ASEXUAL MULTIPLICATION OF TETRATHYRIDIA OF *Mesocestoides corti* HOEPPLI, 1925 (CESTODA), AND ITS ACTIVATION BY SOME CYTOSTATIC AGENTS AND LUCANTHONE

by

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PART I.

QUANTITATIVE STUDIES ON THE GROWTH AND MULTIPLICATION OF TETRATHYRIDIA OF *MESOCESTOIDES CORTI* HOEPPLI, 1925 (CESTODA: CYCLOPHYLLIDEA) IN RODENTS

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ABSTRACT

Tetrythyridia multiply by splitting scolices, regenerating their median portions, and increasing in length. Pinching off of caudal end of daughter tetrathyridium leaves at proliferative side of parental organism a stump with one or two excretory bladders. Such stumps may later bud off acephalic fragments, but not regenerate scolices. Long posterior portions of parental organisms with several stumps may separate from scolex-carrying anterior portion and become acephalic. Such fragments do not produce scolices and later degenerate. Polycephalic forms, with up to 22 scolices, occur mostly in hosts with long-standing infections (over 100 days). Such forms transplanted into new host disintegrate into usual tetrathyridia plus large branching acephalic portions. Separation of oldest parental scolex, situated at non-proliferative side of tetrathyridium, transforms this side into a proliferative side. In usual tetrathyridia, each new daughter tetrathyridium splits off anterior to stump left by previous one. If posterior portion was not cast off, number of stumps indicates number of daughter tetrathyridia produced.

In mice injected 0.03 cc of tetrathyridia, and dissected 50 DPI, total volume of intraperitoneal population varied from 0.15 in deer mice to about 1.0 cc in LDF_1 males. Increase in biomass was faster in male hosts than in females.

INTRODUCTION

Specht and Voge (1965) were the first to study the asexual multiplication of Mesocestoides in laboratory animals. They showed that these organisms, found in naturally infected lizard Sceloporus occidentalis, can be inoculated into SWR mice in which they multiply asexually and can be serially transferred.

These authors stated that the asexual multiplication of tetrathyridia occurs in two ways: (1) by fission of the body, starting at the scolex, and (2) by "lateral outgrowths or buds of the body posterior to the scolex". In the second case the suckers differentiate later. Specht and Voge pointed out that "M. corti (is) a valuable species for laboratory research". Later, Voge and Coulombe (1966) studied the growth and asexual multiplication of these organisms in vitro. Diagrams illustrating the asexual multiplication of tetrathyridia were given by Voge (1969), and by Hart (1968).

Experimental work on the tetrathyridia of M. corti is of both theoretical and practical importance. To study the problems of ecology of proliferating cestode larvae quantitatively, it is preferable to have solid, not cystic, organisms, which can be easily freed from host tissues. These requirements are fulfilled by tetrathyridia of M. corti.

The aim of the present project was to study the morphology of asexual multiplication of these larvae and the increase of their biomass in experimental rodents, and to provide thus a quantitative basis for research in experimental chemotherapy of diseases caused by them.

MATERIALS AND METHODS

In August 1968 a strain of tetrathyridia was received from Prof. M. Voge, UCLA, and inoculated into LDF_1 hybrid mice (C57/L female x DBA male), used by Lubinsky (1967) in his work on Echinococcus multilocularis. The strain was maintained in these hybrids and later in SWR mice by serial transfers made every 3 months.

Experimental animals were infected with larvae obtained from the fifth to twelfth transfers in mice. The inoculum of 0.03 cc of sedimented larvae (200 - 300 tetrathyridia) was injected intraperitoneally, using a tuberculin syringe with an 18 gauge needle. Mice were fed Purina Lab chow and water ad libitum, and kept at a temperature of 20°C and a humidity of fifty per cent. The light cycle was 15 hours simulated daylight (6.00 a.m. - 9.00 p.m.) and 9 hours darkness.

The quantitative experiments were discontinued 26 to 50 days post infection (DPI), though some other mice were dissected much later. The invasion of the liver was estimated semiquantitatively on the basis of the number of larvae per 1 cm^2 of the liver surface as follows: L - Light infection (1 - 10 larvae per 1 cm^2), M - Moderate infection (10 - 50 larvae per 1 cm^2), and H - Heavy infection (more than 50 larvae per 1 cm^2). The total volume of larvae, washed out of the abdominal and thoracic cavity of each mouse, was determined using a tuberculin syringe as a measuring cylinder.

Live larvae were examined in a balanced salt solution with neutral red added. The outlines of tetrathyridia were drawn at x 40 or 80, the surfaces enclosed measured with a zero setting compensating planimeter (Geotec), and their real sizes calculated in mm^2.

RESULTS

Multiplication of tetrathyridia of M. corti in vivo

The asexual multiplication of tetrathyridia of M. corti is very peculiar, and seems to be unique among the cestodes. Though these larvae vary greatly both

in size and shape, depending on the host and the intensity and duration of infection, they can be classified into a small number of basic stages of development (Fig. 1), as observations made on populations from SWR, SEC and LDF_1 mice, as well as from gerbils and cotton rats, show.

Stage 1

(Fig. 1, #1) is a basic tetrathyridium. It varies in length from less than 1 to over 10 mm, but has only four suckers and a single terminal excretory pore. The posterior pointed end is usually curved to one, the future proliferative, side.

Stage 2

(Fig. 1, #2) the beginning of longitudinal splitting is heralded by a widening of the anterior end of the body and the appearance of a longitudinal cleft near the mid line of the scolex. In each half of the cleaving scolex, primordia of a new pair of suckers appear. At this stage the tetrathyridium has 2 pairs of large suckers and 2 pairs of smaller ones. It could be, indeed, called "octothyridium" (Fig. 2).

Stage 3

(Fig. 1, # 3) the new suckers gradually grow, and the cleft between the two developing scoleces deepens. The splitting of the anterior end progresses, producing an Y-shaped organism.

Stage 4

(Fig. 1, # 4) deep splitting into two larvae. Duplication of nerve trunks and excretory ducts is completed. The new larva tends to move away from the parental organism, and this finally leads to a complete separation. The stalk of the daughter larva becomes progressively thinner and finally breaks. The separation of the daughter individual takes place at various levels of the proliferative side of the parental organism, but always at some distance from its posterior end, which remains with the parental tetrathyridium (Fig. 3).

Stage 5

(Fig. 1,# 5) at the site of separation of the daughter tetrathyridium, on the proliferative side of the parental larva, a stump-like excrescence designated as "bud" by Specht and Voge (1965), remains (Fig. 4). At its apex there is a functional excretory bladder (Hart 1967) with its pore and plexus of canals. This stage is followed by growth and elongation of the mother organism, culminating in the initiation of the next splitting.

Stage 6

(Fig. 1, # 6) the splitting, started at stage 5, results in the separation of another daughter individual, which leaves at the posterior end of the proliferative side of the parent one more stump, anterior to the previous one. This stump is also provided with an excretory bladder.

Stage 7

(Fig. 1, #7) separation of the fourth daughter individual. In the process of production of subsequent tetrathyridia the splitting becomes accelerated and the heads may possess only two suckers at the time of separation. The daughter scolex is now mostly smaller than that of the parental organism, and its secondary suckers develop later (Fig. 8). Recently separated larvae often have at their posterior ends small blobs of parenchyma, which later probably separate. With progressing growth of the parental organism some of the lateral outgrowths (stumps) elongate, and their distal portions separate as acephalic fragments, which do not regenerate a scolex (Figs 5, 6).

Stage 8

(Fig. 1, # 8) as results of continued asexual multiplication a large, long larva with many stumps at the proliferative side of the body is produced (Fig. 10). Now the scolex with the anterior portion of the body may separate from the posterior portion, which becomes acephalic, does not regenerate a scolex, and later degenerates (Fig. 9). The anterior tetrathyridium may now start a new cycle of asexual multiplication.

After the separation of the primary scolex, both its posterior end, and the anterior end of the acephalic portion may develop one, or seldom two bladders each, at the site of separation. The acephalic fragments vary in size from a fraction of a millimeter to over 5 mm, and may have up to 10 lateral stumps.

Stage 9

(Fig. 1, # 9) in mice, about 200 DPI, polycephalic forms with up to 22 heads develop (Figs 14 to 18). They are mostly rosette-shaped, and up to 10 mm in diameter. When transferred to other mice, they disintegrate into simple tetrathyridia.

Thus in hosts infected with M. corti larvae, three basic types of tetrathyridial bodies can be found: (1) Single non-dividing tetrathyridia without any stumps (2) Dividing forms and (3) Sterile acephalic fragments.

The great majority of stages found in infected rodents fall into the categories described above (Fig. 1, #1 - 9). But there occur also some other forms which deviate from this basic pattern of multiplication. Fig. 19,#1 - 7 show that the separation of the oldest ("parental") scolex, situated at the non-proliferative side of a tetrathyridium, results in the transformation of this side into a proliferative one. The resulting forms, with stumps and excretory bladders at both sides of the body, occurred but seldom in our material (Fig. 11).

Still more seldom occurred tetrathyridia with two terminal bladders close to each other, and also acephalic tail fragments with two bladders close together (Fig. 7). Such pairs of bladders are probably the result of development of separate bladders at the ends of each of the two lateral pairs of excretory canals.

Growth and multiplication of tetrathyridia in different rodents

To produce a quantitative basis for ecological and biomedical studies on Mesocestoides infections in intermediate rodent hosts, it was necessary in the first place to determine in which species and/or strain of rodents the tetrathyridia multiply fastest.

In the first experiment, which lasted 49 days, an attempt was made to compare the increase in the biomass of tetrathyridia in LDF_1 hybrids with that in SEC and SWR mice, as well as in the few available Peromyscus maniculatus, and to compare the multiplication of tetrathyridia in male and female hosts. The results, graphically represented in Fig. 20, show that the increase in biomass is fastest in LDF_1 hybrids, and slower in SEC and SWR mice, in this sequence. It is still slower in P. maniculatus, though the results obtained with this host are not convincing, because of the small numbers of deer-mice used.

In all types of mice the asexual multiplication of tetrathyridia was faster in males than in females. The livers of both males and females were moderately infected (M), but in P. maniculatus the liver infection was light (L).

At the end of experiments with LDF_1, SEC and SWR mice, which lasted about 50 days, the volumes of tetrathyridial populations collected from the body cavities were measured. These varied from about 0.1 cc in some experiments with SWR females, to about 1.0 cc in some LDF_1 males.

The morphology of 200 tetrathyridia from SWR male mice was studied in measuring their body areas and counting the suckers (Fig. 21). The mean area of the larvae was 0.35 ± 0.02 mm^2, the majority had areas from 0.05 to 0.50 mm^2, and only three had surfaces exceeding 1.0 mm^2. The surface of the largest larva was almost 1.5 mm^2. Eleven and a half per cent of larvae were acephalic, 25 per cent had 2 suckers, 60.5 per cent 4 suckers, and only 3.0 per cent had 8 suckers and usually two scolices.

In Meriones unguiculatus, Sigmodon hispidus and Citellus franklini the tetrathyridia multiplied in the same way as they did in mice. However, larvae recovered from experimentally infected Ondatra zibethicus were much longer than those from other rodents, mostly 5 to 11 mm long (Fig. 12), and showed very little evidence of multiplication.

Acephaly: To study whether the fragments of tetrathyridia can regenerate a scolex, 10 SWR mice of both sexes were infected intraperitoneally, each with 20 live acephalic fragments, both of lateral and of posterior origin. At the autopsy 31 days later no regeneration of scolices was seen in any mice. From the five males 25 motile, acephalic fragments were collected, and from the five females, only 17. Some dead and disintegrating fragments were also present.

Polycephaly: Polycephalic tetrathyridia were observed by Hart (1967) in mice with long standing infections. He suggested, that such tetrathyridia do not separate, because they have only one terminal bladder.

In an attempt to produce polycephaly experimentally, a strain of mice was chosen in which tetrathyridia multiply slowly. Five SWR females received 25 larvae each, and four SWR males 10 larvae each, intraperitoneally. The males were killed 170 DPI, and 37 polycephalic forms with 3 to 20 scolices were collected from them along with numerous usual tetrathyridia (Fig. 16). Three females were dissected 200 DPI and found to contain numerous tetrathyridia, among them 19 polycephalic forms with up to 22 scolices (Figs. 14, 15, 17 and 18).

The polycephalic larvae varied greatly in the number of their scolices, in the shape and size of the bodies, as well as in the number and location of their excretory bladders. Many of these larvae had, besides the terminal bladder, several bladders at the end of the stumps, which strongly suggests that they, just as the ordinary tetrathyridia, may bud off single-headed individuals.

To prove this experimentally, polycephalic larvae with at least 10 scolices but only one bladder each, were implanted intraperitoneally into 8 SWR mice, each mouse received one larva. At the autopsy 38 days later, it was found that all polycephalic larvae have disintegrated into a large number of conventional tetrathyridia, many of which penetrated into the livers. Peculiar branching remnants of polycephalic forms, resembling those from the muskrat (Fig. 13), were found in the peritoneal cavity of these mice.

A similar experiment was made with a muskrat, which received 75 two- to five-headed tetrathyridia intraperitoneally and was dissected 46 days later. At autopsy only single non-multiplying larvae were found, along with peculiar acephalic forms with several stumps and excretory bladders (Fig. 13).

DISCUSSION

My observations on asexual multiplication of tetrathyridia of M. corti in mice showed, that new scolices originate as result of longitudinal splitting of the parental scolex, and confirmed basically the views of Specht and Voge (1965) and of Hart (1967, 1968). However, I was not able to confirm the statement of Specht and Voge (1965), that lateral outgrowths at the proliferative side of tetrathyridia may develop into daughter individuals. It seems that the lateral "buds" are not developing daughter individuals, but stumps, remaining on the parental body after the separation of split off tetrathyridia.

The stumps may bud off at their distal ends small acephalic fragments, but not develop scolices. On the other hand, large fragments with many excretory bladders originate as result of separation of the entire distal end of the parental tetrathyridium. Such transverse division may take place at different levels, separating from the scolex either the entire proliferative region, or only its portion, and, in this case, leaving with the parental organism one or several anterior lateral stumps. Hart (1968) described only one method of production of acephalic fragments, namely separation of the distal end of the parental tetrathyridium.

He showed experimentally, that such fragments, when implanted into new hosts, do not produce scolices and later degenerate.

Hart thought, that the bladders of the proliferative side of the parental organism develop before the splitting is completed and each daughter individual receives thus an already functional bladder. My observations showed that the development of the bladders both on the posterior end of the daughter individual and on the stump of the parental body are the result of separation of a daughter tetrathyridium. Hart also described the presence of two bladders close to each other at the end of the tetrathyridium, and interpreted this as evidence of division of the bladders. Though in my material such pairs of bladders did occur, I feel that they develop independently at each side of the posterior end of tetrathyridium from the corresponding two lateral vessels of the same body-side. The spacial relationship between the position of lateral vessels and the timing of constriction of the peduncle determine whether only one medial bladder or two bladders will develop.

In my material, especially that from SWR mice, I have found many polycephalic forms with 3 to 22 scolices. When such forms were implanted into the peritoneal cavity of a muskrat, a host in which tetrathyridia almost do not multiply, the polycephalic larvae disintegrated into normal tetrathyridia, leaving an irregularly branching posterior portion with many excretory bladders. Similar results were obtained in implanting polycephalic forms into mice. In this case, however, the resulting tetrathyridia multiplied intensively.

The fastest increase in the biomass of tetrathyridia was observed in LDF_1 hybrids, the lowest in muskrats. The intensity of multiplication and the growth of individual tetrathyridia depend both on the species or strain of the host and on its sex. The fastest growth was observed in the LDF_1 mice in which Echinococcus multilocularis grows fastest (Lubinsky 1967). The growth in SEC and SWR mice was slower, as was that of E. multilocularis (Lubinsky, unpublished). But whereas E. multilocularis grows faster in female hosts than in males, the biomass of tetrathyridia of M. corti increases faster in males than in females. In this respect M. corti seems to be closer to E. granulosus, which,

according to Frayha et al (1971), grows faster in male hosts.

I was unable to find in the literature any description of the multiplication of flatworms by longitudinal fission. However, it is well known that planarians, cut along their sagittal plane, will regenerate the missing halves. But longitudinal fission as a mode of reproduction was repeatedly observed in coelenterates. Pasteels (1939) has described sporadic longitudinal fission of Cladonema radiatum (Anthomedusae), which commenced with a longitudinal division of the manubrium, and Lipin (1911) in Polypodium hydriforme, a coelenterate parasite of the eggs of sturgeons. Its free living polyps with 24 tentacles divide medially, producing two individuals with 12 tentacles each, and these divide again, giving rise to polyps with 6 tentacles, which can reconstitute polyps with 12 and 24 tentacles. Later Lipin (1926) found such free polyps with immature gonads, reminiscent of those of medusae. Berrill (1950) regards these "walking" polyps as modified medusae. The life cycle of P. hydriforme was later illustrated by Rajkova (in Petrŭsevskiĭ and Shul'man 1958). In 1950, Berrill wrote: "What is known of Polypodium is fantastic enough; what is left to be discovered cannot be less". This statement may well be applicable to the still incompletely known life cycle of Mesocestoides corti.

