming of the outer layer of the cyst. Soon after detaching of the tail the cercaria begins the rotation movement

at the end of the wake-like substance. With the rotation, this substance surrounds the cercaria forming a spherical thick-layered cyst.

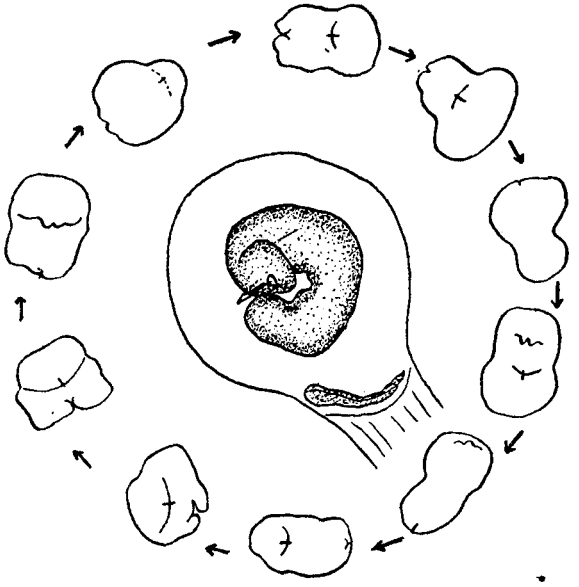

Fig. 9. Change of body shape of cercaria in rotation movement and formation of the outer layer of cyst.

Before rotating, the stylet of the cercaria continues its movement very actively, and from the penetration glands the cercaria secrets actively a granular substance which rapidly dissolves and forms a thin wall in front of the anterior end of the body. However, this wall disappears soon after. The stylet moves actively against this wall as it does when penetrating the intermediate host. When the cercaria is surrounded by the thick layer as above mentioned, the detached tail ceases movement soon.

(*c*) *The third stage.*

This stage extends from the beginning of the occurrence of the inner layer called "true cyst" to the completion of encystation. The cercaria which is surrounded by the thick layer continues the active rotation movement within it, and a thin membranous layer appears at the inner side incontact with the thick layer, surrounding the cercaria as a cyst within about two minutes after the start

of the rotation movement. At the beginning of this encystation process the "true cyst" is soft and varies in shape with the rotation movement, being elliptical in shape usually. After a few minutes the "true cyst" becomes so hard that its shape is not changed easily with the rotation. After a few hours it changes into almost spherical shape which is characteristic to this species.

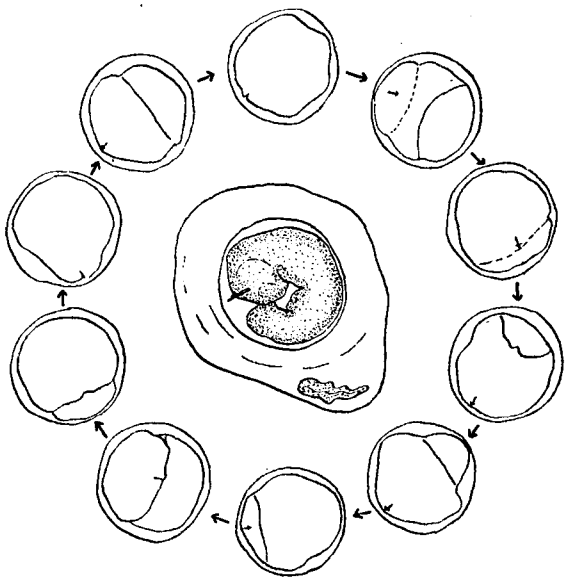

Fig. 10. Change of body shape of cercaria during rotation movement within "true cyst".

The size of the cyst varies with each individual cercaria. This variation is due to the size variation of the body within the cysts, for the cysts are formed by the rotation movement of the cercaria. The size of the cysts is shown in Table 19.

TABLE 19. Size of cyst (true cyst) formed artificially in the serum.

0.083 × 0.082	0.093 × 0.079
0.083 × 0.085	0.079 × 0.077
0.083 × 0.083	0.083 × 0.083
0.079 × 0.079	0.085 × 0.083
0.088 × 0.092	0.083 × 0.083

The resistance of the cyst wall and larva within it against the pressure or chemicals is fairly strong. Even if the cyst is pressed so as to have a diameter twice as much as before, the wall remains unbrocken, and the larva is kept alive within the cyst.

(*d*) *The forth stage.*

This stage extends from the transformation of the body structure to the maturation of the larva. Now the cysts of the cercariae formed in the artificial media survive no longer than three days, even if the serum containing cysts is renewed twice daily. Therefore, no development of encysted larvae is observed. However it is probable that if the larvae survived for one or two days more, at least in this stage, the stylet and penetration glands would also disappear the cyst formed in the intermediate host.

B. Encystation in the blood serum.

(*a*) *Relation between speed of encystation and temperature.*

The speed of encystation in colt serum was observed using a hollow slide glass. The temperature was controlled by the thermostat and thermo-apparatus of the microscope. From the data in Table 20, it is clear that the temperature affects the speed of encystation of the cercaria.

TABLE 20. Encystation speed in various temperature.

Temperature (°C)	Encystation speed (Min.)
20	15—20
22	10—12
24	8—10
26	8—10
30	5—7
37	4—6
40	4—5
45	3—5
48	3—4
50	3—4
52	3—4

At 30°C the encystation was made very rapidly, but at 48°C many cercariae died before encysting. This may be due to the reduction of the resistance of the cercaria at such a temperature.

(*b*) *The speed of encystation in normal serum.*

Fresh blood sera of man, cattle, horse, pig, dog, cat, rabbit, guinea pig, roof rat (*Rattus rattus rattus*), Norway rat (*Rattus norvegicus norvegicus*), white mouse, domestic fowl, salamander (*Hynobius retardatus*), frog (*Rana temporalia*) and fish (*Carassius auratus*) were used. All experiments were carried out at room temperature (24–26°C), excepting only the case of the human blood serum which was done at 19°C.

Detaching of the tail occurs more rapidly in the blood sera of pig, dog, domestic fowl, salamander, frog and fish than in the others. Especially in the sera of frog, salamander and fish it occurs very rapidly. Subsequently, the rotation movement or encystation, occur more rapidly in such blood sera as that of amphibia and fish than in the others. The time required for the encystation in more than one half of the cercariae is almost the same in every kind of sera of mammals at 24–26°C, but at 19°C it is far longer than at 24–26°C.

