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Study on *Centrocestus armatus* in Korea†

II. Recovery rate, growth and development of worms in albino rats

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Abstract: Some biological characteristics of *Centrocestus armatus* were studied using albino rats as its experimental host. The metacercariae were collected from *Zacco platypus* by artificial digestion method. Laboratory rats(Wistar) were fed each 100 or 200 metacercariae and sacrificed on 1, 2, 3, 4, 5, 6, 8, 14 and 28 days after infection to recover worms of various ages. The average recovery rate was 10.7% from 82 rats. The rate decreased rather slowly for the first 8 days but showed a steep decrease thereafter. Of the worms, 35.5% were recovered from the duodenum and 62.5% from the jejunum. At metacercarial stage, body length was 293 μm and body width 144 μm. At adult stage, the length and width reached 382 μm and 214 μm respectively at 14 days after infection. The testes and Mehlis' gland were recognized at metacercarial stage, whereas the ovarian anlage appeared on the 1st day of infection, seminal vesicle and vitellaria on the 2nd day, and seminal receptacle and uterine eggs on the 3rd day. Until 8 days after infection the genital organs developed continuously and the number of uterine eggs increased. The above results show that albino rats are one of useful experimental hosts for *C. armatus* and the worms can develop to adults in 3 days after infection.

Key words: *Centrocestus armatus*, intestinal fluke, growth and development, albino rat, recovery rate

INTRODUCTION

The fluke genus *Centrocestus* is a group of minute intestinal trematodes belonging to the family Heterophyidae, which infect various kinds of avian and mammalian hosts including man (Yamaguti, 1958). *Centrocestus* has circum-oral spines arranged in two rows around its oral sucker characteristically. *Centrocestus armatus* was described firstly by Tanabe (1922) from the dogs, cats and mice fed with freshwater fishes in Japan. Now about 20 species of fishes such as *Zacco platypus*, *Z. temminckii*, *Pseudorasbora parva*, *Carassius carassius* are known as the second intermediate host of *C. armatus* (Tanabe, 1922; Komiya, 1965; Lee *et al.*, 1984). *Semisulcospira* spp. snails are the first intermediate host (Takahashi, 1929).

A natural human infection of *C. armatus* was reported by Hong *et al.* (1988) in Korea. Experimental infection of *C. armatus* in man

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was also reported successful (Tanabe, 1922). As for other species of *Centrocestus*, experimental human infection was proved in *C. formosanus* (Nishigori, 1924) and in *C. asadai* (Mishima, 1959) and natural human infection in *C. formosanus* var. *kurokawai*(Kurokawa, 1929).

The knowledges on chronological development of parasites are essential in understanding parasitism and host-parasite interactions. However, there is only one report concerning the growth and development of *C. armatus* in the final host (Tanabe, 1922), which described briefly on early worm development in several laboratory animals. This study was conducted to observe the habitat, worm recovery, and growth and development of *C. armatus* in albino rats.

MATERIALS AND METHODS

Z. platypus were caught from a stream at Sangbiryang-myun, Sanchung-gun from October, 1987 to August, 1988. Metacercariae of *C. armatus* were collected from the fish by artificial digestion method and grouped into 100 or 200 for experimental infection to rats. For the observation of morphology, metacercariae were excysted under cover glass pressure and fixed in 10% neutral formalin.

A total of 82 albino rats (Wistar strain, 4~6 week old) fed with metacercariae of *C. armatus* were sacrificed on day 1, 2, 3, 4, 6, 8, 14 and 28 post-infection (Table 1). Their small intestine was resected and divided into the duodenum, jejunum and ileum, and opened in 0.85% saline. Under dissecting microscope, the worms were recovered from intestinal contents and fixed in 10% neutral formalin under cover glass pressure.

The worms and excysted metacercariae were stained with Semichon's acetocarmine and mounted in resinous balsam. The growth and development of worms was described based on microscopical findings and measurements.