REFERENCES

Berrill, N. J. 1950. Development and medusa-bud formation in the Hydromedusae. Quart. Rev. Biol. 25: 292-316.

Frayha, G. J. et al. 1971. Echinococcus granulosus in albino mice: effect of host sex and sex hormones on the growth of hydatid cysts. Exp. Parasitol. 29: 255-262.

Hart, J. L. 1967. Studies on the nervous system of tetrathyridia (Cestoda : Mesocestoididae). J. Parasitol. 53: 1032-1039.

_____ 1968. Regeneration of tetrathyridia of Mesocestoides (Cestoda : Cyclophyllidea) in vivo and in vitro. J. Parasitol. 54: 950-956.

Lipin, A. 1911. Die Morphologie und Biologie von Polypodium hydriforme Uss. Zool. Jb. Anat., 31: 317-426.

_____ 1926. Geschlechtliche Form, Phylogenie und systematische Stellung von Polypodium hydriforme Uss. Zool. Jb. Anat., 47: 541-635.

Lubinsky, G. 1967. Accelerating growth of a vegetatively propagated strain of larval Echinococcus multilocularis Leuck., 1863. Can. J. Zool. 45: 175-180.

Pasteels, J. 1939. Scissiparite sporadigue chez l'hydromeduse Cladonema radiatum Duj. Bull. Mus. R. Hist. nat. Belg., 15: 1-7.

Petruševskiĭ, G. K., and Shul'man, S. S. 1958. Parasitic diseases of fish in commercial bodies of water in SSSR (Russian text). In:"Osnov. Probl. Parazitol. Ryb" (Dogiel, Petrušcevskiĭ and Polianskiĭ, editors). pp. 301-320. German summary, p. 341.

Specht, D., and Voge, M. 1965. Asexual multiplication of Mesocestoides tetrathyridia in laboratory animals. J. Parasitol. 51: 268-272.

Voge, M., and Coulombe, L. S. 1966. Growth and asexual multiplication in vitro of Mesocestoides tetrathyridia. Am. J. Trop. Med. Hyg. 15: 902-907.

Voge, M. 1969. Systematics of cestodes, present and future. In: Schmidt, G. D. (edit.) "Problems in systematics of parasites". pp. 49-73 University Park Press: Baltimore, Md. U.S.A. and Manchester, England.

Figure Legends

Fig. 1. Basic pattern of asexual multiplication of tetrathyridia of Mesocestoides corti, Hoeppli 1925. Explanation in text.

Plate 1, Figs. 2 to 7

Fig. 2. Head of a tetrathyridium with eight suckers; early stage of splitting; x 55.

Fig. 3. Constriction of the peduncle preceding the complete separation of a new individual. Both scolices have two bigger and two smaller suckers each; x 35.

Fig. 4. Tetrathyridium with a lateral stump, provided with an excretory bladder; x 60.

Fig. 5. An acephalic fragment separates from a lateral stump; x 40.

Fig. 6. Completely separated acephalic fragment; x 120.

Fig. 7. Distal end of an already separated acephalic fragment with a pair of bladders; x 95.

Plate 2, Figs. 8 to 13

Fig. 8. Separation of the 5th or 6th daughter tetrathyridium from a larva with 4 or 5 stumps; x 20.

Fig. 9. Separation of the last daughter tetrathyridium from the anterior end of the proliferative side. The parental scolex separated at the site indicated by the arrow; x 25.

Fig. 10. Tetrathyridium with five lateral stumps; x 20.

Fig. 11. A tetrathyridium in the process of separation from its long posterior portion. Note the presence of stumps on both its margins; x 25. Compare with Fig. 19, 6-7.

Fig. 12. A long tetrathyridium from the abdominal cavity of a muskrat, 50 DPI; x 20.

Fig. 13. Acephalic posterior fragment of a polycephalic larva implanted into a muskrat, 46 DPI, with stumps remaining after the separation of many tetrathyridia; x 25.

Plate 3, Figs. 14 to 18

Fig. 14. Several stumps on both sides of the posterior portion of an eight-headed tetrathyridium from an SWR female, 200 DPI; x 10.

Fig. 15. A seven-headed tetrathyridium with multiple stumps both on its posterior portion and on its side branches, from an SWR female, 200 DPI; x 10.

Fig. 16. A tetrathyridium from an SWR male, 170 DPI. This peculiar larva has 20 suckers, of which 8 belong to the two well differentiated and invaginated scolices on its right side; x 30.

Fig. 17. Twenty-two-headed branching tetrathyridium with a single terminal bladder from an SWR female, 200 DPI; x 10.

Fig. 18. A branching tetrathyridium with all its 10 scolices invaginated. From an SWR female, 200 DPI; x 10.

Fig. 19. Inversion of the proliferative side of a tetrathyridium of M. corti.

1. First splitting, and 2. separation of a daughter tetrathyridium. 3. Second splitting, and 4. separation of the parental scolex from the non-proliferative side of the body. The non-proliferative side now becomes the proliferative side. 5 and 6. Separation of another tetrathyridium from the new proliferative side. 7. Separation of the new parental scolex leaves a long acephalic remnant with stumps and excretory bladders at both its lateral margins.

Fig. 20. Growth and multiplication of tetrathyridia of M. corti in SEC, SWR, LDF_1 mice, and in Peromyscus maniculatus. The numbers above the bars represent the number of animals used.

Fig. 21. The variability of the body areas and of the number of suckers of two hundred tetrathyridia from SWR male mice, 50 DPI.

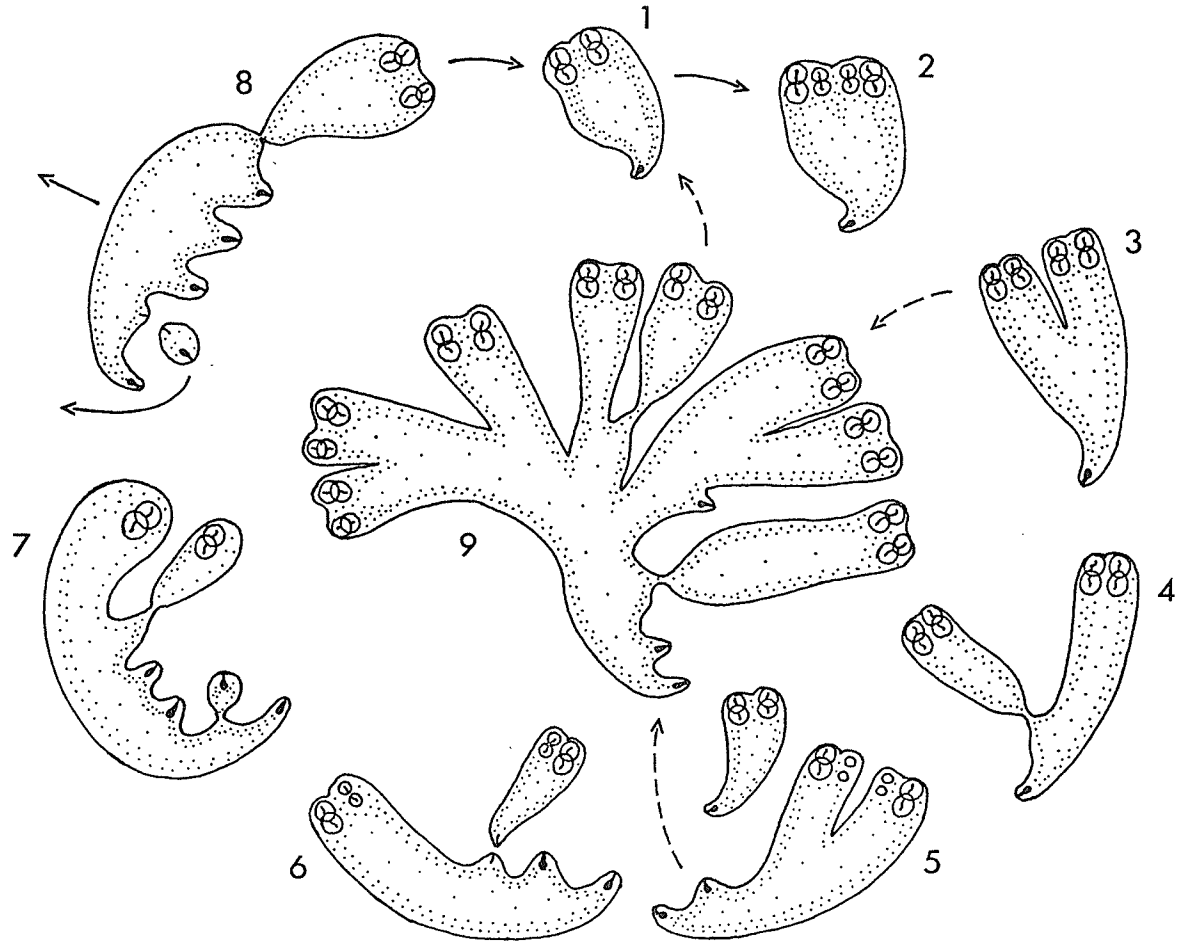
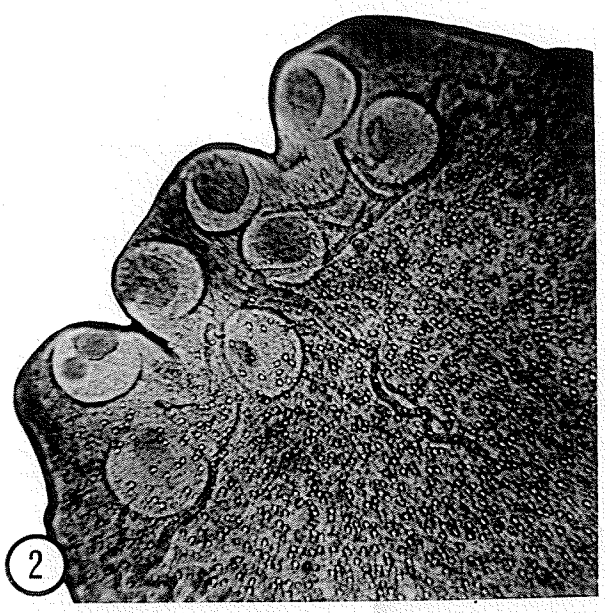
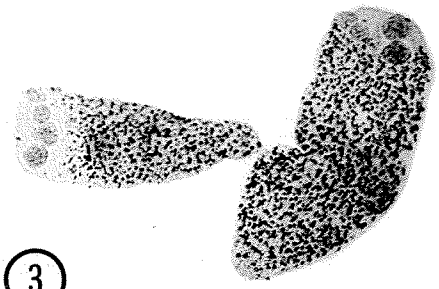
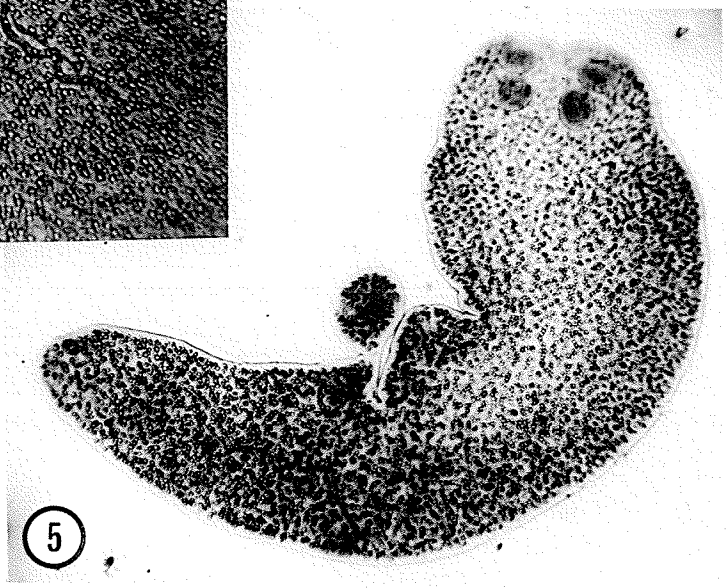


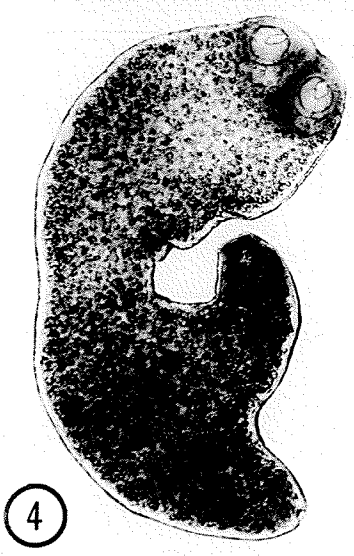
Fig. 1

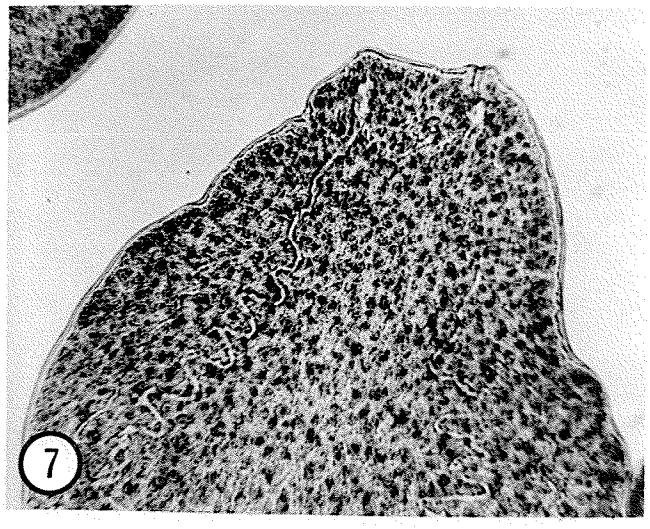
Plate I

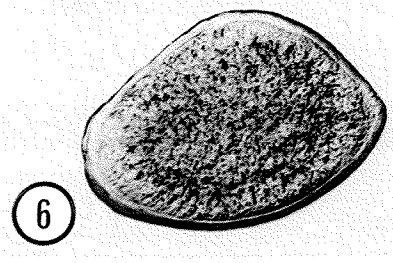












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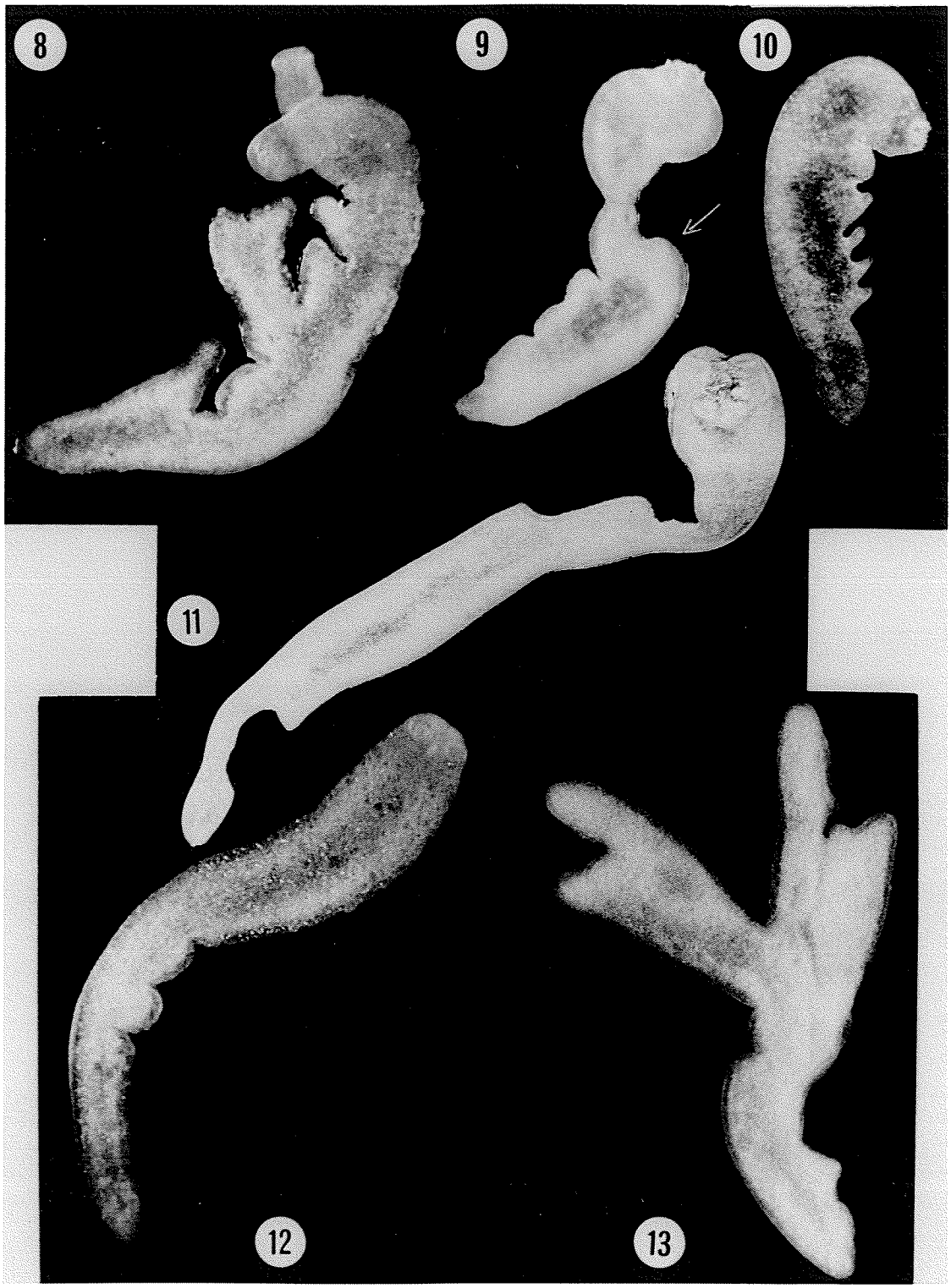