The speed of the encystation differs with the individual cercaria, and the required time from the beginning of rotation to the detaching of the tail is about 2 minutes in every cercaria. As above mentioned the encystation of the cercaria occurs very rapidly in the blood sera of amphibia and fish, but many cercariae die before "true cyst" appears. The faster encystation brings about the higher frequency of abnormal individuals. Consequently it is probable that the stimulus of the sera is so strong as to prevent the cercaria from encysting.

TABLE 21. Encystation speed in normal sera of animals.

Kinds of sera	Required time (Min.)			
	Tail detaching	Beginning of rotation	Encystation	
Man	20—22	23—24	24—26	Average 25
	6—7	7—9	8—10	8
Cattle	8—9	10—11	12—13	12
	5—6	6—7	7—8	8
	6—7	7—8	8—9	8

Kinds of sera	Required time (Min.)			
	Tail detaching	Beginning of rotation	Encystation	
Horse	5—6	6.5—8	8—10	Average 8
	5—6	7—9	8—11	9
	7—10	14—17	15—18	16
Pig	3—4	4—5	7—11	9
	7—8	8—15	10—17	11
	5—6	7—8	8—10	9
Dog	4—5	6—8	8—10	9
	4—5	7—9	8—10	9
Cat	8—9	10—12	12—14	12
	8—10	10—12	12—15	13
	5—7	6—8	7—9	8
Rabbit	8—9	9—11	10—12	11
	7—9	8—11	9—11	10
Guinea pig	8—9	9—11	10—12	10
	7—8	9—11	10—12	10
Norway rat	7.5—8	8—9	9—10	9
Roof rat	7—8	8—9	9—10	9
White mouse	7—8	9—10	10—12	10
Domestic fowl	3—4	5—8	6—9	8
	3—4	5—7	6—8	7
Salamander	2—3	5—6	6—8	6
	3—4	6—8	7—9	7
Frog	3—4	4—6	5—7	6
	1—2	2—4	4—6	5
	1.5—3	2—4	3—5	4
Fish	3—4	4—5	5—6	5
	3—4	5—6	6—7	6
	3—4	4—6	5—7	5

(*c*) *The speed of encystation in heated serum.*

The encystation of the cercaria was observed in heated blood sera of mammals as shown in the following table. The normal sera of the animal which served as the control induced the encystation

within twelve minutes.

Now it has become clear that the encystation of the cercaria in the heated serum occurs slowly as compared with that in the normal serum and added to this there appear much differences of encysting speed between individuals. Among all kinds of sera heated at 50°C for 30 minutes, only the cattle serum is unfavorable to encystation. Excepting the cattle serum, the other kinds of sera heated at 54°C for 3o minutes still provide suitable media for encystation, although the strength is exceedingly reduced, especially in the sera of dog and guinea pig. When they are heated at 56°C for 30 minutes, all sera lose susceptibility to encystation absolutely except the horse serum which still retains it indistinctly even at 58°C. The horse serum, however, becomes ineffective, when heated at 60°C for 10 minutes.

TABLE 22. Encystation speed in heated sera of mammals.

Kinds of sera	Temperature (°C)	Heated time (Min.)	Encystation time (Min.)	Number of cysts after three hrs.
Man	50	30	7—60	many
	52	"	20—60	many
	54	"	40—60	few
	56	"	—	none
Cattle	50	30	30—100	many
	52	"	—	none
	54	"	—	none
Horse	56	30	15—20	all
	58	"	120—150	very few
	60	10	—	none
	60	30	—	none
	62	5	—	none
Pig	46	30	10—20	all
	50	"	20—40	almost all
	52	"	30—40	many
	54	"	24—50	almost a half
	56	"	—	none
Dog	52	30	30—50	many
	54	"	120—150	very few
	56	"	—	none

Kinds of sera	Temperature (°C)	Heated time (Min.)	Encystation time (Min.)	Number of cysts after three hrs.
Cat	52	30	30—60	many
	54	"	60—150	few
	56	"	—	none
Rabbit	50	30	10—25	all
	52	"	20—30	almost all
	54	"	50—100	very few
	56	"	—	none
Guinea pig	50	30	15—50	all
	52	"	20—50	many
	54	"	100—150	very few
	56	"	—	none

Detailed observations were made on the encystation of the cercaria in the sera of man, rabbit and guinea pig heated 50°C for 30 minutes. Twentyfour cercariae were used in each kind of serum, and the number of cysts were recorded after 20, 25, 30, 40, 50 or 60 minutes. The results are shown in Table 23.

TABLE 23. Compraison of encystation speed between three kinds of sera heated at 50°C for 30 minutes.

Kinds of sera	Time after placed in serum and number of encysted cercariae.						Unencysted cercariae
	20 min.	25 min.	30 min.	40 min.	50 min.	60 min.	
Man	4	8	8	10	12	16	8
Rabbit	14	24					0
Guinea pig	5	16	18	20	24		0

All cercariae encysted within 25 minutes in the rabbit serum, and all cercariae encysted within 50 minutes in the guinea pig serum. In the human serum one half of them encysted after 50 minutes, and only two-thirds of them had encysted even after one hour. It is clear that the speed of encystation occurs far more rapidly in the rabbit serum, or in guinea pig serum than in the human serum. But the data in Table 21 have shown that after three hours at a temperature of 54°C the number of encysted cercariae is far greater in the human serum than in the rabbit and

guinea pig serum. From the above it is clear that the reduction of the encystation susceptibility of rabbit and guinea pig serum occurs more rapidly than that of human serum, when the affected temperature is over a certain degree.

(*d*) *The speed of encystation in diluted serum.*

1. On unheated serum.

As shown in the following table, the serum of the horse, dog or rabbit diluted sixteen-fold still retains susceptibility to encystation, though some other sera lose it. In the serum which is diluted to the maximum so as not to destroy the encystation efficacy, the number of encysted cercaria is all the time less than a half of the total.

TABLE 24. Encystation speed in diluted sera of various kinds of animals.