RESULTS

1. Metacercariae of *C. armatus*

Cyst ellipsoidal, with pointed ends, 229 μm (198~278 μm) in length and 127 μm(110~141 μm) in diameter, and yellowish brown. Cyst wall thin, 3~4 μm in thickness. Oral sucker at anterior terminal. Total 44 circumoral spines arranged in two rows alternately around oral sucker. Ventral sucker, about half size of oral sucker, located in the middle portion of the body. Anlage of two testes ovoidal and parallel to each other. Excretory bladder 'X'-shape (Figs. 1 & 2).

2. Worm Recovery Rate from Rats

A total of 1,697 worms(10.7%) were recovered from 82 rats infected with 15,800 meta-

Table 1. The recovery rate of *C. armatus* from experimentally infected rats

Day after infection	No. rats	No. Mc* given	No. of worms from			Subtotal (%)
			duodenum	jejunum	ileum	
1	10	1,700	120	167	8	295(17.4)
2	11	1,900	148	152	6	306(16.1)
3	8	1,600	63	114	5	182(11.4)
4	12	2,400	122	201	4	327(13.6)
6	9	1,800	63	194	7	264(14.7)
8	8	1,600	53	188	3	244(15.3)
14	14	2,800	30	42	0	72 (2.6)
28	10	2,000	4	3	0	7 (0.4)
Total	82	15,800	603	1,061	33	1,697(10.7)

* Mc : metacercaria

Table 2. Measurements of *C. armatus* recovered from albino rats from 1 to 14 days after infection (unit : μm)

Organ	Age of worms after infection(days)							
	EMC*	1	2	3	4	6	8	14
No. of worms measured	11	17	15	14	15	12	20	13
Body Length	293±34**	291±27	279±20	359±49	288±41	326±46	373±44	382±28
Width	144±14	147±18	154±8	170±10	170±14	197±15	208±15	214±23
Oral Sucker								
Length	53±5	56±4	52±2	58±6	46±5	52±5	57±14	57±4
Width	60±6	57±3	55±4	62±5	52±3	58±3	67±4	65±4
Prepharynx								
Length	12±5	12±4	6±2	8±6	5±4	4±2	4±2	5±3
Pharynx								
Length	29±2	32±3	31±3	35±3	31±3	36±3	39±2	41±2
Width	27±3	27±3	29±2	32±5	29±2	34±3	36±3	36±3
Esophagus								
Length	17±6	10±3	11±4	13±5	7±3	7±5	9±5	10±4
Ventral sucker								
Length	31±3	33±3	34±2	37±5	33±3	37±4	41±4	42±3
Width	38±2	41±3	42±2	46±3	41±3	46±3	50±3	51±3
Seminal vesicle								
Length	—	—	24±7	56±8	64±14	71±22	76±14	85±17
Width	—	—	55±8	65±7	68±10	78±8	82±8	89±16
Ovary								
Length	—	22±5	30±5	55±8	52±8	58±10	69±9	66±10
Width	—	39±7	55±8	65±7	68±10	78±8	82±8	89±16
Mehlis' gland								
Length	16±2	21±4	23±3	36±6	33±3	41±8	42±7	45±11
Width	37±6	37±5	49±6	52±14	55±10	56±9	63±7	73±9
Seminal receptacle								
Length	—	—	—	31±7	28±7	44±11	53±12	42±12
Width	—	—	—	37±9	35±9	59±20	63±14	52±16
Testis(Right)								
Length	33±5	49±7	55±5	53±10	45±8	54±8	63±7	60±7
Width	49±6	61±8	72±6	72±7	70±6	81±5	91±10	89±10
Testis(Left)								
Length	32±5	43±6	50±4	48±7	42±5	46±5	56±7	52±10
Width	43±6	54±7	60±5	62±4	59±5	67±5	74±7	77±12
No. of circumoral spines	44 (42～44)	44 (42～48)	44	44 (44～46)	44	44 (42～44)	44 (42～44)	44 (42～44)
No. of intrauterine eggs	—	—	—	8.7±2.6	18±10	24.8±14.3	4.3±7.0	25.5±14.8