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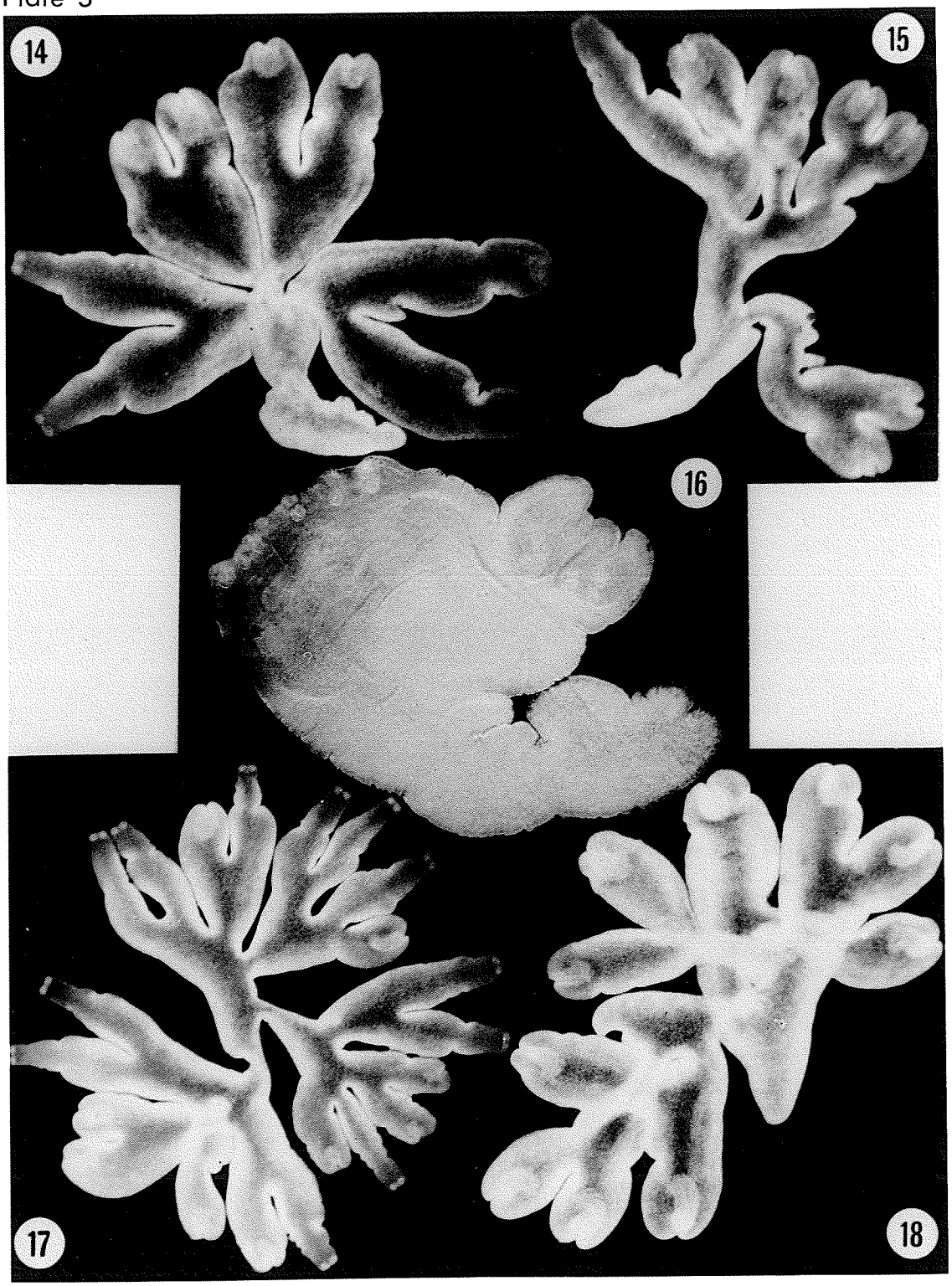


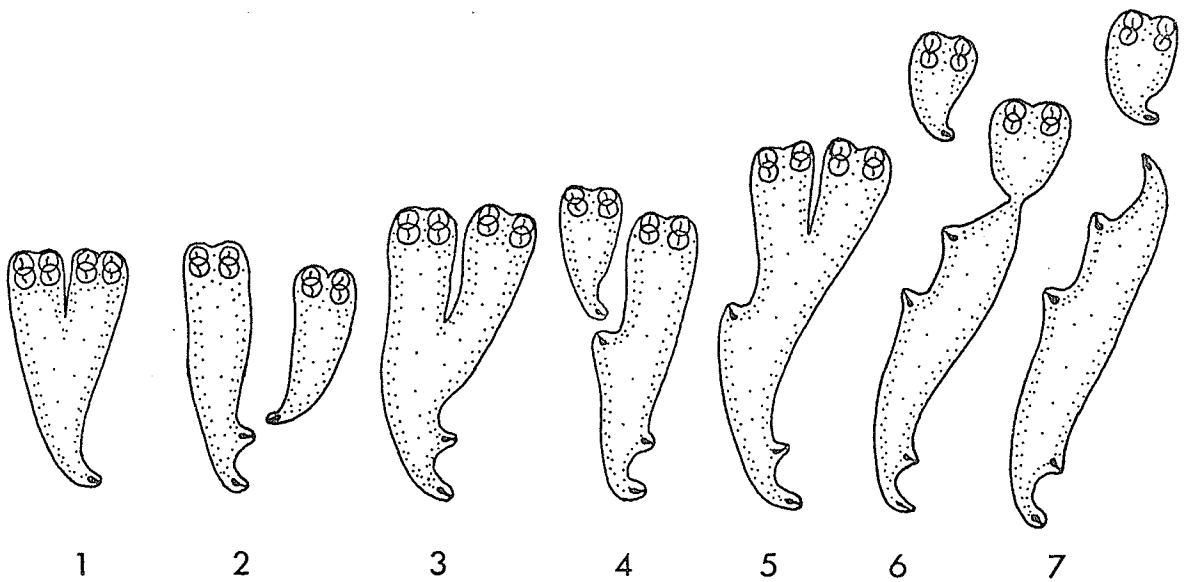
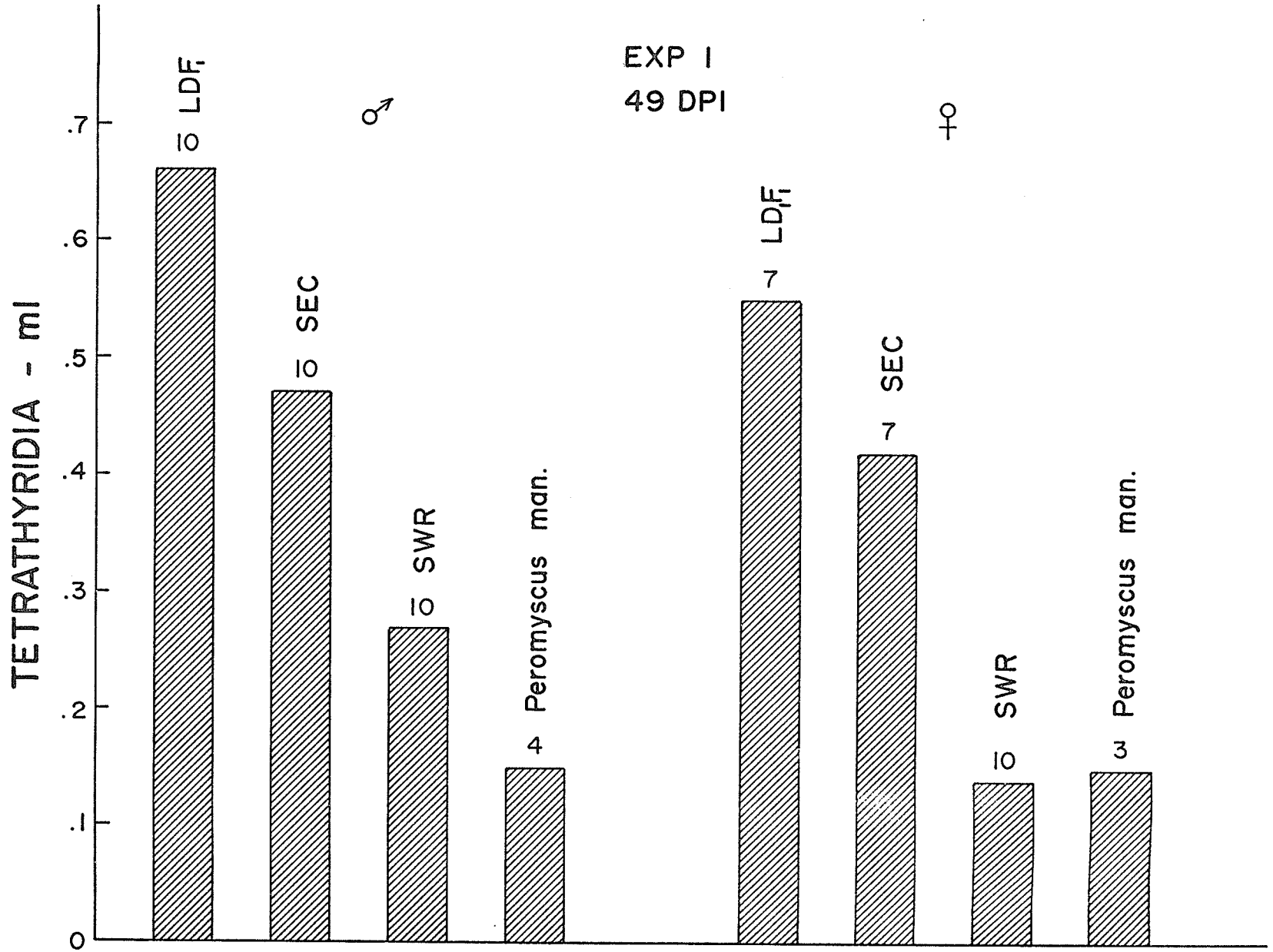


Fig. 19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7 LDF1
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3 Peromyscus man.

Fig. 20

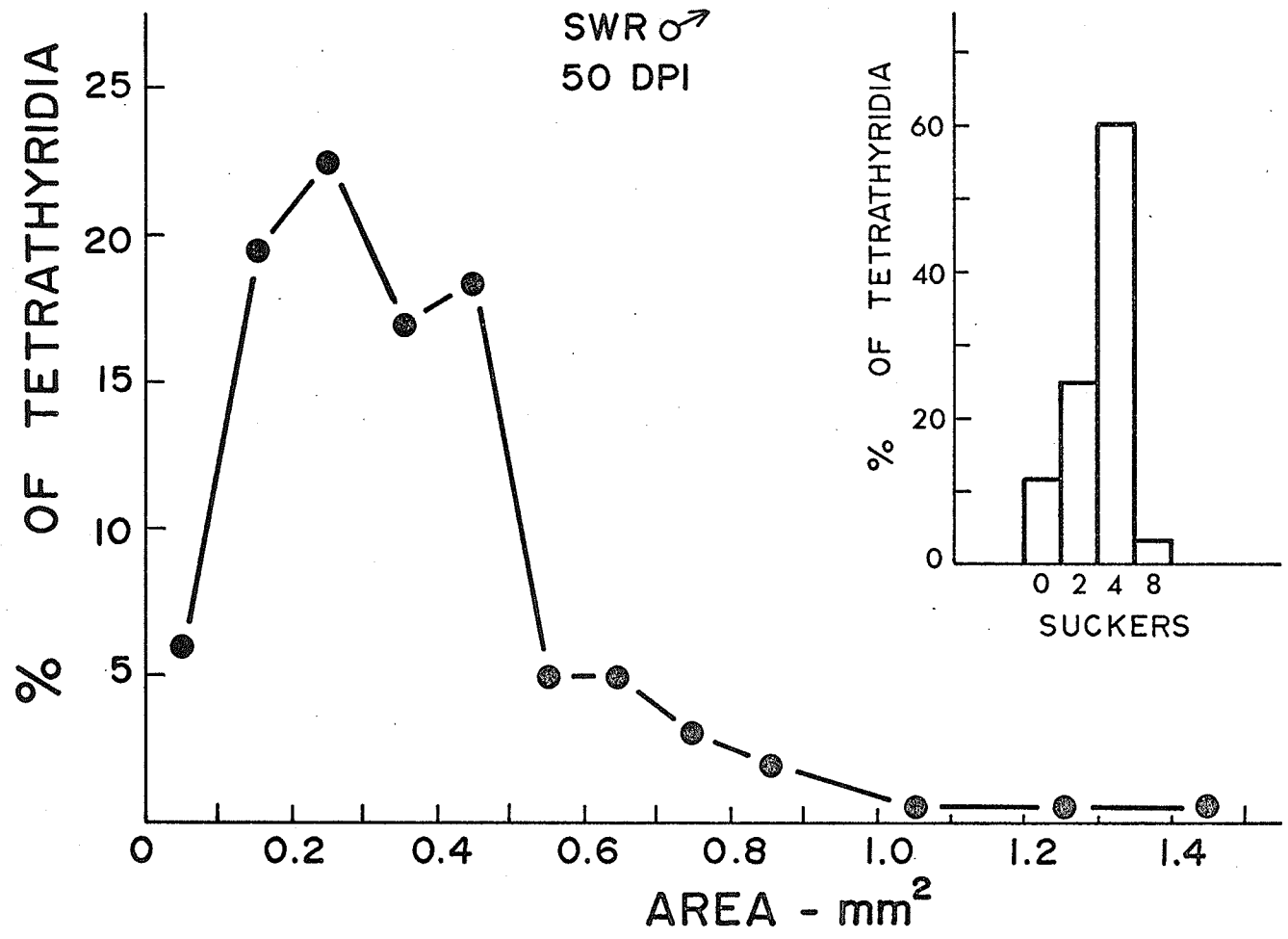
SWR ♂
50 DPI
% OF TETRATHYRIDIA
25
20
15
10
5
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
AREA - mm²
% OF TETRATHYRIDIA
60
40
20
0
0 2 4 8
SUCKERS

Fig. 21

PART II.

ACCELERATION OF THE GROWTH AND MULTIPLICATION OF TETRATHYRIDIA OF MESOCESTOIDES CORTI HOEPPLI, 1925 (CESTODA: CYCLOPHYLLIDEA) BY CYCLOPHOSPHAMIDE AND DACTINOMYCIN

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ABSTRACT

The cytostatic agents cyclophosphamide and dactinomycin, which inhibit the growth of Echinococcus multilocularis cysts, accelerate the increase in biomass of tetrathyridial populations of Mesocestoides corti in mice by 50 to 200 per cent, and the parasiticide lucanthone, by 30 to 110 per cent. Demecolcine and quinacrine are almost inactive. The active compounds cause a decrease in the average size of larvae, and an increase in the percentage of tetrathyridia with two suckers. All compounds, except cyclophosphamide, cause an increase in the number of acephalic forms.

INTRODUCTION

Several cytostatic agents are known to inhibit the growth of non-proliferating cestode larvae. Thus Hinz in 1964 demonstrated, that cyclophosphamide ("Endoxan") inhibited the growth of "Cysticercus fasciolaris", the larva of Hydatigera taeniaeformis, and sometimes killed it. Mettrick and Parnell (1967) showed, that thio-TEPA strongly inhibits the development of cysticercoids of Hymenolepis diminuta in Tribolium confusum, and may kill them.

In 1967 Lubinsky and Galaugher, and later Lubinsky (1969) showed, that cyclophosphamide strongly inhibits the growth of the proliferating cysts of Echinococcus multilocularis in rodents. Similar observations were made on dactinomycin and demecolcine (Lubinsky, et al 1971). It may be of interest therefore to study the influence of cyclophosphamide and of some other cytostatic agents on proliferating cestode larvae other than E. multilocularis.