Kinds of serum	Control (normal serum)	Grade of dilution and encystation speed (Min.)				
		2 fold	4 fold	8 fold	16 fold	32 fold
Man	24—26	30—35	37—40	—	—	—
	8—10	10—12	14—24	30—50	—	—
Cattle	12—13	11—27	12—34	20—90	—	—
	7—8	7—8	10—12	20—60	—	—
	8—9	8—9	10—12	20—60	—	—
Horse	8—10	9.5—19	10—15	15—40	30—80	—
	8—11	10—12	12—16	18—40	30—80	—
	15—18	17—20	20—25	26—50	35—90	—
Pig	7—11	7—12	9—15	17—30	—	—
	10—17	10—17	12—25	20—50	—	—
	8—10	8—12	10—15	17—35	—	—
Dog	8—10	8—11	10—16	20—30	40—60	—
	8—10	9—12	10—15	20—30	38—70	—
Cat	12—14	12—15	15—20	18—40	—	—
	12—15	12—16	15—20	20—40	—	—
	7—9	10—12	12—15	20—40	—	—
Rabbit	10—12	20—26	30—60	90—100	100—150	—
	9—11	18—26	30—50	90—100	120—150	—

Kinds of serum	Control (normal serum)	Grade of dilution and encystation speed (Min.)				
		2 fold	4 fold	8 fold	16 fold	32 fold
Guinea pig	10—12	15—30	16—35	30—50	—	—
	10—12	16—30	20—37	30—60	—	—
Norway rat	9—10	9—16	16.5—20	35—60	—	—
Roof rat	9—10	9—16	18—20	30—60	—	—
White mouse	10—12	10—17	20—30	30—60	—	—
Domestic fowl	6—9	6—10	7—11	11—50	—	—
	6—8	6—8	7—10	15—70	—	—
Salamander	6—8	6—10	8—12	20—60	—	—
	7—9	7—10	8—12	20—70	—	—
Frog	5—7	6—10	8—12	18—60	—	—
	4—6	6—10	8—15	20—70	—	—
	3—5	6—10	8—16	19—60	—	—
Fish	5—6	7—8	14—20	26—100	—	—
	6—7	7—8	10—20	30—50	—	—
	5—7	7—9	10—18	25—60	—	—

2. On heated serum.

In this experiment the horse serum heated at 56°C for 30 minutes and the pig serum heated at 46°C for 30 minutes were used. The heated horse serum still retains susceptibility to encystation at the eight-fold dilution. The encystation in the unheated serum is faster than in the heated (compare with Table 24). The number of encysted cercariae in the former is less than that in the latter which is at eight-fold dilution. The difference in number is more clear at the sixteen-fold dilution.

The pig serum heated at 46°C for 30 minutes is entirely unfavorable to encystation at the eight-fold dilution.

As shown in Tables 21 and 22, the difference of the speed of encystation and the number of encysted cercaria between the horse serum heated at 46°C for 30 minutes and the unheated are distinct. Both sera show clearly the difference of the encystation power by the dilution as shown in Tables 25 and 26. These results show that the encystation power is reduced by heating.

TABLE 25. Comparison of encystation speed between diluted serum heated at 50°C for 30 minutes and diluted normal serum of horse.

Serum diluted	Encystation speed (Min.)		Number of encysted cercariae (after 3 hrs.)	
	Heated serum	Unheated serum	Heated serum	Unheated serum
no	16—20	8—11	all	all
2-fold	18—21	10—12	all	all
4-fold	20—50	12—16	almost all	all
8-fold	35—120	18—40	one third	almost all
16-fold	—	30—80	none	one third
32-fold	—	—	none	none

TABLE 26. Comparison of encystation speed between diluted serum heated at 46°C for 30 minutes and diluted normal serum of pig.

Serum diluted	Encystation speed (Min.)		Number of encysted cercariae (after 3 hrs.)	
	Heated serum	Unheated serum	Heated serum	Unheated serum
no	10—18	10—18	all	all
2-fold	17—35	10—13	all	all
4-fold	25—40	12—25	one third	all
8-fold	—	20—50	none	one half
16-fold	—	—	none	none

(*e*) *Eecystation power in the serum in the preserved condition.*

As already mentioned before, many cercariae die before forming "true cyst", though the outer layer of cyst forms very rapidly in fresh serum of amphibia or fish, and every cercaria encysts normally when these sera are used after preserving for one day or diluting two-fold. In the serum of cattle or horse which is prepared soon after the collection of the blood, the same phenomenon occurs. The blood serum preserved for one or two days unseptically is effective for encystation just as the fresh serum, but the serum preserved for three days becomes less effective reducing the speed. Even after five days the encystation power

of the serum is not greatly weakened as compared with the normal one. Therefore the writer says that the serum retains the encystation power, in so far as it is not corrupted.

(*f*) *Encystation in abnormal serum.*

As investigation was caried out on the encystation of the cercaria in the serum of rabbits which had been injected 1 cc. of 20% ethyl alcohol per 1 Kg. of body weight, in the ear vein for six weeks daily. Two rabbits were sent to the author from Dr. T. TANABE of the Insutitute of Pharmacology, Faculty of Medicine, Hokkaido University. The quantity of alcohol included in the serum of the rabbit was 130.23 mg/dl and 140.58 mg/dl respectively. The sera showed the same trend in the encystation process.

TABLE 27. Encystation speed of cercaria in abnormal serum of rabbit injected with alcohol.

Serum diluted	Observation time and rate of encysted cercaria showed in brackets.
no	10 min. (1/3) 15 min. (all)
2-fold	16 min. (1/5) 19 min. (2/3) 21 min. (all)
4-fold	27 min. (1/5) 40 min. (all)
8-fold	53 min. (1/10) 65 min. (1/5) 90 min. (1/2) 3 hrs. (1/2)
16-fold	90 min. (very few) 3 hrs. (very few)
32-fold	none even after 3 hrs.

From the above table it is clear that the encystation in the serum of the animal which has been treated with alcohol, occurs normally. The encystation in the serum of the rabbit, guinea pig or man to which is added 0.001 or 0.003% HCl is also normal. The sera of white mice which were infected heavily with the parasite showed the normal encystation too.

C. Encystation in the body fluid of insects.

In this observation two kinds of body fluid were used, namely that of the dragonfly larva which is an intermediate host and that of the silk worm which is not an intermediate host.

TABLE 28. Encystation speed in body fluid of dragonfly larva.