* EMC : excysted metacercaria ** Mean and standard deviation

cercariae. The recovery rate was 17.4% on average on the day 1 post-infection and the rate ranged 11.4～16.1% until the day 8. Thereafter, however, the rate decreased remarkably until the day 28 (Table 1). Among the worms 35.5% was recovered from the duodenum, 62.5% from the jejunum and 2.0% from the ileum.

3. Growth and Development of Worms in Rats

The excysted, living metacercaria was ovoid, spoon-like and concave ventrally. The average length and width of the excysted metacercariae were 293 μm and 144 μm respectively. The growth of worms was relatively slow, to be 382 μm in length and 214 μm in width at 14 days after infection (Table 2). The growth pattern of non-genital organs such as the oral and ventral suckers and the pharynx was similar to that of the whole body.

Mehlis' gland and two testes were observed at their metacercarial stage(Fig. 3). Ovary was ellipsoidal and appeared in front of the right testis on day 1 after infection (Fig. 4) and became darker in stainability than Mehlis' gland on day 2 (Fig. 5). Ovary developed very rapidly up to 3 days and became ovoidal with 4~5 lobes in shape (Fig. 6), and enlarged slowly to 8 days. On day 2 after infection, seminal vesicle full of sperms, 'S'-shape, and divided into anterior and posterior sacs, appeared transversely anterior to ovary and Mehlis' gland in 30% of worms observed(Fig. 5). Increase in size of seminal vesicle was remarkable until 4 days and continued steadily to 14 days.

Follicular vitellaria were observed in subtegument of lateral field from the level of pharynx to posterior extremity in 2-day-old worms and increased in number and size with the age of worm. On the third day, seminal receptacle with sperms, round to ovoid, appeared at median part between ovary, testes and Mehlis' gland (Figs. 6 & 7), and enlarged remarkably up to the 8th day but reduced on the 14th day. On the 3rd day intrauterine eggs were formed in all worms observed (Fig. 6) and 8.7 in average number. In 6-day old worms, eggs were increased in number. Eggs in terminal part of uterus were ovoidal and considered to be matured. (Figs. 8 & 10). Circumoral spines revealed more distinctive contour and arrangement at 6 days (Fig. 9).

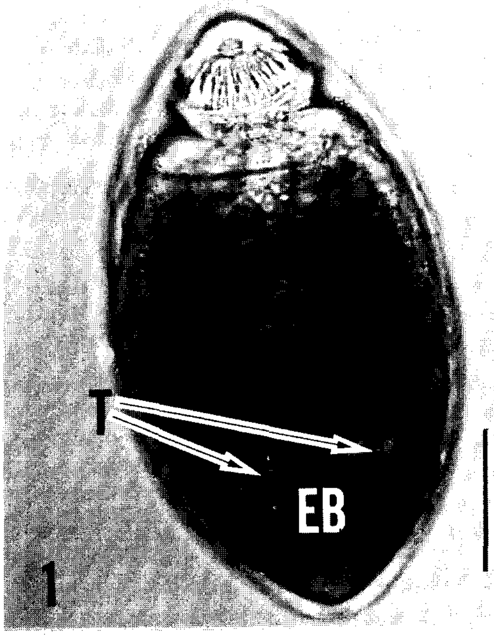
Dimension of Mehlis' gland was increased steadily through the course of experiment. Testes grew remarkably up to 2 days and the lobulation was progressed to be 4(4~5) in right testis and 3(2~4) in left testis at 8 and 14 days after infection (Figs. 11 & 12). Growth of genital organs continued for 14 days and their dimension became over two times that of excysted metacercariae.