Interesting in this respect are tetrathyridia of Mesocestoides corti, because this cestode does not belong to Taeniidae, and even its placement into the order Cyclophyllidea is questionable. The solid, not cystic, tetrathyridia from the body cavities of the hosts can be easily collected and washed free from host's tissues. Their total volume is therefore a

good measure of their biomass. Methods for the quantitative study of their populations in experimental rodents were recently given by M. Novak (in press).

In the present series of experiments we attempted to study the influence on the growth and multiplication of Mesocestoides tetrathyridia of the antiproliferative agents cyclophosphamide, dactinomycin, and demecolcine, and of the parasiticides quinacrine and lucanthone.

MATERIALS AND METHODS

A strain of tetrathyridia received from Prof. M. Voge, UCLA, in 1968, and maintained in LDF_1 and SWR mice was used in the present study.

The larvae from the peritoneal cavities of the donor mice were collected in Petri dishes with balanced salt solution. Each recipient animal was inoculated intraperitoneally with 0.03 cc of sedimented tetrathyridia (200 to 300 larvae), using an 18 gauge needle. Over 500 mice of the SEC/1 ReJ, SWR/J strains and of LDF_1 hybrids, as well as 90 jirds ("gerbils"), Meriones unguiculatus, were used. The animals were kept at 15 hours simulated day light, at a temperature of 20 °C and fed Purina laboratory chow ad libitum.

The treatment was started one day after infection and continued for 4 to 5 weeks. All drugs were injected

subcutaneously. The experiments were discontinued 26 to 55 DPI (days post infection), the larvae washed out from peritoneal and thoracic cavities, and the volumes of populations from each individual host determined using a tuberculine syringe as a measuring cylinder.

The intensity of infection of the liver was recorded semiquantitatively as: "L"-light (1-10 larvae per 1 cm^2 of liver surface), "M"-moderate (10-50 larvae per 1 cm^2), and "H"-heavy (over 50 larvae per 1 cm^2).

The morphology of populations was studied in drawing outlines of the larvae at x40 or x80, measuring their areas with a compensating planimeter, and computing the real areas in mm^2. Further details of technique were described in a previous paper (M. Novak, in press).

RESULTS

Cyclophosphamide. (Fig. 1) In all experiments with mice and jirds, the animals were divided into one control and two experimental groups, and infected with tetrathyridia. The next day experimental animals of both groups received an injection of cyclophosphamide, 200 mg/kg. The first group received no further treatment, whereas the second got another such injection a fortnight later. All animals of each experiment were dissected in the course of one day and the volumes of larval populations for each

individual host determined. The results of these experiments are summarized in Table I. They show that in mice 35 DPI, the increase of biomass caused by cyclophosphamide varied from 50 to 60 per cent, and 50 DPI, from 46 to 208 per cent. The liver infection was always heavier in treated animals than in the controls.

In jirds the infection ran a very acute course, and the tetrathyridia tended to accumulate in the liver. In some treated animals the liver seemed to contain more tetrathyridia than parenchyma. The experiment with jirds was discontinued 26 DPI, after a spontaneous mortality set in.

In experiments with mice, the second injection of cyclophosphamide, made a fortnight after the first, did not enhance the effect of the first injection. To the contrary, the biomass of tetrathyridial populations in the peritoneal cavity of mice which have received two injections was 8 to 27 per cent less than in mice injected only once. Though the differences between the two groups were not statistically significant they occurred in all 4 experiments and were probably real. It may be interesting to note that, in LDF_1 mice infected with Echinococcus multilocularis cysts, the second injection of cyclophosphamide does not prolong the survival time of experimental animals beyond that of mice which have received only one injection (Lubinsky, 1969b).

In experiments 6 to 9 an attempt was made to study the

influence of dactinomycin and demecolcine, and to compare it with that of the parasiticides lucanthone and quinacrine. The data, summarized in Table II show, that dactinomycin, 0.35 mg/kg, once a week for 5 weeks, increased the biomass of intraperitoneal populations by 48 to 144 per cent, and lucanthone, 100 mg/kg, twice a week also for 5 weeks, by 30 to 111 per cent. But demecolcine, 0.5 mg/kg, once a week for 6 weeks, increased the parasitic biomass by only 22 per cent, and the antimalarial, quinacrine, 100 mg/kg, twice a week for 5 weeks, by 13 to 26 per cent, the differences for these two compounds being mostly on the boundary of statistical significance. In all experiments the livers of treated animals were more heavily infected than those of the controls.

Morphological changes in populations of tetrathyridia from treated hosts.

Cyclophosphamide: (Fig. 3) The areas of 200 tetrathyridia from each of the three groups of mice in Exp. 2 (controls, those injected once, and those injected twice) were measured, and their suckers counted. The majority of larvae from all groups of hosts covered areas from 0.1 to 0.4 mm^2. The mean area of larvae from controls was 0.35 ± 0.02 mm^2, that from mice injected once, 0.25 ± 0.01 mm^2, and from those injected twice, 0.31 ± 0.01 mm^2. Thus the larvae from mice of the last group were intermediate in size between those

from controls, and those from mice injected once.

Larvae from each population were classified into four groups: acephalic, two-suckered, four-suckered, and those with eight suckers. No polycephalic forms were encountered in the populations examined, probably because they appear late in the course of infection. The population from controls had 11.5 per cent of acephalic forms, 25 per cent of two-suckered larvae, 60.5 per cent of those with four suckers, and 3.0 per cent larvae with eight suckers. The population from mice injected once also had 11.5 per cent of acephalic forms, but the percentage of two-suckered was 42.5, as compared to 25 in controls. Larvae with four suckers comprised 46 per cent of this population, and those with eight suckers were absent. Thus the percentage of two and four-suckered larvae in this group was almost equal, whereas in the controls the number of four-suckered larvae was more than twice that of the two-suckered. Unexpectedly, in mice which got two injections of cyclophosphamide, the distribution of larvae among these different groups closely resembled that in the controls.

A similar increase in the percentage of tetrathyridia with two suckers was observed in jirds treated with cyclophosphamide, 200 mg/kg, and dissected 26 DPI.

As in the case of cyclophosphamide, dactinomycin decreased the average size of larvae and increased

considerably the percentage of tetrathyridia with two suckers (Fig. 4); so did lucanthone, but to a much lesser extent.

Demecolcine and quinacrine did not increase the percentage of larvae with two suckers, but more than doubled that of the acephalic fragments. None of the compounds tested increased the percentage of polycephalic forms.

DISCUSSION

In the present series of experiments an attempt was made to inhibit the increase in the biomass of populations of Mesocestoides larvae in experimental rodents, in using cytostatic agents known to inhibit the growth of Echinococcus multilocularis cysts. Paradoxically, cyclophosphamide, in the same dosage as in experiments with E. multilocularis, (Lubinsky, 1969b) accelerated the growth of tetrathyridial populations by at least 60 per cent, and in some experiments tripled it.

Dactinomycin, which in repeated doses of 0.17 to 0.35 mg/kg inhibited the growth of E. multilocularis by about 40 per cent (Lubinsky et al, 1971) accelerated the increase in biomass of tetrathyridia by 50 to 140 per cent. Demecolcine, which at LD_{50} inhibited the growth of Echinococcus cysts by up to 30 per cent (l.c.)

accelerated the growth of tetrathyridial populations by about 20 per cent. Thus the three cytostatic agents tested can be arranged according to their efficiency in inhibiting the growth of *Echinococcus* and in activating the growth of *Mesocestoides* into the following series: cyclophosphamide > dactinomycin > demecolcine. Whether the similarity of the inhibitory series for *Echinococcus* with the activating series for tetrathyridia of *Mesocestoides* is accidental or depends on some basic biochemical or physiological differences between these two cestodes, can not be at present decided.

It is tempting to ascribe the acceleration of the growth of tetrathyridial populations by cytostatic agents to their immunosuppressive activity. Yet the considerable fall in the total leucocyte count in mice treated with cytostatic agents obviously does not explain the *difference* in the response to these agents of tetrathyridial populations on one hand, and of *Echinococcus* cysts on the other.

The interpretation of these observations becomes still more difficult if we consider that both parasiticides tested, lucanthone and quinacrine, also accelerated the growth of tetrathyridial populations, the first by 30 to 100 per cent, the second by up to 26 per cent. Of these two compounds only lucanthone inhibits the growth of *Echinococcus* cysts. Again a compound active against these cysts caused a more pronounced acceleration of

tetrathyridial growth than an inactive compound.

Populations of tetrathyridia changed morphologically under the influence of treatment, especially in mice which got one injection of cyclophosphamide. In these, the proportion of two-suckered larvae almost doubled and larvae with four-suckered scolices disappeared completely. This can be construed either as an acceleration of splitting with a relative lag in the differentiation of suckers, or as an inhibition of differentiation of new suckers. The rapid increase in the total biomass of tetrathyridial populations despite a decrease in the average size of individuals under the influence of cyclophosphamide and of some other compounds speaks in favor of the first explanation.

Korotkova and Tokin (1969) observed a stimulation of somatic embryogenesis in Laomedea flexuosa Hinks (Coelenterata: Hydrozoa) by colchicine in concentrations of 0.001 and 0.01 per cent, the colchicinized hydrants developing more buds than the controls. The increase in the number of tetrathyridia with two suckers in our experiments with cyclophosphamide may also be construed as an acceleration of somatic embryogenesis by a cytostatic agent.

What is the physiological basis of the acceleration of growth of tetrathyridial populations by cyclophosphamide and lucanthone? Does this last act also as a cytostatic agent (and it is a cytostatic, at least in

respect of some schistosomes)? To what extent is the action of these agents mediated by the host? Some of these questions may be answered on the basis of *in vitro* studies, others will require extensive animal experimentation. It seems that experiments described in the present paper have posed more questions than they have answered.

REFERENCES

Hinz, E. 1964. Chemotherapeutische Untersuchungen mit Endoxan an der experimentellen Finneninfection der weissen Mauss. Z. Tropen med. Parasit. 15: 332-336.

Korotkova, G.P. and Tokin, B.P. 1969. Stimulation of the processes of somatic embryogenesis in some Porifera and Coelenterata. II. Provocation of somatic embryogenesis in Laomedea flexuosa Hinks by colchicine and mechanical injuries. Acta Biol. Acad. Sci. Hung. 20: 35-42.

Lubinsky, G. and Galaugher, W. 1967. Inhibition of the growth of Echinococcus multilocularis cysts by cyclophosphamide. Can. J. Zool. 45: 1302-1303.

Lubinsky, G. 1969a. Cyclophosphamide and the survival time of mice infected with Echinococcus multilocularis cysts. Can. J. Zool. 47: 144-145.

Lubinsky, G. 1969b. Attempts at chemotherapy of Echinococcus multilocularis infections in rodents. Can. J. Zool. 47: 1001-1004.

Lubinsky, G. et al. 1971. Attempts at chemotherapy of Echinococcus multilocularis infections in rodents. II. A study of some parasiticides and cytostatic agents. Can. J. Zool. 49: 1301-1304.

Mettrick, D.F. and Parnell, J.R. 1967. Exposure of the beetle Tribolium confusum to thio-TEPA and its effects upon the development of Hymenolepis diminuta cysticercoids. Exper. Parasitol. 20: 17-26.

LEGENDS

Fig. 1. Acceleration of the growth of tetrathyridial populations of Mesocestoides corti in LDF_1, SWR mice and in jirds by cyclophosphamide. Numbers of animals in each group are given above the corresponding columns.

Fig. 2. Acceleration of the growth of tetrathyridial populations of M. corti in SEC and LDF_1 mice by dactinomycin and lucanthone. Numbers of mice in each group are given above the corresponding columns.

Fig. 3. The variability of the body areas and of the number of suckers of two hundred tetrathyridia from SWR male mice, 50 DPI, injected cyclophosphamide.

Fig. 4. The variability of the body areas and of the number of suckers of two hundred tetrathyridia from SEC male mice, 55 DPI, injected dactinomycin and lucanthone.

TABLE I.

Increase in the biomass of tetrathyridial populations of M. corti in mice and jirds injected cyclophosphamide.

Exp.	Duration (days)	Type of animal	No. animals at autopsy	Compound Dosage	Liver inf'n.	Tetra-thyridia ml.	Increase in biomass %	P
1	35	LDF_1 ♂	10	Control -	M	0.32 ± 0.05	-	-
			10	Cycloph. 200mg/kg (x1)	H	0.52 ± 0.03	62.5	<0.01
		6 mos.	10	Cycloph. 200mg/kg (x2)	H	0.48 ± 0.03	50.0	<0.01
2	50	SWR ♂	8	Control -	M	0.19 ± 0.03	-	-
			10	Cycloph. 200mg/kg (x1)	H	0.54 ± 0.07	184.0	<0.001
		5 mos.	10	Cycloph. 200mg/kg (x2)	H	0.43 ± 0.05	126.0	<0.01
3	49	SWR ♂	10	Control -	M	0.12 ± 0.02	-	-
			9	Cycloph. 200mg/kg (x1)	H	0.37 ± 0.06	208.3	<0.001
		5 mos.	9	Cycloph. 200mg/kg (x2)	H	0.29 ± 0.06	141.7	<0.001
4	26	Jirds ♂	12	Control -	H	0.21 ± 0.04	-	-
		12 mos.	11	Cycloph. 200mg/kg (x1)	H	0.36 ± 0.06	71.4	<0.05
		Jirds ♀	9	Control -	H	0.19 ± 0.04	-	-
		12 mos.	16	Cycloph. 200mg/kg (x1)	H	0.05 ± 0.06	163.1	<0.01
5	50	SWR ♀	19	Control -	M	0.13 ± 0.01	-	-
			16	Cycloph. 200mg/kg (x1)	H	0.26 ± 0.02	100.0	<0.001
		3 mos.	16	Cycloph. 200mg/kg (x2)	H	0.19 ± 0.01	46.1	<0.001

Table II.

Increase in the biomass of tetrathyridial populations of M. corti in mice injected dactinomycin, lucanthone, quinacrine and demecolcine.

Exp.	Duration (days)	mice	No. mice at autopsy	Compound	Dosage	Liver inf'n.	Tetra-thyridia ml.	Increase in biomass %	P
6	55	SEC ♂ 4 mos.	9	Control	-	M	0.59 ± 0.11	-	-
			17	Dactino.	0.35mg/kg (x5)	H	1.13 ± 0.09	91.5	<0.001
			15	Lucanth.	100 mg/kg (x10)	H	1.06 ± 0.13	79.6	<0.05
		SEC ♀ 4 mos.	9	Control	-	L	0.26 ± 0.05	-	-
			12	Dactino.	0.35mg/kg (x5)	M	0.54 ± 0.13	107.6	>0.05
			14	Lucanth.	100 mg/kg (x10)	M	0.55 ± 0.06	111.5	<0.01
7	50	LDF_1 ♂ 4 mos.	25	Control	-	M	0.27 ± 0.01	-	-
			25	Dactino.	0.35mg/kg (x5)	H	0.66 ± 0.05	144.4	<0.001
			23	Lucanth.	100 mg/kg (x10)	H	0.37 ± 0.02	40.7	<0.001
		LDF_1 ♀ 4 mos.	25	Control	-	L	0.23 ± 0.02	-	-
			27	Dactino.	0.35mg/kg (x5)	M	0.34 ± 0.02	47.8	<0.001
			26	Lucanth.	100 mg/kg (x10)	M	0.30 ± 0.03	30.4	<0.02
8	50	SWR ♂ 4 mos.	22	Control	-	M	0.15 ± 0.01	-	-
			22	Quinacr.	100 mg/kg (x10)	H	0.19 ± 0.01	26.0	<0.001
9	50	LDF_1 ♂ 3 mos.	14	Control	-	M	0.46 ± 0.03	-	-
			14	Quinacr.	100 mg/kg (x10)	H	0.55 ± 0.02	19.5	<0.02
			14	Demecol.	0.5 mg/kg (x6)	H	0.56 ± 0.04	21.7	<0.05
		LDF_1 ♀ 3 mos.	14	Control	-	L	0.39 ± 0.03	-	-
			13	Quinacr.	100 mg/kg (x10)	M	0.44 ± 0.04	12.8	>0.05