No. of body fluid	Tail detaching (Min.)	Reginning of rotation (Min.)	Encystation (Min.)	Number of encysted cercaria
1	6—7	7—14	8—15	all
2	3—5	5—14	6—16	all

As shown in Table 28, in the body fluid of the dragonfly larva the encystation of the cercaria occurs rapidly similar to that in the serum of the other animals especially such as domestic fowl, amphibia or fish. The colour of the body fluid of the dragonfly larva is lemon yellow just after collection, and then becomes ochreous yellow after 15 minutes, light sepia after 20 minutes and grey after 35 minutes.

The encystation power of the senile body fluid which had become ochreous yellow or grey was examined. In the ochreous yellow fluid the cercaria loses the tail after 3 or 4 minutes, begins the rotation movement after 4 to 10 minutes, and encysts after 6 to 15 minutes. It is clear that the body fluid of the dragonfly larva turned ochreous yellow is as normal so far as the encystation is concerned as is the fresh body fluid. In grey fluid the creeping movement of the cercaia becomes very slow after 45 minutes, and all movement ceases after 50 minutes, showing no rotation movement even after 3 hours. So it is clear that in the body fluid turmed grey the cercaria already lost the encystation power. The body fluid of the insect loses the encystation power when diluted twice as much.

In the body fluid of the silkworm the cercaria swims around for 20 minutes continuously, and then stops swimming to creep slowly after 3 hours without rotation movement or encystation. In repeated experiments with the silk worm serum the results have proved all negative.

D. Encystation in the coelomic fluid of Ascaris.

The behavior of the cercaria in the coelomic fluid of *Ascaris lumbricoides* or *Parascaris eguorum* which gives a characteristic stimulus to the other animals, was observed. The cercaria ceased movement after 3 hours without encystation in these samples.

E. Encystation of the cercaria affected by some other factors.

In Section F Chapter V it was reported that the cercariae affected by 0.05% CH_3COOH for 10 minutes, 5% $NaHCO_3$ for 13.5 minutes or 50°C for 5 minutes are unable to penetrate the intermediate host, but there remained a question whether these cercariae retain the encystation power or not. In the following experiment the artificial encystation method in vitro was applied in order to solve the problem.

After the recovering of the movement of the cercariae affected by these media, they were transfered into fresh horse serum. The cercariae affected by CH_3COOH began the rotation movement after 8 minutes, and encysted after 9 or 10 minutes. The body fluid of the dragonfly larva was likewise effective. The cercariae encysted after 12 to 15 minutes. It took a longer time than in the case of subjection to acetic acid. The cercariae affected by high temperature showed almost the same tendency as the above concerning both the movement and the encystation. It thus has become clear that even though the penetration power of the cercariae affected by these factors is lost, the potency of encystation is still retained.

However, the cercariae exposed to the temperature at 50°C for 10 minutes lose encystation power. The influence of the undesirable medium upon the cercariae will be discussed in detail in Chapter VII.

The encystation of the cercariae having been stained by 0.02% neutral red or nile blue was observed in cattle serum. The cercariae stained by neutral red for 30 or 40 seconds encysted after 7 to 10 minutes. Only a few cercariae which were stained for 50 seconds, encysted, and the most of them died without forming "true cyst". The cercariae stained by nile blue for 10 seconds showed the same tendency as those affected by neutral red for 50 seconds.

F. Encystation and components of serum.

An attempt was made to determine what component of the serum is concerned with the encystation of the cercaria. At first the albumin, euglobulin and pseudoglobulin were tested. These components were separated from the serum by means of HESTOEN

& COLE's methode modified by OGATA using the saturated solution of ammonium sulphate. The cercaria swam actively, regardless of the various concentrations of these components. The creeping movement was normal, but no cercaria encysted even after 3 hours. There was no cercaria which detached the tail. Encystation did not take place in a mixture of the three components. It is possible that the method of preparation of the component may change the character. So the writer separated the component from the serum by another method. Seven sacks of the cellophane each having 1 cc. of the serum, were put in running water in order to dialyse the component. After some time of dialysis the contents of each sack was tested. The results are given below.

TABLE 29. Relation between time of dialysis and encystation susceptibility of cattle serum.

No. of cellophan sack	Time of dialysis (Min.)	Quality of serum before dialysis (cc.)	Quality of content in sack after dialysis (cc.)	Time of tail detaching (Min.)	Time of encystation (Min.)	% of encysted cercariae after 3 hrs.
1	30	1	0.85	5—13	7—15	100
2	60	"	1.2	5—13	7—15	100
3	90	"	1.25	15—30	18—60	50
4	120	"	1.6	40	45	8
5	240	"	1.6	90	100	6
6	300	"	1.65	—	—	0
7	360	"	1.7	—	—	0

As seen in the above the encystation susceptibility of the sack content dialyzed for 30 minutes or one hour shows no difference from that of the normal serum, but 1.5 hours dialysis reduces the power by half. Dialyzing for 2 or 4 hours the susceptibility falls exceedingly, and 5 hours' dialysis destroys susceptibility entirely. In the normal serum of the cattle, the cercariae encyst within 7 to 15 minutes, and even in the two-fold dilution they can encyst within 7 to 15 minutes. In the four-fold dilution, they encyst within 18 to 30 minutes. So it is clear that the reduction of the encystation susceptibility of the serum is not caused by the intruding water in the case of dilution, as the diluted serum is suitable in spite of the concentration. The disappearance of some components

during dialysis may be responsible. It is noted that the salt separated frok the serum is unfavorable to encystation.

VII. Discussion.

The cercariae of *Plagiorchis muris* emerge from Limnaea snail during the night particularly in large number in Summer. The swimming movement of the cercaria is most active in the low basic water. After escaping from the snail host the cercaria swims near the surface of the water for a while and gradually sinks to the bottom. This occurs within 5 hours after escaping. The tail of the cercaria is removed from the body, when about one-third of the body penetrates into the host's body. This penetration into the host body is made by the physical function of the stylet being assisted by the chemicals from the secreta of the penetration glands of the cercaria. The movement of the stylet is seen at the time of penetration, in both serum and in water. No secretion of the penetration gland occurs in the water though it does occur in the serum, The granular secretory substance is found at the opening of the penetration gland only in the serum. Therefore, it is probable that the secretory function of the penetration gland may be induced, when the anterior end of the body in which the penetration gland opens touches with the body fluid running out of the host body through the physical function of the stylet.

The writer has found first that a wake-like substance is produced behind the body of the cercaria which put into the serum. This substance may surely be produced in the host's body, though it has not been recognized clearly. The substance is of the same character as the outer layer of the cyst.