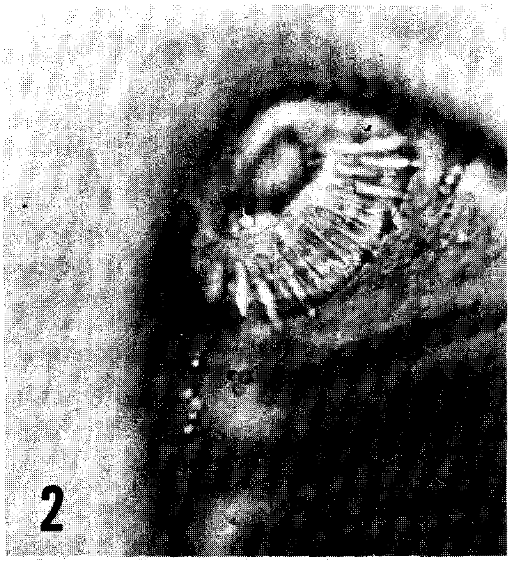
Morphology of the adult worm(8-day old): Body ovoidal with broad posterior half, 373 μm long and 208 μm wide, covered with tegumental spines from anterior to posterior ends (Fig. 13). Oral sucker terminal, and 44(42~44) circumoral spines arranged in two alternate rows (Fig. 14). Prepharynx very short. Some glandular cells were observed in posterior one-fifth of pharynx (Fig. 11). Esophagus was short and bifurcated in front of ventral sucker and ceca reached to ovary and Mehlis' gland on each side. Ventral sucker smaller than oral sucker and located at median, two-fifth of body. Seminal vesicle, 'S'-shape with anterior and posterior sacs, adjacent to posterior border of ventral sucker. Ovary lobulated into 4~6 lobes, right to seminal vesicle. Mehlis' gland ellipsoidal, left to seminal vesicle. Seminal receptacle, round to ovoid, in median between ovary and testes. Testes, ellipsoidal, parallel at posterior extremity of body. Right testis with 4~5 lobes slightly larger than left testis with 2~4 lobes. Vitellaria, follicular, from the level of pharynx to posterior end. Excretory bladder 'X'-shape.

Eggs collected from feces of experimental rats were ovoidal, symmetrical or more concave in one side, 31.2±1.7 μm long and 16.4±0.5 μm wide, and more pointed at opercular end. Operculum shallow, 6.3±0.7 μm in width and

(→)

Fig. 1. A metacercaria showing 'X'-shape excretory bladder(EB) and testes(T). Bar=50 μm.
Fig. 2. Circumoral spines around oral sucker of an excysted metacercaria. Bar=25 μm.
Fig. 3. Excysted metacercaria, acetocarmine stained, showing Mehlis' gland(M). Bar=100 μm.
Fig. 4. A 1-day old worm showing ovarian anlage(OA). Bar=100 μm.
Fig. 5. A 2-day old worm showing seminal vesicle(SV) with sperms. Bar=200 μm.
Fig. 6. Seminal receptacle(SR) and intrauterine eggs in a 3-day-old worm. Bar=100 μm.