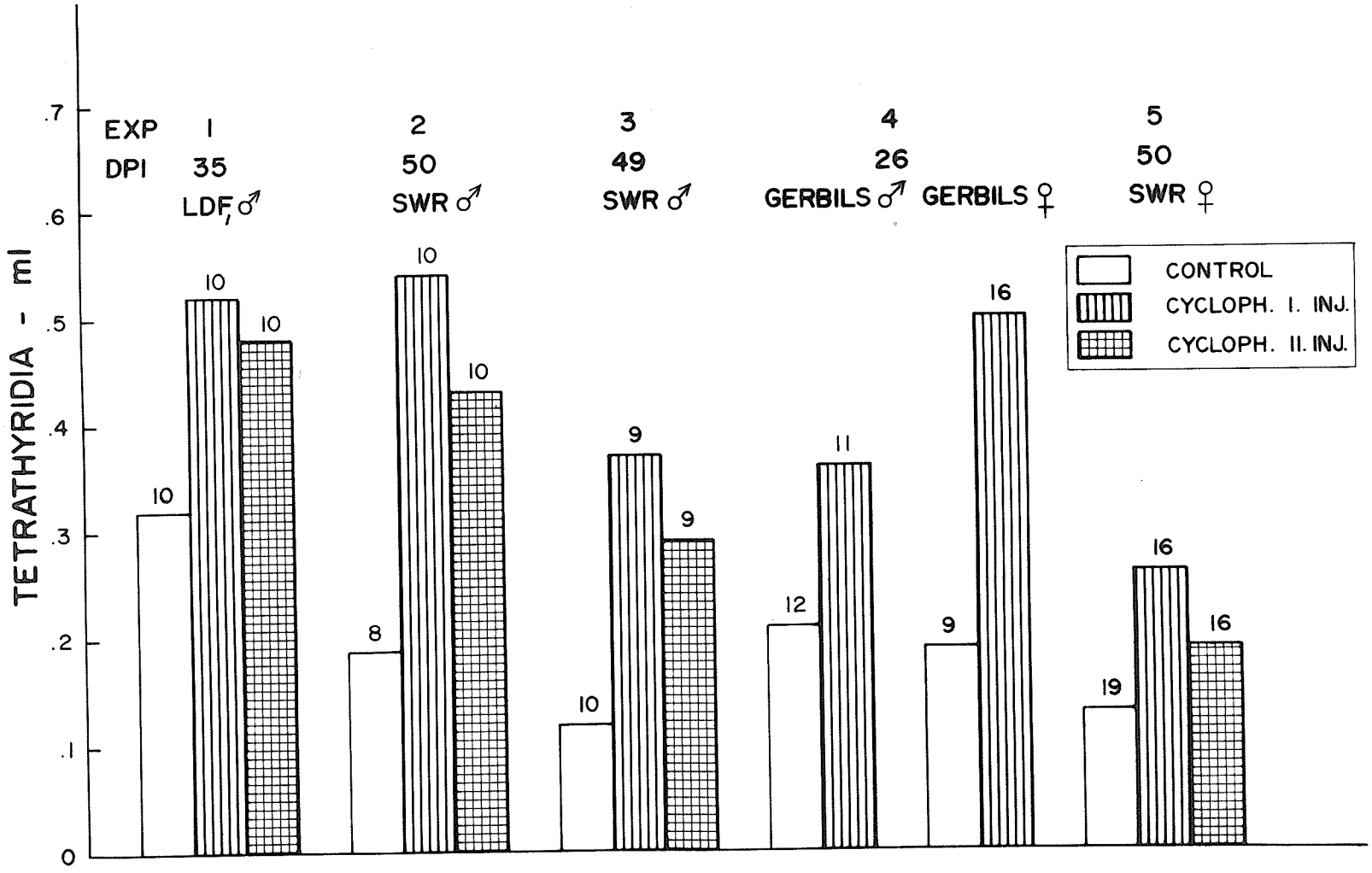

TETRATHYRIDIA - ml
.7
.6
.5
.4
.3
.2
.1
0
EXP 1 2 3 4 5
DPI 35 50 49 26 50
LDF1 ♂
SWR ♂
SWR ♂
GERBILS ♂
GERBILS ♀
SWR ♀
CONTROL
CYCLOPH. I. INJ.
CYCLOPH. II. INJ.
10
10
10
8
10
10
10
9
9
12
11
9
16
19
16
16

Fig. I

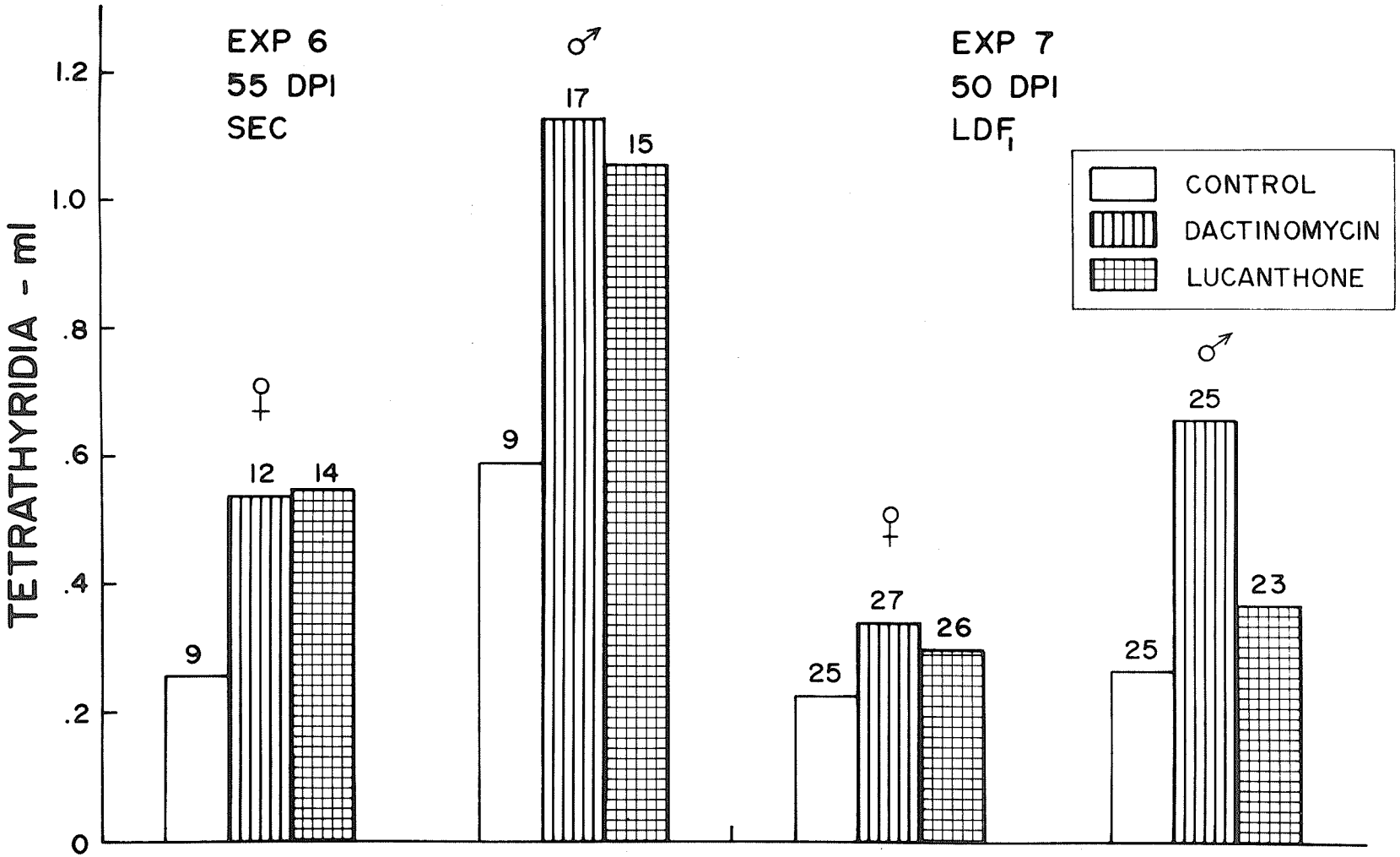

EXP 6
55 DPI
SEC
EXP 7
50 DPI
LDF1
CONTROL
DACTINOMYCIN
LUCANTHONE
TETRATHYRIDIA - ml
1.2
1.0
.8
.6
.4
.2
0
♀
♂
9
12
14
9
17
15
25
27
26
25
25
23

Fig. 2

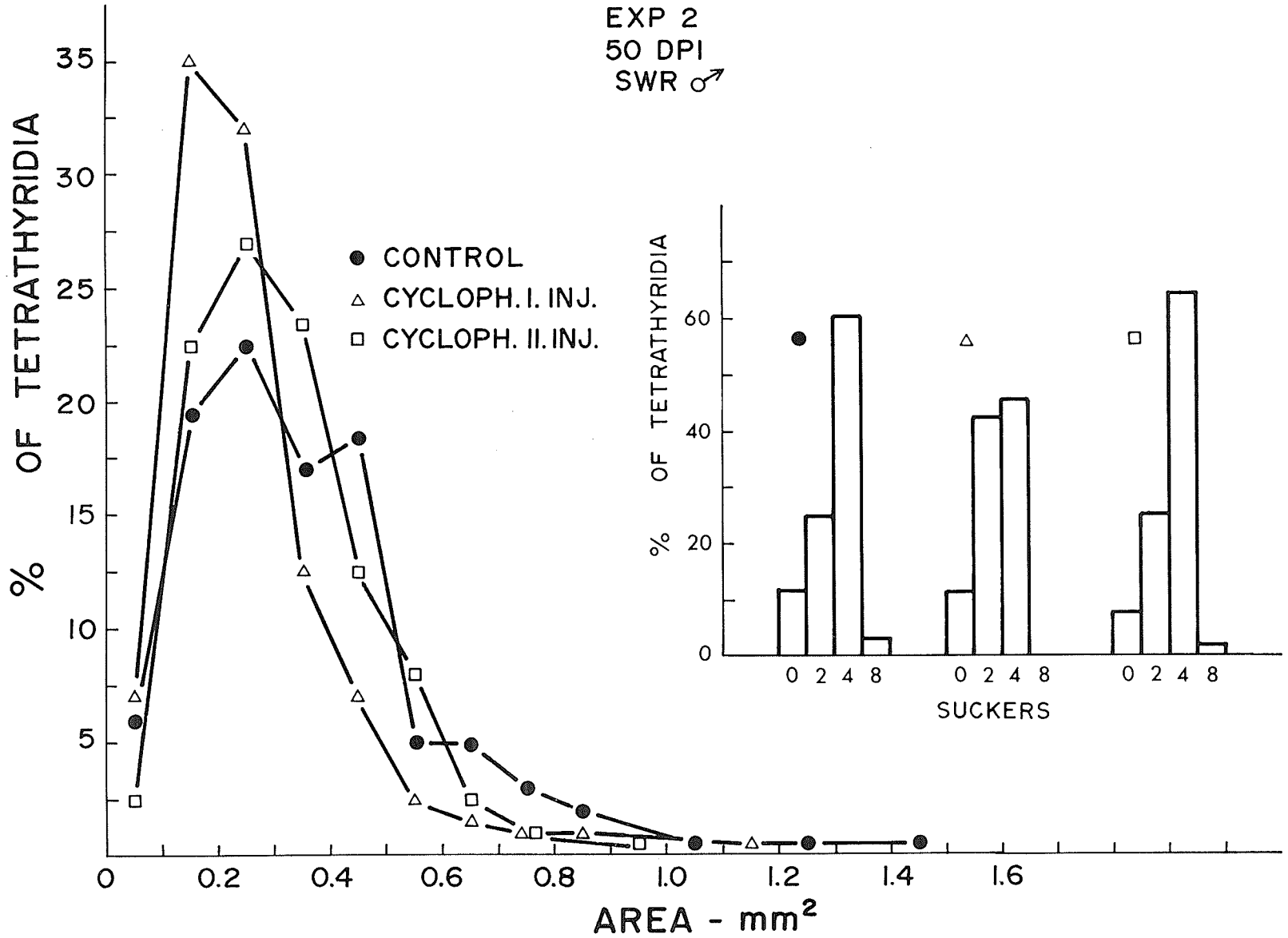

EXP 2
50 DPI
SWR ♂
CONTROL
CYCLOPH. I. INJ.
CYCLOPH. II. INJ.
% OF TETRATHYRIDIA
AREA - mm²
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
5
10
15
20
25
30
35
% OF TETRATHYRIDIA
0
20
40
60
0 2 4 8
0 2 4 8
0 2 4 8
SUCKERS

Fig.3

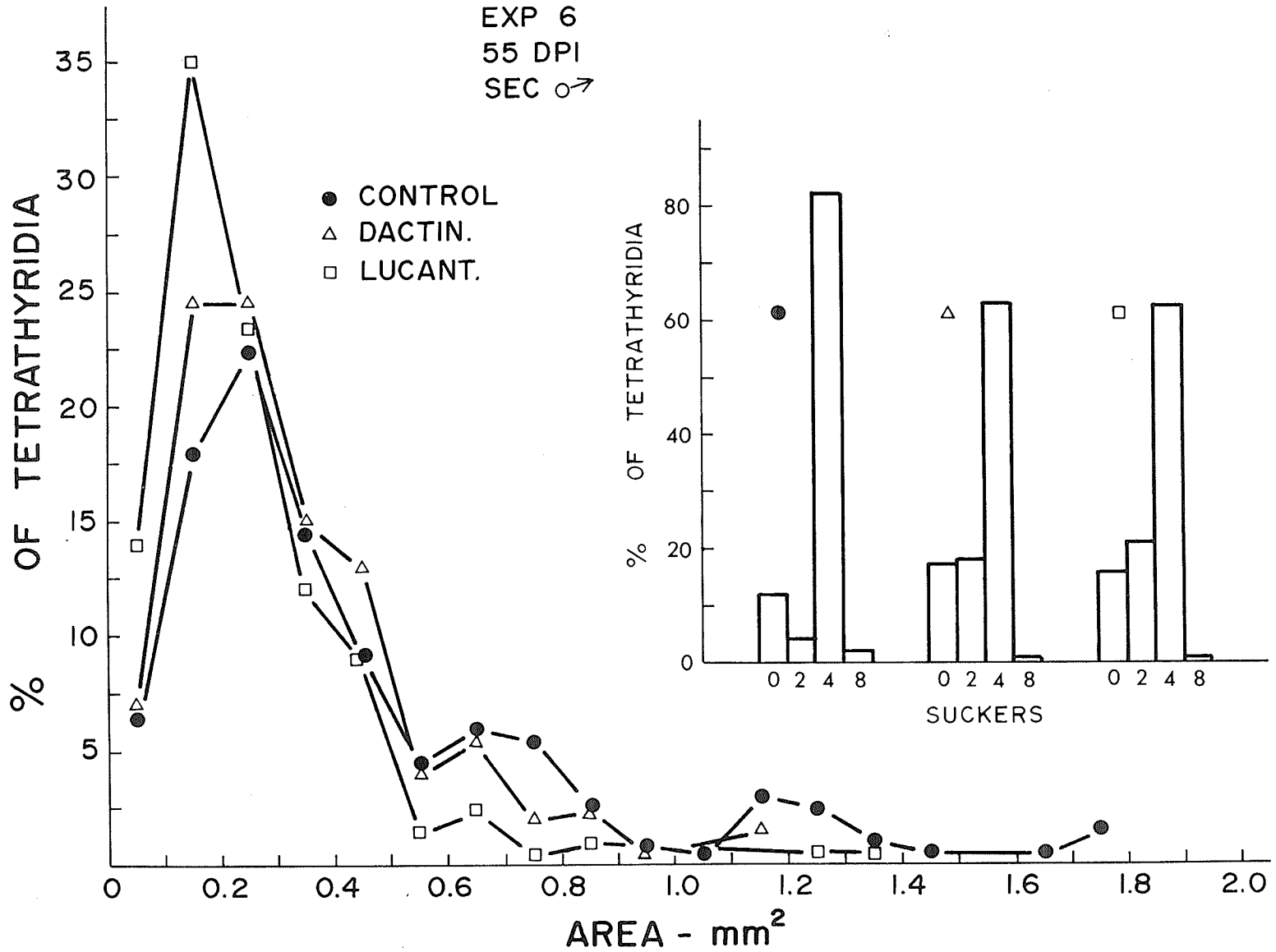
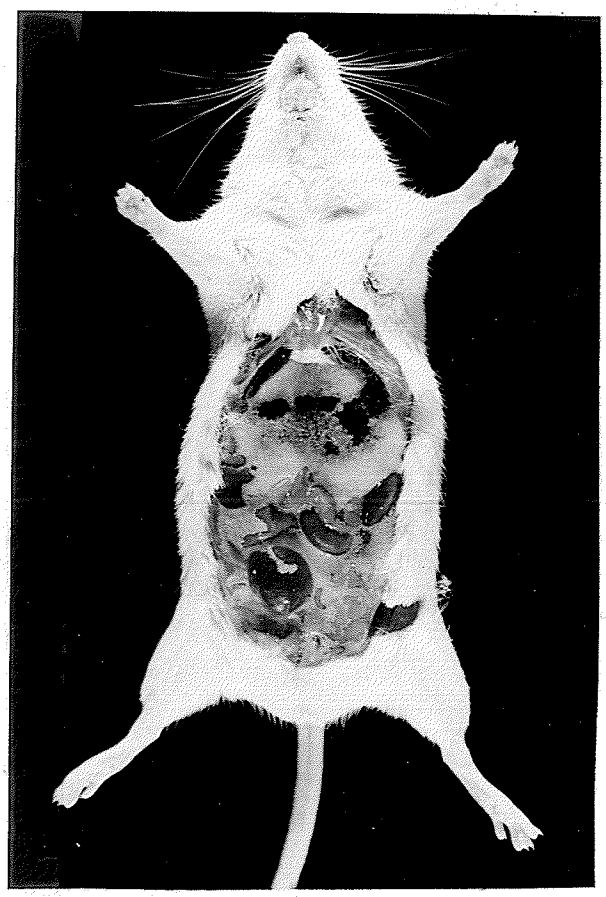