As for the reasons why the cercaria can not penetrate the intermediate host, three factors were discussed, viz. (1) the reduction of power of attaching to the host, or the reduction of the other movements of the body, (2) the reduction of the penetration power of the stylet and (3) the secretory impairment of the penetration gland or change of the character of its excreata. The writer has described how the cercaria affected by some factors loses penetration power, without losing encystation power. In such case the cercaria creeps on the body surface of the host, just showing occasionally the penetration behavior with the movement of the stylet

and body as seen in the normal. So the third reason of the above is especially worthy of note.

When the cercaria shows the penetration behavior against the soft part of the host's body, the secreta of the penetration gland appears in the opening, even if it cannot penetrate into the host. The secreta is recognized more clearly, when the cercaria is put in the serum. In other words the secretory function of penetration gland of the cercaria is normal in both cases. Therefore, the change of the character of secreta of the penetration gland is considered to be the reason why the cercaria affected by some factors cannot penetrate into the host's body.

Up to the present it has been known that the secreta has lytic action upon the host tissue, though pH of this secreta has not been made clear yet. If the reduction of the penetration power is due to the change of the character of the secreta, the change of pH may influence the histolytic character of the secreta. From other studies it has been already learned that the enzyme-like substance of the larva of the warble fly has a lytic action upon the cattle tissue in a weak acid but loses its power in high basic or high acid. The most high lytic action is in weak basic. The pH of the 0.05% CH_3COOH is 4.2, and that of 5% $NaHCO_3$ is more than 9.6. The penetration power of the secreta of the cercaria may be lost in high basic or high acid. That is to say, the effective pH range of the secreta of the penetration gland is very narrow as in the warble-fly larva. Also the decrease of the penetration power of the cercaria subjected to 50°C temperature for 5 minutes may be ascribed to the destruction of the histolytic action of the secreta. This fact that the penetration power is lost because of some factors without destroying the encystation power has been first made clear by using the artificial encystation method in vitro. The secreta of the penetration gland differs in character from that of the cystogenous gland. Various investigators have suggested that the penetration gland is related not only to hypolysis, but also to the encystation. However, it has become clear from the present study that this gland accounts for only hypolysis.

It is highly probable that the cercaria may encyst for protection. When the cercaria is put in the cattle or horse serum separated soon from fresh blood, it forms only the outer layer of the cyst, and dies before the formation of the inner thin cyst called

"true cyst". However, it completes normal encystation by diluting the serum; the cercaria without "true cyst" dies within a short time. The stains pass through the outer thick layer of the cyst very rapidly, but they cannot rapidly pass through the inner thin layer, the so-called "true cyst". So the writer thinks that the cercaria having penetrated into the host's body cannot protect itself only by the outer layer without the inner layer of the cyst. The meaning of the "true cyst" which has been used by other investigators is confirmed in the present study.

The cyst of the cercaria formed in the artificial media survives no longer than three days, even if the serum containing cyst was renewed twice a day. Naturally no development of the encysted larvae is observed. So it is clear that the serum only stimulates the cercaria to form cyst, having no effect on the development.

None of the euglobulin, pseudoglobulin and albumin of the serum or the mixture of them stimulates cyst formation. The salts contained in the serum are also proved ineffective. So it is probable that the serum is favorable to the encystation so far as it remains as a complete form. In a future study the essential function of the serum upon the encystation of the cercaria will be made clear.

VIII. Summary and conculusions.

In the vicinity of Sapporo the large-sized Limnaea snail, *Limnaea japonica*, is very common. The snail is found severely infected with the larvae of *Plagiorchis muris*. Though the general outline of the life-history of this parasite has already been made clear by TANABE (1922), HIRASAWA and ASADA (1929) and MCMULLEN (1937), the fundamental study on the behavior of the cercaria has remained almost untouched. In the present research, first the morphological study of the cercaria has been made. Then the seasonal fluctuation of infestation of the cercaria into the snail and the behavior of the larvae in different media have been observed. A series of experiments on the encystation of the cercariae have been carried out employing a second intermediate host and also using the blood serum of different animals including mammalia, aves, amphibia and pices. The body fluid of the dragon-fly larvae has been used. From these experiments the author has obtained some new facts which are to be noted. The results are summarized as follows:

(1) The morphological observation of the cercaria was made mainly by the intravitam staining using neutral red and nile blue which are particularly advisable for the study of living cercaria. There were found penetration glands opening each separately near the ventral side of the stylet of the head.

(2) The cercariae emerge from Limmaea during the night particularly more in number at temperatures from 20 to 27°C. The emergence of the cercariae does not occur constantly in a large number, but they release discontinuously with short intervals of rest. After escaping from the snail host they swim near the surface of the water for a while and then gradually sink to the bottom. This occurs within 5 hours. However the length of life of the cercaria which does not encyst or misses the host is less than 48 hours. In the direct sun light it dies within 7 hours.

(3) The speed of the movement of cercariae was measured. They use from 23.8 to 53 seconds for an upward movement of 1 cm. in the water (temperature 22°C), while they sink without body movement in 22 to 56.5 seconds. They creep a distance of 1 mm. for 10.2 to 12 seconds.

(4) The effect of several media and of the different grades of temperature on the longevity and infectivity of the cercariae was observed. The swimming movement of the cercariae becomes more active in basic solutions than in acid. When treated with 0.05% acetic acid for 10 minutes or 5% water solution of sodium bicarbonate, the infectivity of cercariae disappears, while the ability of encystation still remains. Subjection to a temperature of 50°C for 5 minutes also affects the animal as the above.

(5) The percentage of natural infection of Limnaea by this parasite is 49.65% on an average, and it increases gradually from May to August showing 92.72% in August.

(6) Besides known second intermediate hosts, viz. *Anax parthenope, Ephemera strigata, Asellus aquaticus*, etc., *Chironomus dorsalis* and *Anisogammarus annandalei* were newly found as hosts. The most frequent parasitic position of cercariae in these hosts is on the hind part of the body including the 11th and 12th segments. The cercariae penetrate the soft parts between the body segments of the host. In gammarus they penetrate also between the leg segments. They develop in the host into the encysted cercariae, being endowed with infectivity as long as five or seven days. In *Anisogam-*

marus the condition of parasitic cyst differs from that of the other host appearing as black points which were covered with a soot-like layer. The mature metacercariae given to the mouse experimentally becomes adult which discharge eggs in the mouse faeces on the fifth day after infection.