T
EB
1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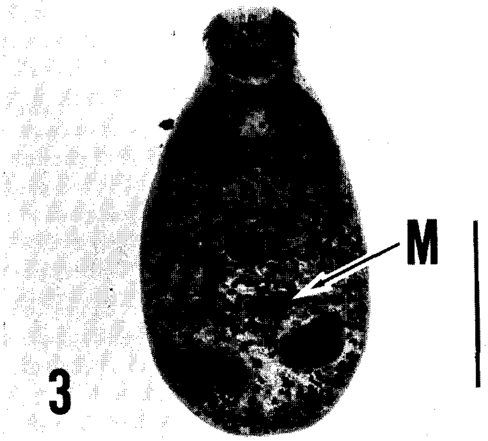
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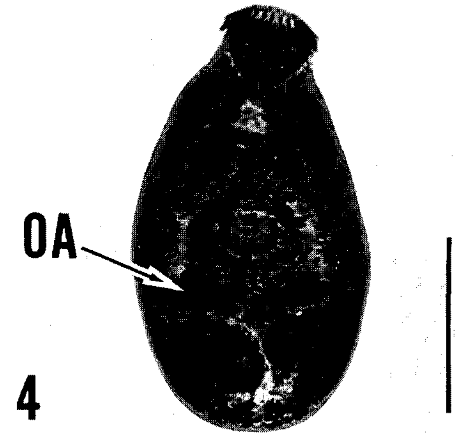
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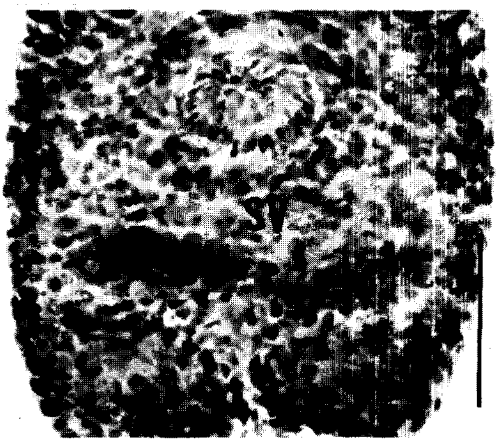
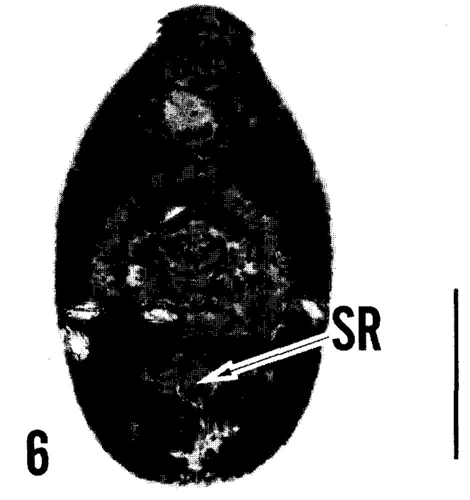
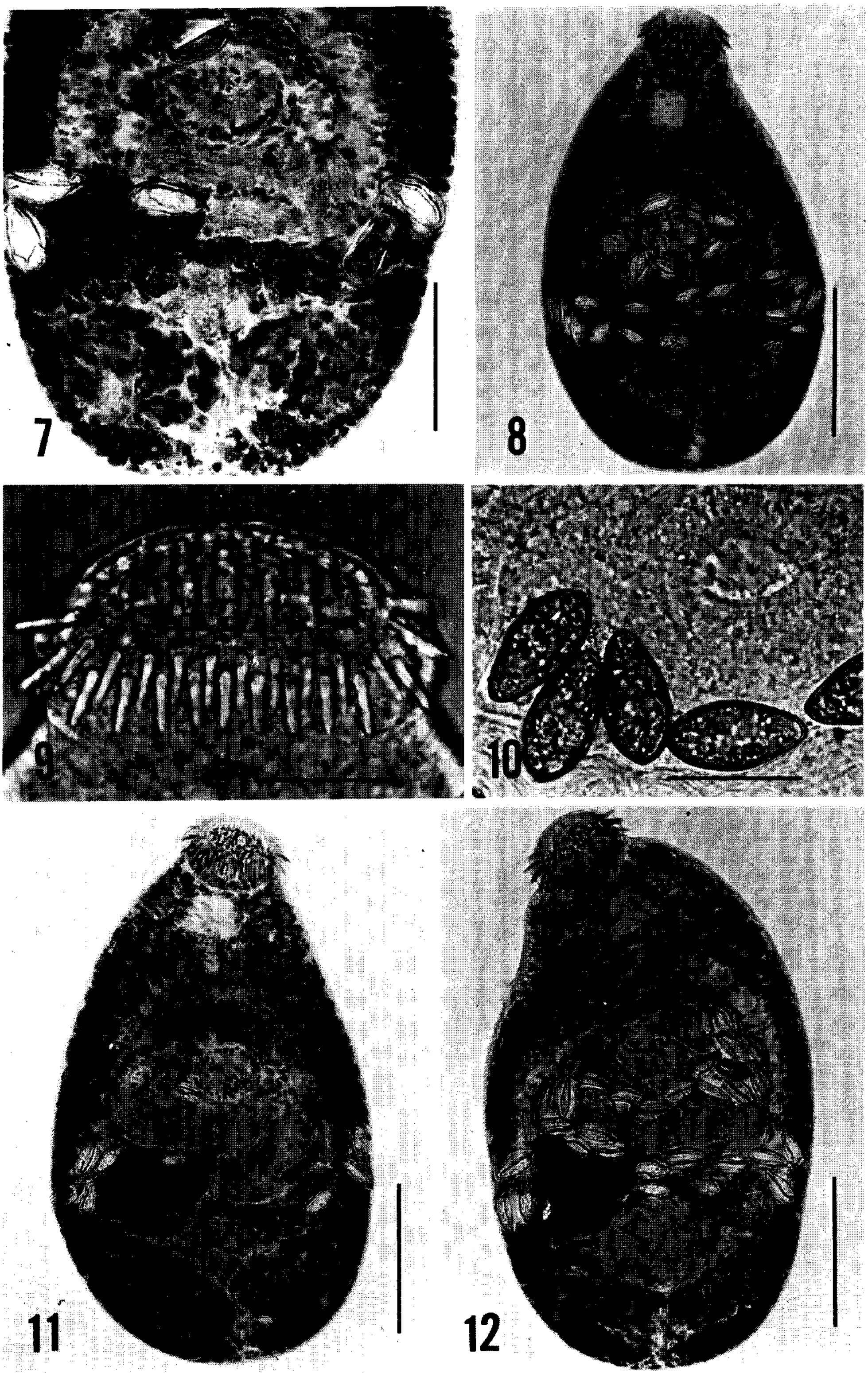