EXP 6
55 DPI
SEC ♂
CONTROL
DACTIN.
LUCANT.
% OF TETRATHYRIDIA
AREA - mm²
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
5
10
15
20
25
30
35
% OF TETRATHYRIDIA
0
20
40
60
80
0 2 4 8
0 2 4 8
0 2 4 8
SUCKERS

Fig.4

APPENDIX A. Figures

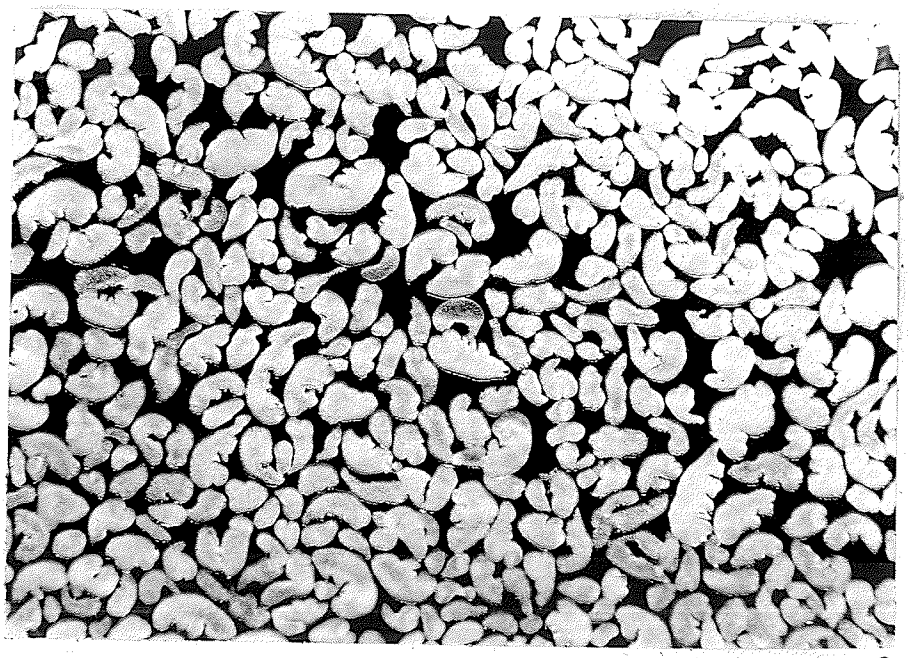
Fig. 1. SWR male infected with tetrathyridia, 170 DPI.



1

Fig. 2. Tetrathyridia from the peritoneal cavity of an SWR mouse. Fixed in 4% formaldehyde; x 10.

Fig. 3. Tetrathyridium with four suckers and an excretory opening near the posterior end of the body (arrow). Lactic acid. Phase contrast; x 90.



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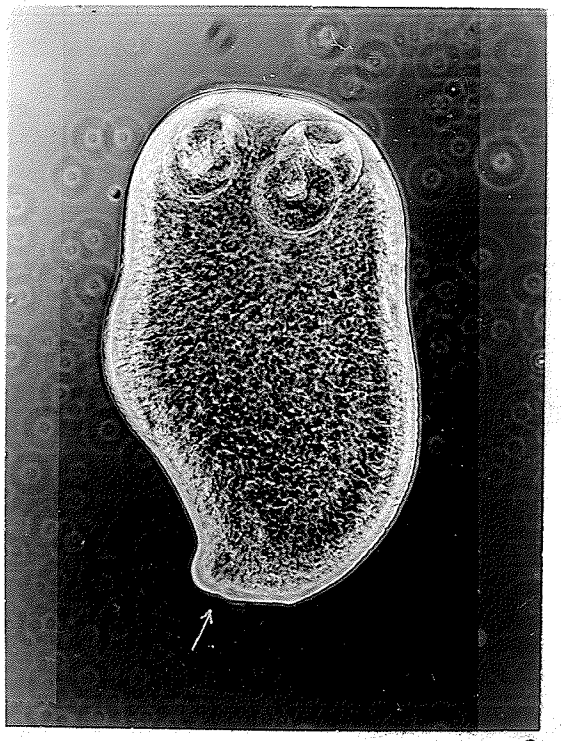


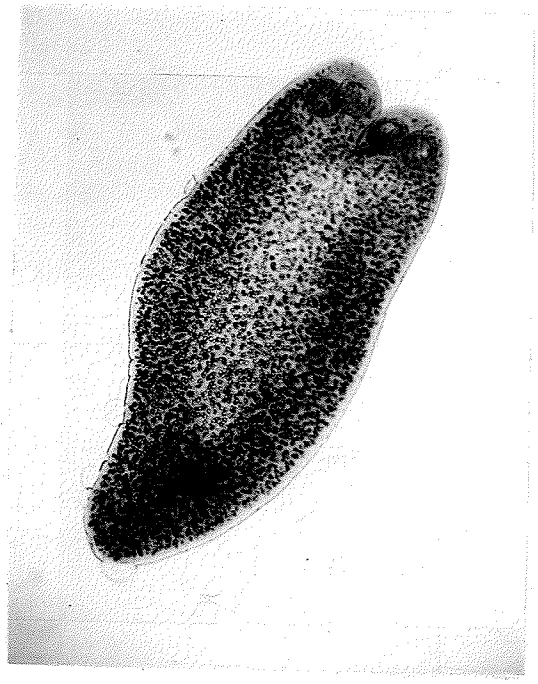
3

Fig. 4. Early splitting, before the developmant of "accesory suckers". From SWR mouse. Saline; x 40.

Fig. 5. Advanced stage of splitting into 2 two-suckered tetrathyridia. Saline; x 40.

Fig. 6. Late splitting into two very long individuals, each with 4 fully developed suckers. From the peritoneal cavity of a muskrat, 106 DPI. Fixed in 4% formaldehyde; x 30.

Fig. 7. Very late stage of longitudinal splitting of a tetrathyridium from the abdominal cavity of an SWR mouse, 50 DPI. Live specimen in saline; x 40.



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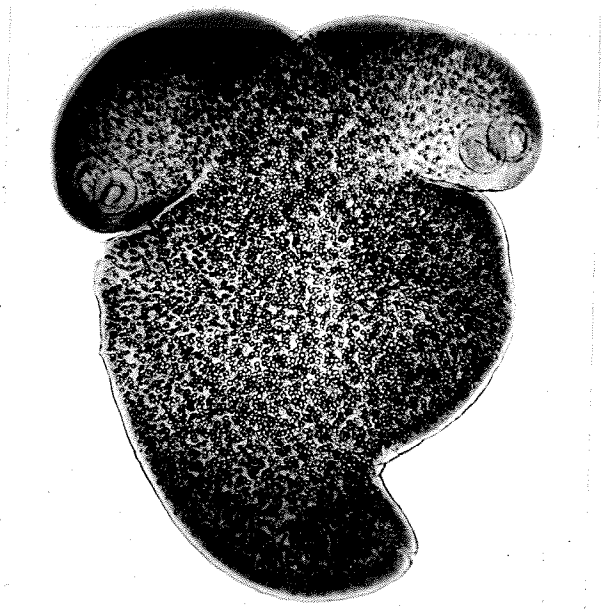


5

6

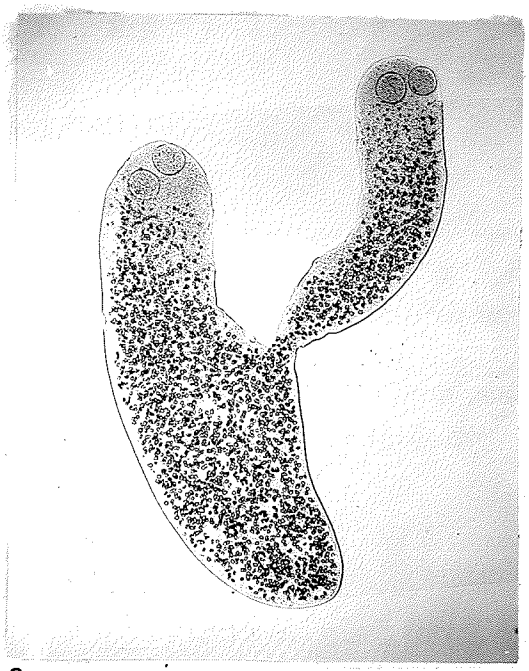
7

Fig. 8. Constriction of the peduncle following a separation of a new individual, in this case with two parental suckers only. Fixed in 4% formaldehyde; x 35.

Fig. 9. A blob of parenchyma at the posterior pole of a larva with only 2 suckers, soon after separation. Lactic acid. Phase contrast; x 90.

Fig. 10. The fifth daughter tetrathyridium in the process of separation from the proliferative side of the parental organism, which already lost the parental scolex. Four per cent formaldehyde; x 25.

Fig. 11. Close-up of the constriction area; x 100.



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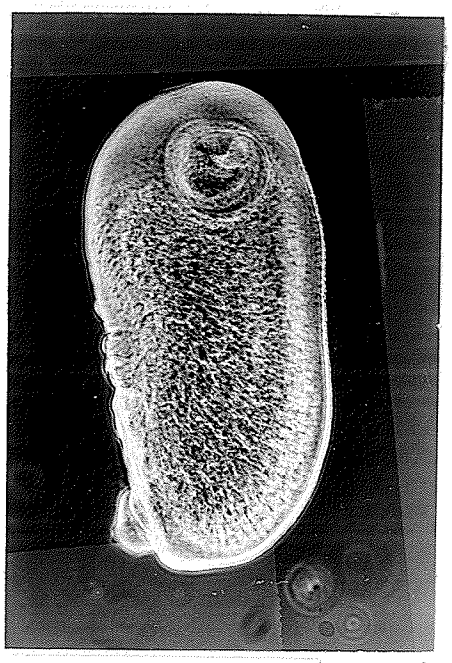

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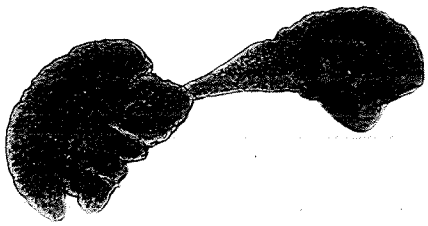

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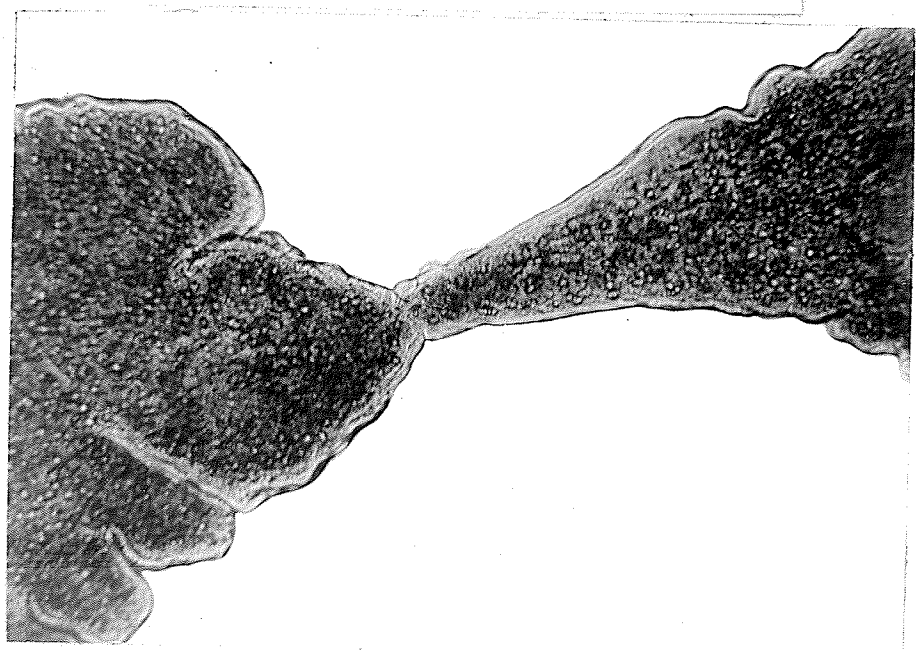
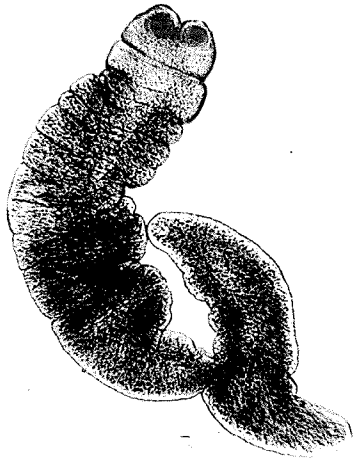


11

Figs 12 - 13. Constriction near the middle of the body, resulting in the separation of the posterior acephalic portion. Fixed in 4% formaldehyde; x 25.

Fig. 14. Close-up of Fig.12; the region of constriction. Lactic acid. Phase contrast; x 100.

12



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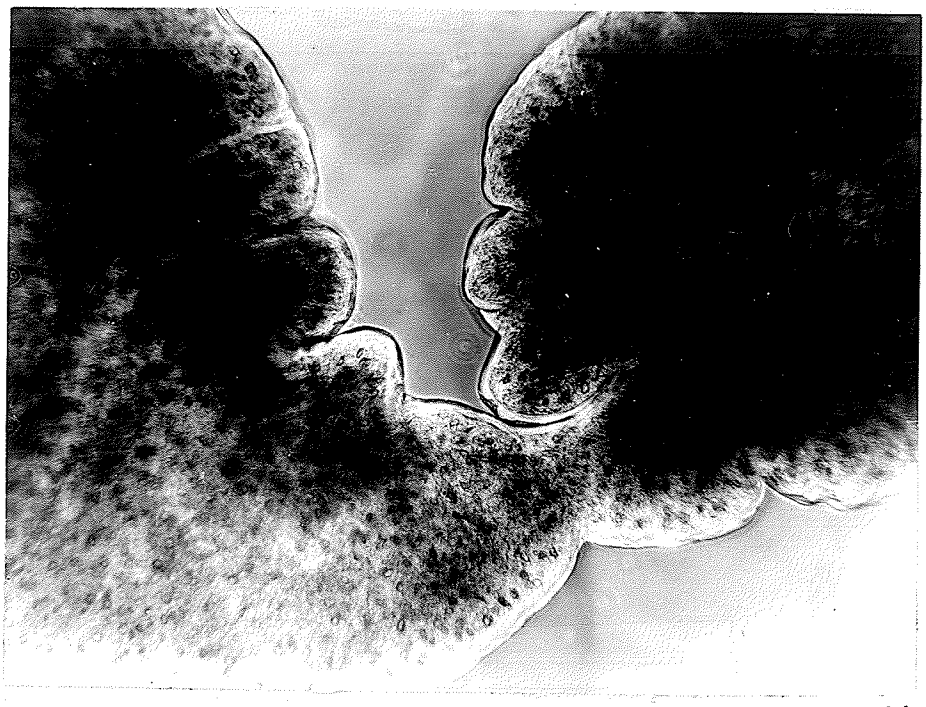
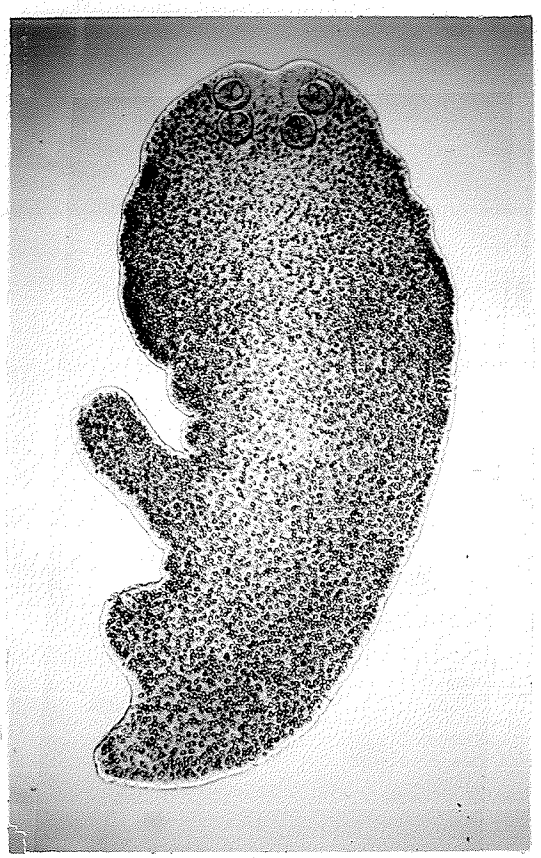


14

Fig. 15. Growth of two a finger-like lateral stumps. Saline; x 40.

Fig. 16. Incipient separation of a small posterior portion of the body. Lactic acid. Phase contrast; x 100.