(7) The cercariae were seen cysting in vitro in media including the normal blood serum of man, cattle, horse, pig, dog, cat, rabbit, guinea pig, roof rat, Norway rat, white mouse, domestic fowl, salamander, frog or fish. The normal body fluid of the dragon-fly larvae is also favorable. In the normal serum and body fluid the cercariae, on the whole, slough off their tails after 5 or 6 minutes, and begin the rotative movement after 7 or 8 minutes. The time varies with the temperature or with the kind of serum used.

(8) The influence of various sera upon encystation was tested in heated or diluted condition. The horse serum was proved most favorable in spite of the above condition.

(9) In the blood serum of the rabbit in which 1 cc. of 20% alcohol had been injected daily for six weeks, the encysting of the cercariae occurs normally.

(10) The encystation of the cercariae which were stained vitally with 0.02% neutral red solution for 50 seconds or 0.02% nile blue solution for 10 seconds takes place poorly.

(11) The resistance of the cyst wall and accordingly the larvae within the cysts against pressure and chemicals are fairly strong. Even if the cysts were flattened so as to have a diameter twice as much as before, the wall remained unbroken, and the larvae kept alive in the cysts.

(12) The cysts of cercariae formed in the artificial media survive no longer than three days, even if the serum containing cysts was renewed twice a day. However no development of the encysted larvae was observed. So it is clear that the serum only stimulates the cercaria to form cysts, but does not affect the development.

(13) The euglobulin, pseudoglobulin or albumin of the serum does not stimulate the formation of cysts. The salts contained in the serum are also proved inactive.

The above mentioned facts have not been acertained previously by other investigators. In the future investigation, the behavior of other kinds of cercariae in the serum as used in this experiment

together with the essential function of the serum as connected with encystation will be made clear. These is need to devise a method to keep alive the larvae encysted artificially in vitro.

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References

(1) ABE, T. (1941): Studies on the metacercaria in *Rhodeus lanceolata* I. Okayama Igakkai Zasshi, 53, 5, 956.

(2) ——— (1941): Ibid. II. Ibid., 53, 6, 1206.

(3) ANDO, R. (1917): On the distribution of Paragonimus westermanii in the second intermediate host and destiny. Iji Shinbun, 1038.

(4) ——— et U. IWAIASHI (1924): The escaping experiments of the cercaria of Metagonimus yokogawai and some trematodes from Melania. Trans. Jap. Path. Soc., 1, 2.

(5) ARCHIBALD, R. G, et A. MARSHALL (1937): A technique for the study of cercaria. Trans. Roy. Soc. Trop. Med. & Hyg., 24, 6, 629.

(6) ASADA, J. (1939); Studies on a new trematode, *Echinostoma hortense* n. sp. and its life-history. Vol. Jub. pro Prof. S. YOSHIDA, 1, 39.

(7) CORT, W. W. (1922): A study of the escape of cercaia from their snail hosts. Journ. Parasitol., 8, 177.

(8) DUBOIS, G. (1929): Les cercaires de la region de Neuchatel. Bull. Soc. Neuchateloise Sci. Nat., 53, 2, 3. (Biol. Abst., 5, 10, 2540).

(9) FAUST, E. C. et O. R. KHAW (1925): Experiment phenomena in *Clonorchis sinensis*. Pro. Soc. Exp. Biol. & Med., 23, 245.

(10) FUKUNISHI, S. (1942): Studies on the tropisms and development of the cercaria of *Schistosoma japonicum*. Osaka Kotoigakusenmon Gakko Zasshi, 9, 1, 3.

(11) GIOVANNOLA, A. (1936): Inversion in the periodicity of emission of cercariae from their snail hosts by reversal of light and darkness. Journ. Parasitol., 22, 3, 292.

(12) HIRASAWA, I. et J. ASADA (1929): Studies on the life history of *Lepoderma muris*. Tokyo Iji Shinshi, 53, 2614, 507.

(13) Ito, A. (1926): Studies on *Schistosoma japonicum*. I. On the resistance of the cercaria of *S. japonicum* in the liver extract, body fluid and secreta of the host. Jap. Journ. Microbiol., 20.

(14) ——— (1926): Ibid. II. On the speed of penetration of the cercaria and the partial change of the host skin, especially the relation of the host age. Jap. Journ. Microbiol., 22.

(15) Ishii, Y. (1934): Studies on the life history of *Fasciolopsis buski* III. Taiwan Igakkai Zasshi, 33, 3, 391.

(16) Isobe, Y. (1922): Biological observations on the cercaria of *Schistosoma japonicum*. Ibid., 21, 2, 225.

(17) Ito, K. (1926): Biological studies on the cercaria of *Clonorchis sinensis*. Aichi Igakkai Zasshi, 33, 2, 197.

(18) Khalil, B. B. (1939): On the bionomics of the free living phase of *Cercaria heterophyes*. Vol. Jub. pro Prof. S. Yoshida, II, 161.

(19) Kobayashi, H. (1916): On the habits of the intermediate host and cercaria of *Schistosoma japonicum*. Jap. Journ Bact., 247.

(20) ——— (1918): Studies on the cercaria. Chosden Igakkai Zasshi, 21.

(21) Komiya, Y. (1938): Die Entwicklung des Exkretionssystem einiger Trematodenlarven aus Alster und Elbe, nebst Bemerkungen über ihren Entwicklungsziklus. Zeitschr. Parasitkd., 10, 3, 340.

(22) Kosaka, K. (1938): Biological studies on the cercaria of *Schistosoma japonicum*. I. On the swimming of the cercaria Taiwan Igakkai Zasshi, 37, 12, 1952.

(23) Kuntz, R. E. (1947): Effect of light and temperature on emergence of *Schistosoma mansoni* cercariae. Trans. Amer. Mic. Soc. 66, 1, 37.

(24) Kuyama, S. (1938): On the seasonal fluctuations of the trematode larvae in the first and second intermediate host. Okayama Igakkai Zasshi, 50, 2, 327.

(25) McCoy, O. R. (1928): Seasonal fluctuation in the infestation of *Planorbis trivolvis* with larval trematodes. Journ. Parasitol., 15, 2, 121.

(26) McMullen, D. B. (1937): An experimental infection of *Plagiorchis muris* in man. Journ. Parasitol., 23, 1, 113.