SR
6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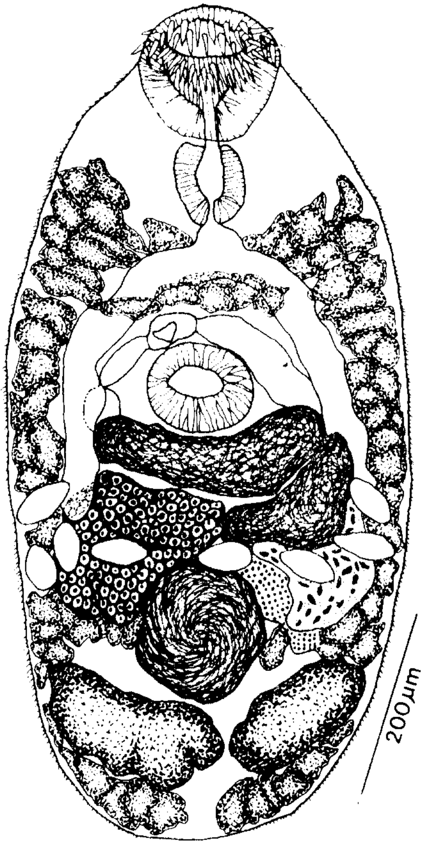

Fig. 13. Drawing of an adult *C. armatus*(8-day old).

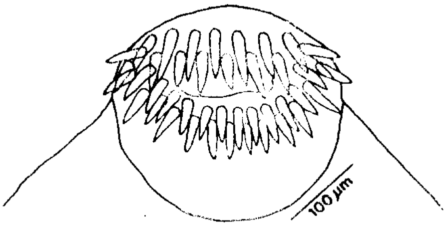

Fig. 14. Circumoral spines around oral sucker of *C. armatus*.