Figs 17 - 18. Lateral remnants (stumps) with excretory ducts and bladders. Saline with neutral red; x 95.



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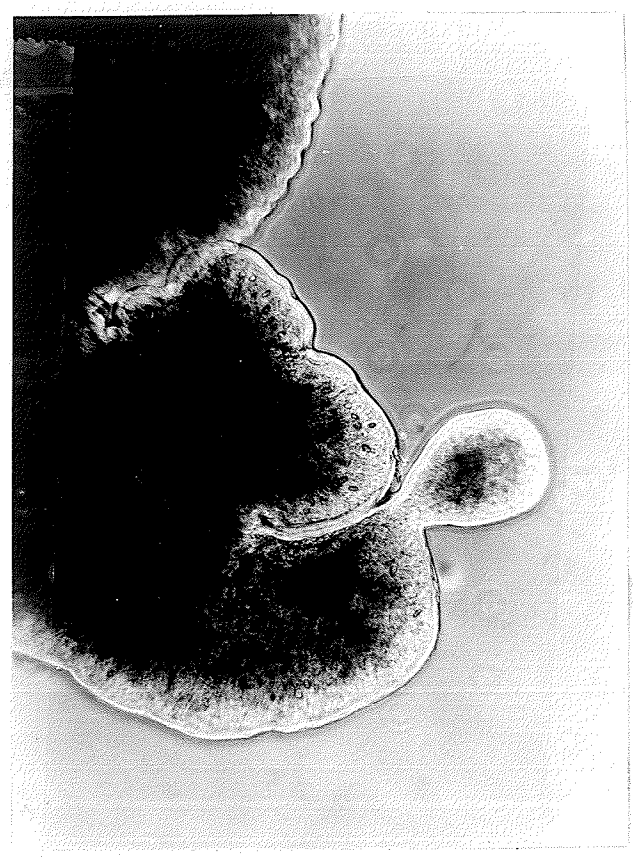

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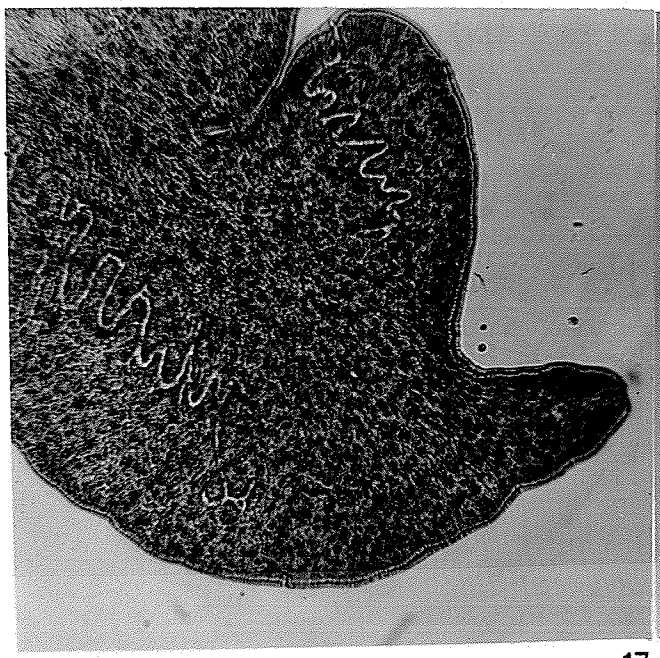

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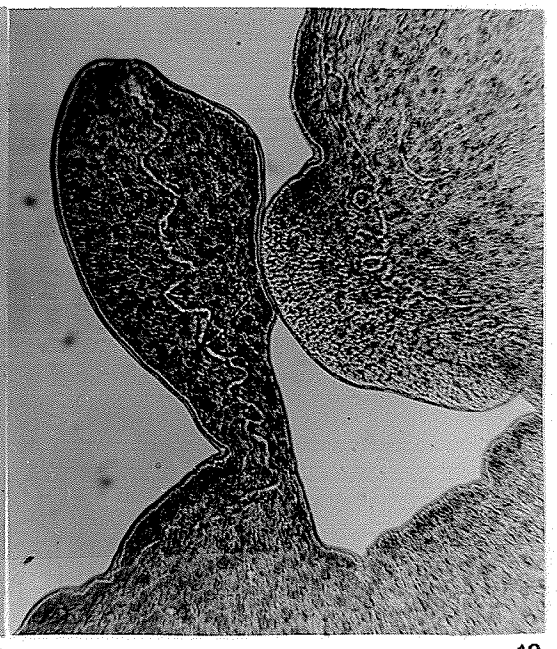
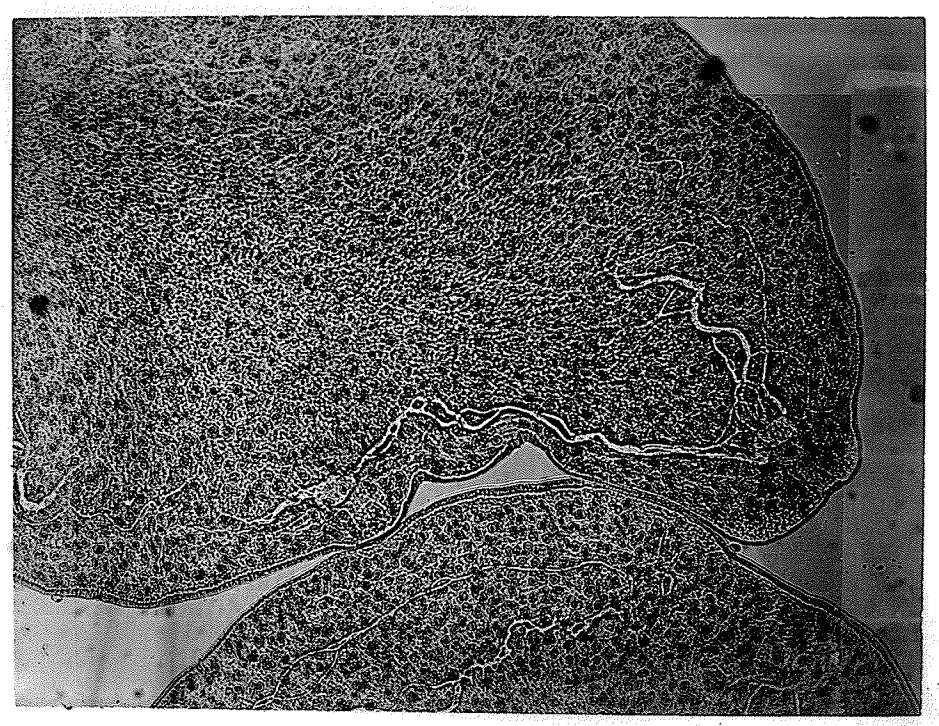


18

Fig. 19. Posterior end of a larva with 2 excretory bladders with their plexuses. On the right - the terminal bladder plexus, on the left - the lateral bladder plexus, which developed as result of separation of a new individual. The bladders are interconnected by a pair of excretory ducts. Saline with neutral red; x 90.

Fig. 20. Two lateral bladders connected by ducts. Saline with neutral red; x 90.



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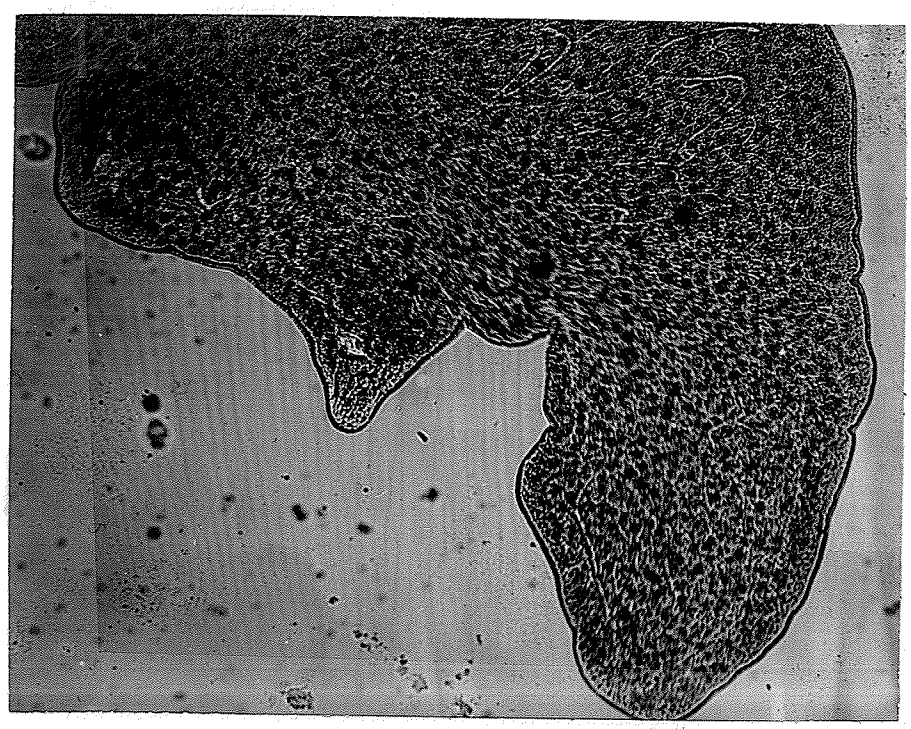


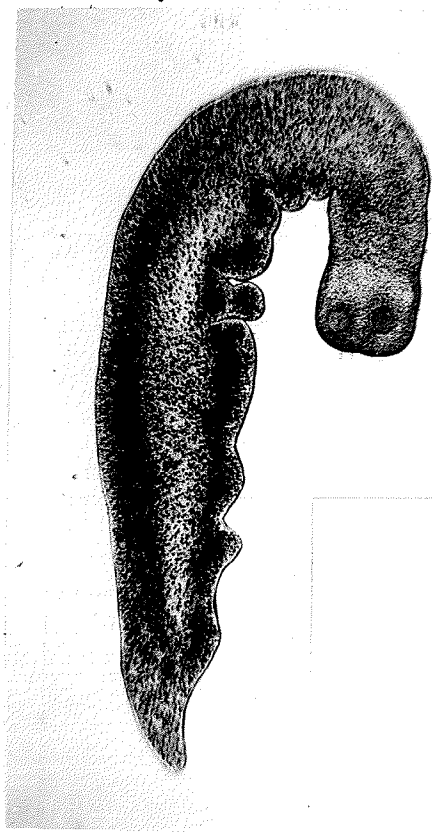
20

Figs 21 - 23. Alongated larvae from the abdominal cavity of a muskrat, 50 DPI. Fixed in 4% formaldehyde; x 20.

21. Larva with one lateral stump.

22 - 23. Larvae with many lateral stumps.

21



22

23

Figs 24 - 25. Completely separated lateral stumps ("buds").

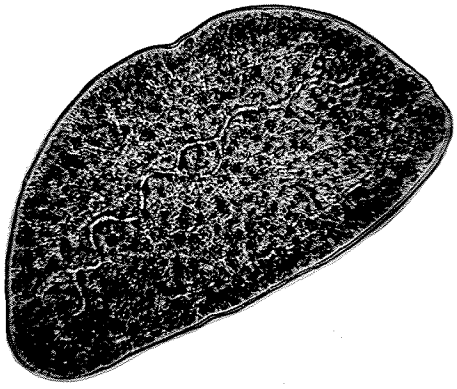
24. Saline with neutral red; x 90.

25. Fixed in 4% formaldehyde; x 40.

Figs 26 - 27. Acephalic fragments (tail remnants), after complete separation.

26. Fixed in 4% formaldehyde. Phase contrast; x 120.

27. Saline with neutral red; x 90.



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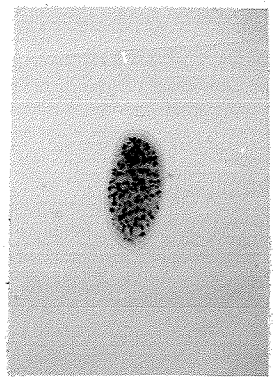

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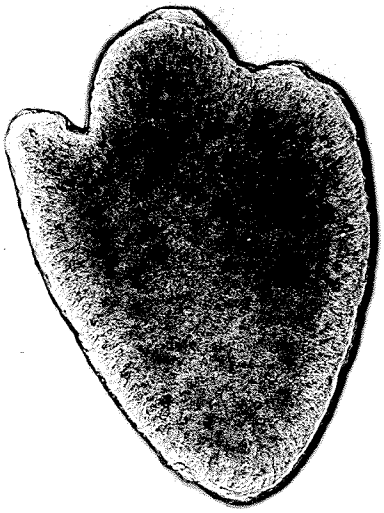

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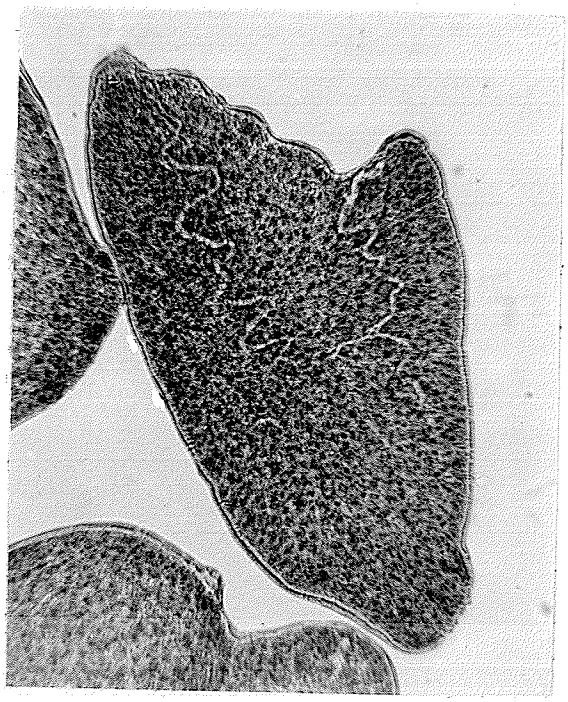


27

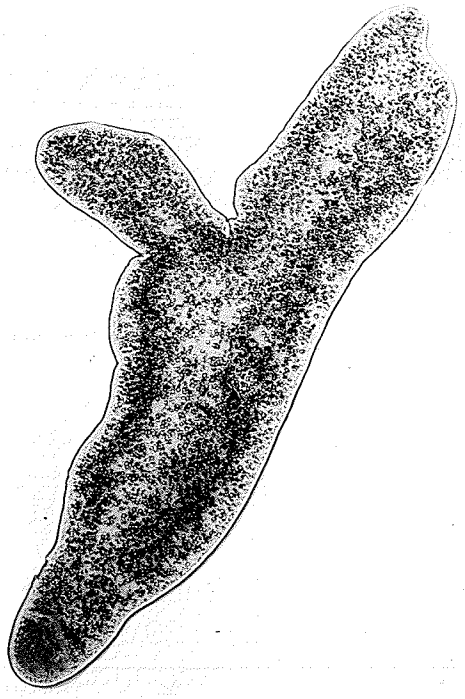
Figs 28 - 30. Acephalic fragments.

28. Fixed in 4% formaldehyde; x 30.

29. Fixed in 4% formaldehyde; x 35.

30. From a muskrat, 46 DPI. Two scolices were probably pinched off from the anterior portion of this tail remnant. Fixed in 4% formaldehyde; x 25.