(27) Miller, H. M. J. et O. R. McCoy (1930): An experimental study of the behavior of *Cercaria floridensis* in relation to its fish intermediate host. Journ. Parasitol., 16, 4, 185.

(28) Miller H. M. J. (1927): Behavior studies on larval trematodes. Journ. Parasitol., 13, 3, 224.

(29) Miyata, I. (1940): On *Plagiorchis muris* and *Opisthoglyphe canis* n. sp. found in the dog. Zool. Mag., 52, 10, 367.

(30) Ogata, T. (1947): The experimental methods of serology. Tokyo.

(31) Olivier, L. (1951): The influence of light on the emergence of *Schistosomatium douthitti cercariae* from their snail host. Journ. Parasitol., 37, 2, 201.

(32) Ono, S. (1934): On the life history of Plagiorchis and Prosthogonimus of dragonflies found in the vicinity of Mukden. Journ. Jap. Soc. Vet. Sci., 13, 4, 267.

(33) ——— (1935): Studies on the trematodes, invading Limnaea snails as the first intermediate hosts, found in the vicinity of Mukden. Ibid., 14, 1, 24.

(34) Rees, W. J. (1935): The anatomy of *Cercaria buccini* Lebour, 1911. Proc. Zool. Soc. London, 2.

(35) ——— (1948): A study of the effect of light, temperature and slinity on the

emergence of *Cercaria purpurea* LEBOUR from *Nucella lepillus* (L.) Journ. Parasitol., 38, 228.

(36) SANDGROUND, J. H. (1940): *Plagiorchis javensis* n. sp., a new trematode parasitic in man. Rev. Med. Trop., Parasitol., Bakt., Clin. Labor., 6, 4, 207.

(37) SCHULZ, R. E. et A. A. SKWORGOW (1931): *Plagiorchis arvicola* n. sp. aus der Wasserratte. Zeitschr. Wiss. Zool., Abt. F. Zeitschr. Parasitkd. 3, 765.

(38) SHIRAI, K. (1928): Studies on the infection of *Fasciola hepanica*. Jap. Journ. Exp. Med., 10, 2.

(39) STUNKARD, H. W. (1930): An anaysis of the methods used in the study of larval trematodes. Journ. Parasitol., 22, 2, 268.

(40) SUDA, K. et M. OZAWA (1927): On the oral infection of *Schistosoma japonicum* and the resistance of the cercaria of this parasite against the sliva, bile and some chemicals. Jap. Journ. Exp. Med., 11, 3.

(41) TANABE, H. (1922): Studies on the life-history of Digenea. On a new trematode, *Lepoderma muris* n. sp. Okayama Igakkai Zasshi, 385, 1.

(42) WUNDER, W. (1932): Untersuchungen über Pigmentierung und Encystierung von Cercarien. Zeitschr. Wiss. Biol., Abt. A, Zeitschr. Morph. u. Oecol. Tiere, 25, 2–3, 336.

(43) YAMAGUTI, S. (1943): Cercaria of *Plagiorchis muris* (TANABE, 1922). Annot. Zool. Jap., 22, 1, 1.

(44) YAMASHITA, J. (1935): On *Plagiorchis maculosus* (RUDOLPHI), a trematoda from Eastern-sandmartin. Botany and Zool., 3, 10, 1861.

(45) ———— (1950): Experimental studies on the behavior and artificial encystation in vitro of the cercaria of *Plagiorchis muris* (TANABE). (Summary of the lecture in the 20th meeting of Zool Soc. Jap., in 1949). Zool. Mag., 59, 2–3, 4.

(46) ———— (1951): Experimental studies on the penetration glands and cystogenous glands of the cercaria of *Plagiorchis muris* (TANABE). (Summary of the lecture in the 20th meeting of Parasitol. Soc. Jap. in 1951, and this summary was published in Trans. Parasitol. Soc. Jap., no. 20).

(47) YOSHINO, K. (1940): On the metacercaria of *Carassius auratus* (LINN.) and the seasonal fluctuation of its infection rate. Okayama Igakkai Zasshi, 52, 2, 5.

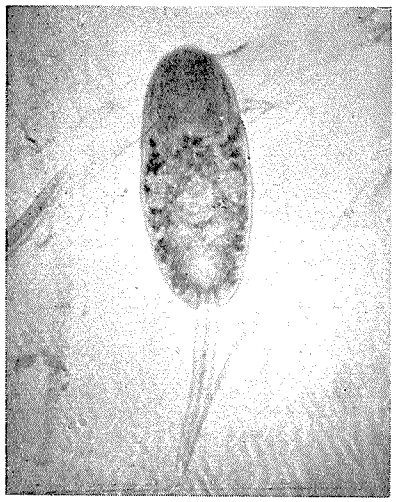
Explanation of Plates

Plate IX.

Fig. 1. Total body of cercaria of *Plagiorchis muris*. Dorsal view. × 130.
Fig. 2. Body of cercaria which detached tail. Ibid. × 270.
Fig. 3. Penetration glands and cystogenous cells. Ventral view. × 240.
Fig. 4. Ibid. Lateral view. × 240.
Fig. 5. Penetration cells and ducts. × 600.
Fig. 6. Stylet. Dorsal view. × 750.
Fig. 7. As above. Lateral view. × 750.
Fig. 8. Opening of penetration gland. × 750.
Fig. 9. Relation between excretory bladder in body and central excretory tube at root of tail. × 380.

Plate X.

Fig. 1. Cyst formed by artificial encystation method using horse serum. After 9 minutes. × 60.
Fig. 2. Cercaria died during artificial encystation process. × 160.
Fig. 3. Encysted cercaria formed artificially in horse serum and detached tail. Pendulous movement of stylet on posterior end. After 12 minutes. × 380.
Fig. 4. Encysted cercaria formed artificially in horse serum. Detached tail contracts much. After 20 minutes. × 150.