3.2±0.4 μm in depth. Shell surface smooth. Abopercular protuberance observed in half of eggs observed. Maximum width at equator or shifted to abopercular side. Eggs remained in single cell stage.

DISCUSSION

Tanabe(1922) reported that the cysts of *C. armatus* are thin-walled and ellipsoidal, 180~280 μm long by 100~168 μm wide, and ruptured at one end under slight pressure where the metacercaria was released. The excysted metacercaria showed 44 circumoral spines in two alternate rows, 'X'-shape excretory bladder, two tandem testes, and irregularly elliptical anlage anterior to the excretory bladder. In this study, the metacercariae excysted under cover glass pressure moved with sluggish motility. Their morphological findings were identical with the description by Tanabe (1922).

In the speciation of flukes belonging to the genus *Centrocestus*, morphological findings such as the number of circumoral spines, shape of the ovary, comparative size and shape of right and left testes, number of intrauterine eggs, and the presence or absence of latticed design on the egg shell were adopted as useful keys for classification (Tanabe, 1922; Nishigori, 1924; Chen, 1942; Yamaguti, 1958). Among these characters, the number of circumoral spines was accepted as the most reliable one. Kobayasi(1968) classified the flukes into 4 groups by the number of spines; those with 26~30 circumoral spines into ***C. yokogawai***, 32~40 into ***C. formosanus***, 42~48 into ***C. armatus*** and 50~60 into ***C. polyspinosus***. According to his suggestion, ***C. nycticoracis*** which has 42 circumoral spines be regarded as a synonym of ***C. armatus***. The adult flukes recovered from the rats after infection with the metacercariae from ***Z. platypus*** had 44(42~46) circumoral spines in this study and their mor-

(←)

Fig. 7. Higher magnification of Fig. 6 showing sperms in seminal vesicle and receptacle. Bar=200 μm.
Fig. 8. A 6-day old worm. Bar=100μm.
Fig. 9. Circumoral spines around oral sucker of a 6-day old worm, unstained. Bar=30 μm.
Fig. 10. Intrauterine eggs in a 6-day old worm. Bar=30 μm.
Fig. 11. A 8-day old worm. Bar=100 μm.
Fig. 12. A 14-day old worm. Bar=100 μm.

phology was compatible with the description of *C. armatus* made by Tanabe (1922). Through this investigation it was confirmed that *C. armatus* is distributed in Korea, with *Z. platypus* taking as a second intermediate host.

With regard to the genital organs in the metacercaria of *C. armatus*, Tanabe (1922) recognized a primitive genital anlage with irregular margin adjacent to the anterior border of the excretory bladder, which is suggested by our results to be Mehlis' gland. On the other hand, Takahashi (1929) and Komiya (1965) observed that the ovary appears as a cell mass in front of the right testis.

The present observation on the organogenesis of genital organs of *C. armatus* in rats was different in part from that observed in the dog, cat or mouse by Tanabe (1922). He reported that only testes were recognized in worms on the 1st day after the experimental infection, whereas anlage of female genital organs were revealed on the 2nd day, the seminal receptacle containing sperms, vitellaria, ovary and intrauterine eggs (12 in number) on the 3rd day, and on the 4th day the intrauterine eggs increased up to 20 in number. He considered 7-day old worms as adults. According to his report, until the third day all genital organs developed very rapidly, and intrauterine eggs were formed. As to *C. formosanus*, in several laboratory animals, the ovarian anlage appeared in front of the right testis in 12 hours after infection; ovary, seminal vesicle and seminal receptacle became distinctive in 24 hours; intrauterine eggs were observed in 48 hours (Nishigori, 1924).