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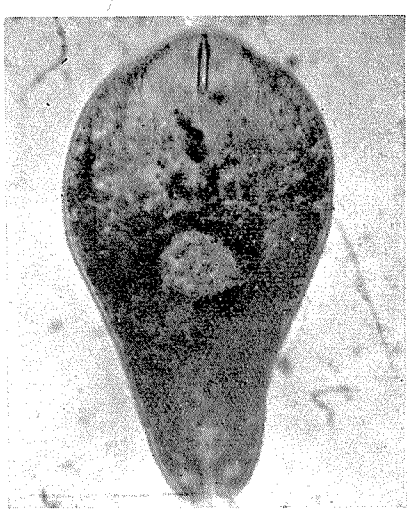

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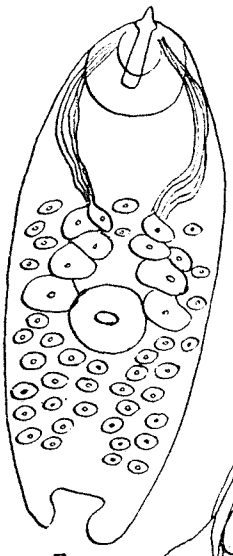

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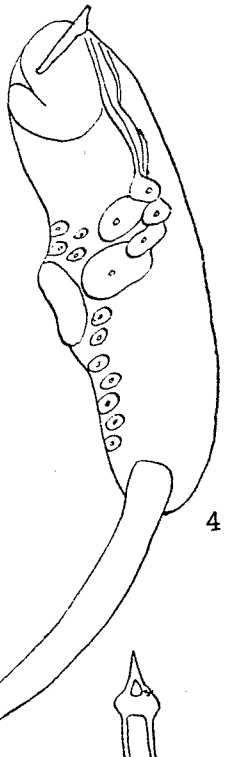

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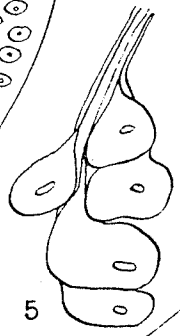

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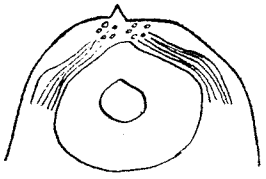

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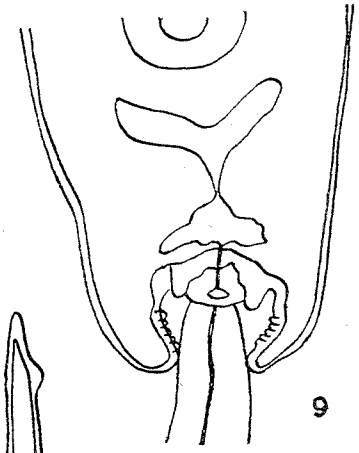

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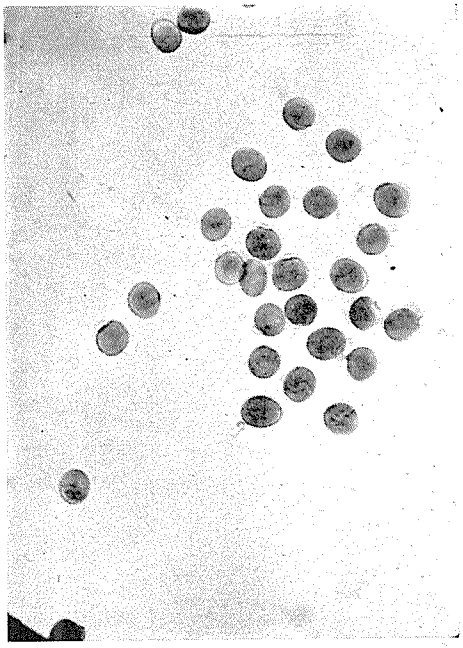

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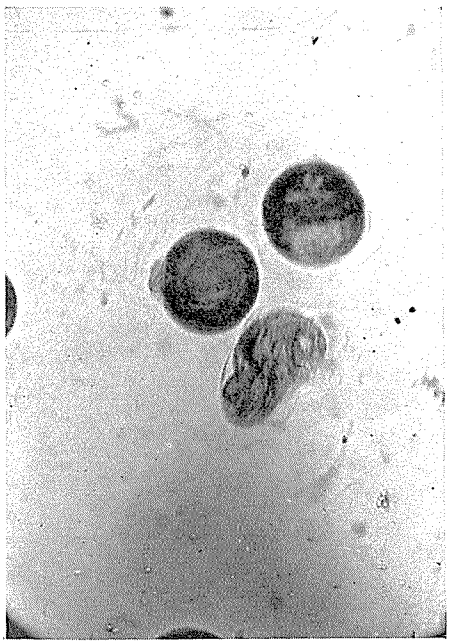

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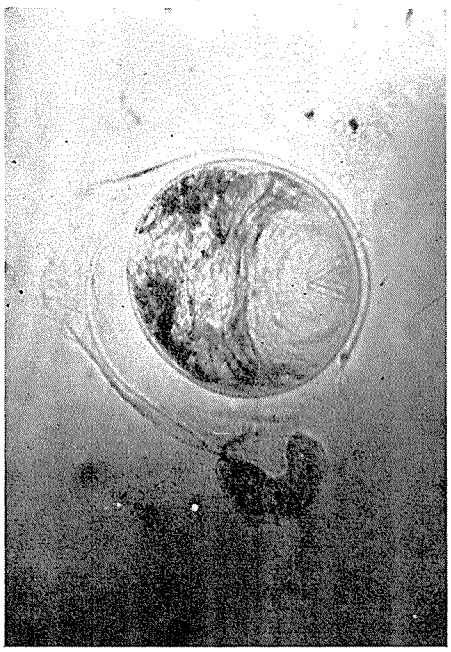

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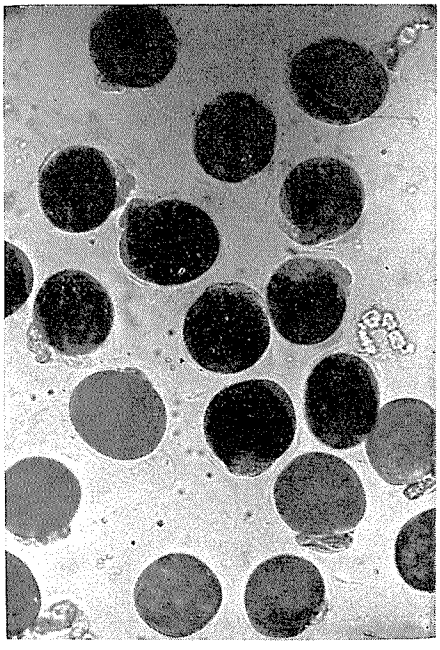

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