The developmental patterns of genital organs of *C. armatus* in early stages differ from that of non-genital organs in this study. Based on the measurement of the specimens, the development of genital organs was remarkable up to 3 days when intrauterine eggs were formed and the development continued to 8 days after infection and was reduced thereafter. However, the seminal vesicle and Mehlis' gland developed continuously during the course of whole experiment. The developmental pattern of *C. armatus* observed in the present study was much similar to those of *Echinostoma hortense* in the reports of Saito (1984) and Seo *et al.* (1985). The growth curves were expressed by straight line up to 10～14 days and progressed slowly in the entire experimental period. In this respect, it is considered in the present study that in *C. armatus*, the appearance and early development of genital organs are well synchronized with the production of uterine eggs at 3 days after infection in albino rats. The worms recovered after 4 days of infection are regarded mature ones.

The recovery rate of *C. armatus* from the rats was in the range of 11.4～17.4% until 8 days but decreased rapidly to 0.4% at 28 days. The rate of *C. formosanus* in rats was 20.0, 20.6 or 3.1% at 2, 3 or 31 days after infection respectively (Chen, 1942). There were also reports on the decreasing tendency of recovery rate in other intestinal trematodes such as *Metagonimus yokogawai* (Chai, 1979) and *Pygidiopsis genata* (Mansour *et al.*, 1981). It is accepted in general that if the susceptibility of a host animal to an intestinal fluke is low, their recovery rate would be decreased more rapidly than other animals which have fairly higher susceptibility, and at later stage the host be cured naturally from the infection. In comparison, Mishima (1959) stated that the rate of *C. asadai* from albino rats was maintained within the range 21.0～36.7% and averaged 31.3% from the 3rd day to the 20th day after infection. In this connection, it is considered that the albino rat is not a highly suitable host for *C. armatus*.

The habitat of *C. armatus* in final hosts was reported either upper (Takahashi, 1929) or lower (Tanabe, 1922) parts of the small intestine. In the present study, almost all worms were collected in the duodenum and jejunum, the upper part of the small intestine.

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=국문초록=

*Centrocestus armatus*에 관한 연구

Ⅱ. 흰쥐에서의 성장 발육 및 충체회수율

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인하대학교 의과대학 기생충학교실**
홍성종 · 우호춘 · 채종일* · 정세우* · 이순형* · 서병설**

*Centrocestus armatus*의 성장 발육 과정을 관찰하기 위하여 피라미(*Zacco platypus*)를 인공소화시켜 획득한 피낭유충을 100 혹은 200개씩 흰쥐(Wistar strain)에 경구 감염시키고 1, 2, 3, 4, 6, 8, 14 및 28일 후에 경추탈구법으로 희생시키고 소장을 적출해 내었다. 적출한 소장을 십이지장, 공장, 회장으로 분리하여 0.85% 생리식염수 내에서 열고 장내용물로부터 충체를 회수하였다. 회수된 충체를 10% formalin으로 cover glass 압력하에서 고정하고 Semichon's acetocarmine으로 염색한 다음 광학현미경으로 관찰하고 계측하였다.

그 결과는 다음과 같다.

1. 충체회수율은 평균 10.7%이었다. 감염 1일부터 8일까지는 회수율이 서서히 감소하였고 그 이후에는 28일까지 관찰한 바 급속히 감소하였다.
2. 회수된 충체의 35.5%는 십이지장에, 62.5%는 공장에 분포하였다.
3. 탈낭된 피낭유충은 길이 293 μm, 폭 144μm이었으나, 감염 후 14일에는 길이와 폭이 각각 382 μm 및 214 μm로 자라 있었다.
4. 피낭유충은 생식기관 중 고환과 Mehlis 선을 가지고 있었으며, 감염 제 1 일에는 난소가, 제 2 일에는 저정낭과 난황소가 출현하였다. 제 3 일 된 충체에는 수정낭이 나타났으며 충란이 형성되었다.
5. 생식기관은 감염 제14일까지 빠른 속도로 발달을 계속하였으며 자궁 내 충란수도 증가하였다.

이상의 결과로 보아 *C. armatus*는 흰쥐에서 소장 상부에 기생하며 감염 후 3일 만에 성충으로 발육한다는 것을 알았다.