28



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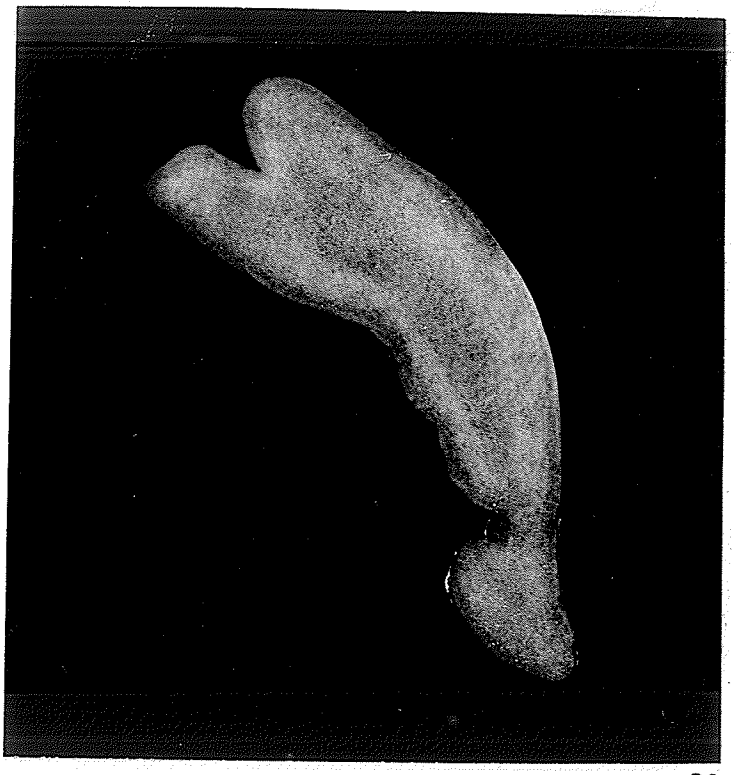
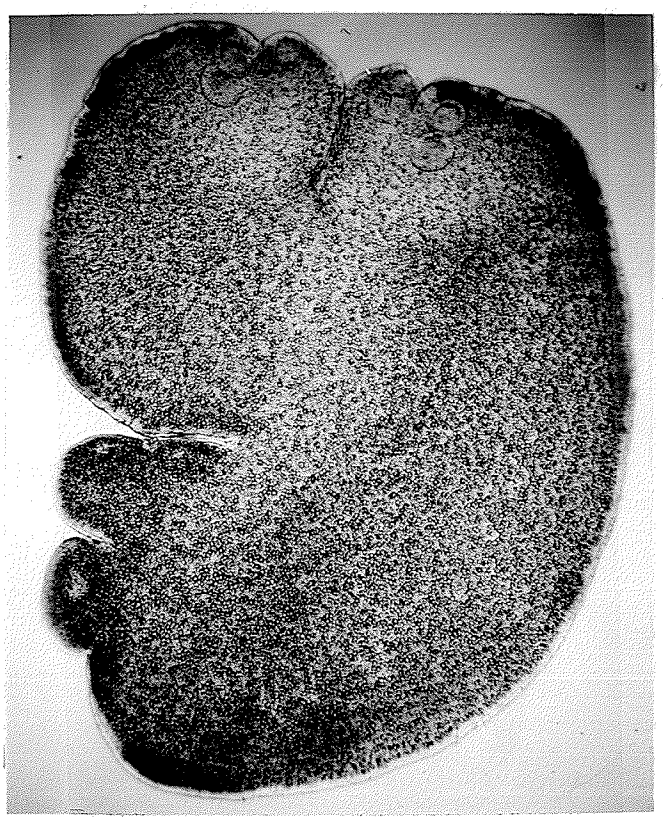


30

Figs 31 - 33. Tetrathyridia with two, three and four scolices and four suckers on each scolex. Saline; x 45.

Fig. 34. Polycephalic tetrathyridium from an SWR female, 200 DPI. Fixed in 4% formaldehyde; x 10.



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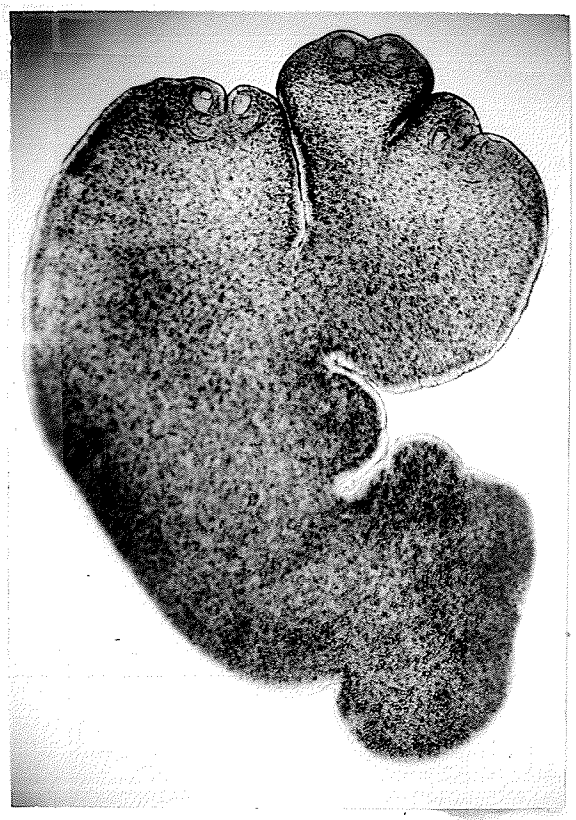

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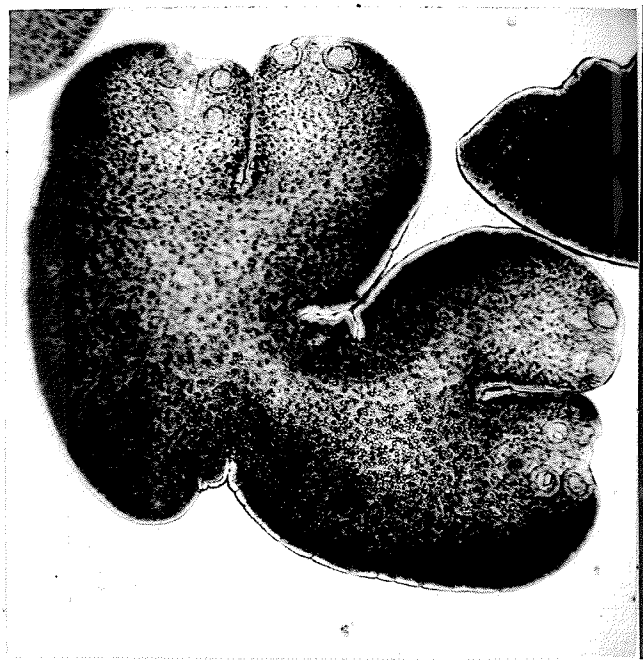

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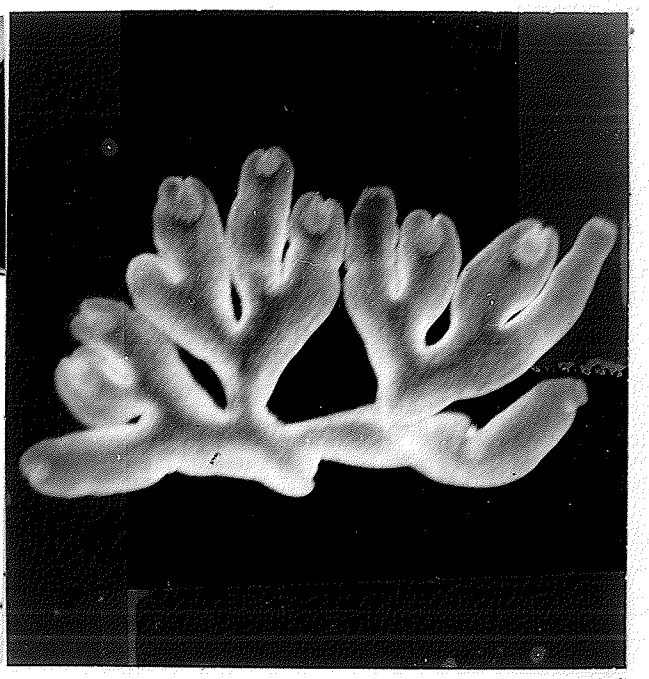
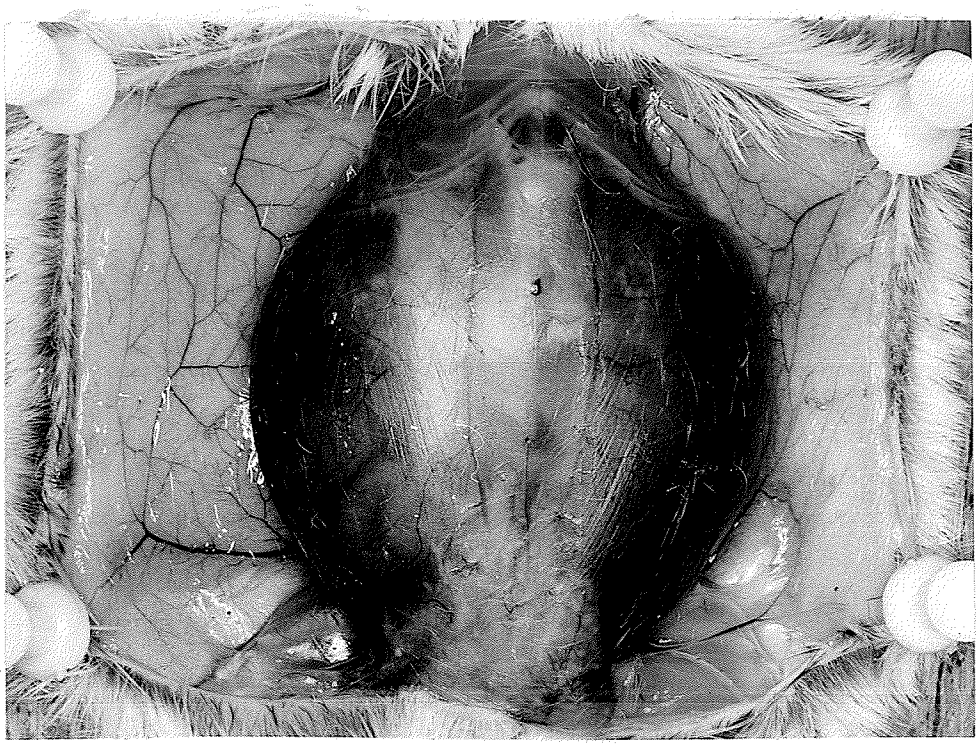


34

Fig. 35. Female jird, 26 DPI, with ascites. This animal had 9 cc of ascitic exudate.

Fig. 36. Close-up of viscera of the same jird. The infection of lungs and a very heavy infection of the liver with the development of scar tissue is apparent. Note the enlarged spleen and two large clusters of larvae under the peritoneum (arrows).



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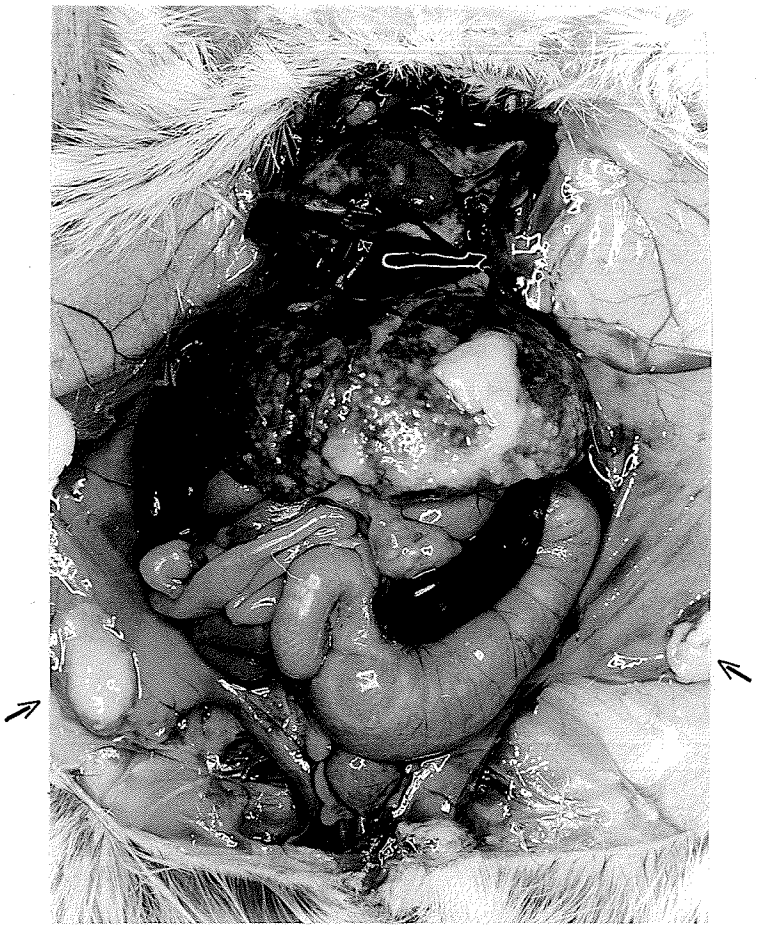


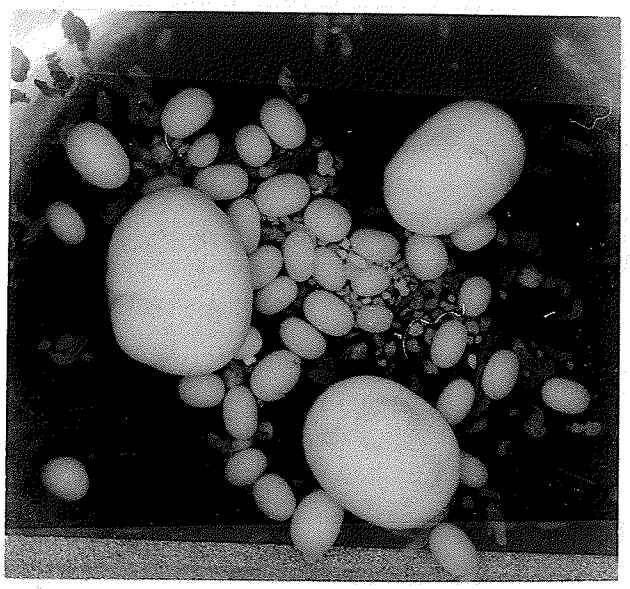
36

Fig. 37. Ellipsoidal clusters of dead and partially calcified larvae from the peritoneal cavity of an SWR female, 205 DPI. Among them are live tetrathyridia. Saline; x 3.

Fig. 38. Liver of a male ground squirrel Citellus franklini, infected intraperitoneally with 0.1 cc of tetrathyridia, 55 DPI; x 1.5

Fig. 37. Ellipsoidal clusters of dead and partially calcified larvae from the peritoneal cavity of an SWR female, 205 DPI. Among them are live tetrathyridia. Saline; x 3.

Fig. 38. Liver of a male ground squirrel Citellus franklini, infected intraperitoneally with 0.1 cc of tetrathyridia, 55 DPI; x 1.5



37

38

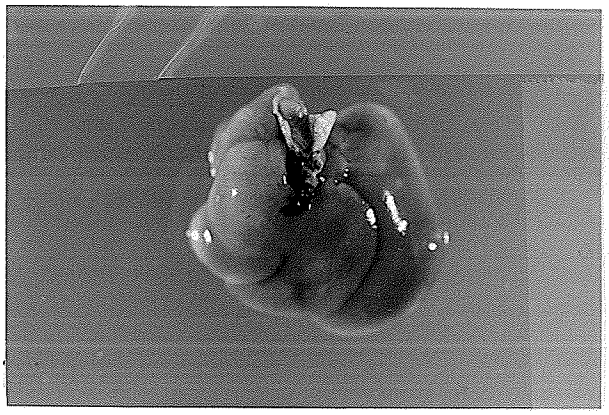
Figs 39 - 42. Infection of the liver in the experimental SWR mice.

39. Normal liver

40. Light infection (L)

41. Moderate infection (M)

42. Heavy infection (H)



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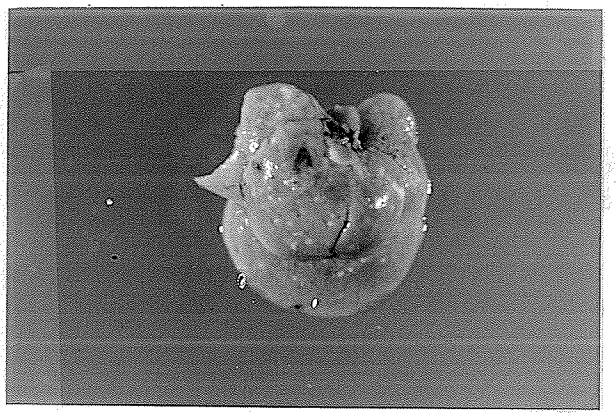

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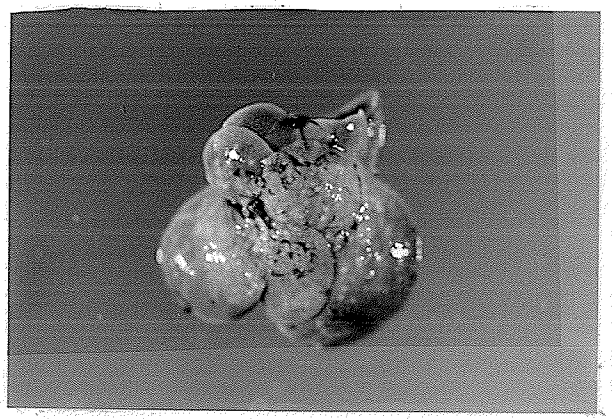

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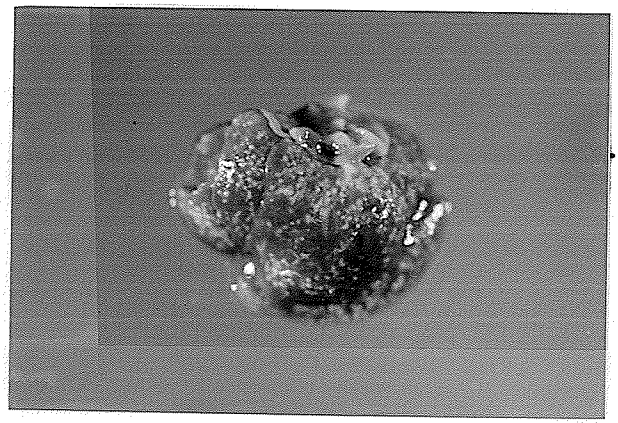
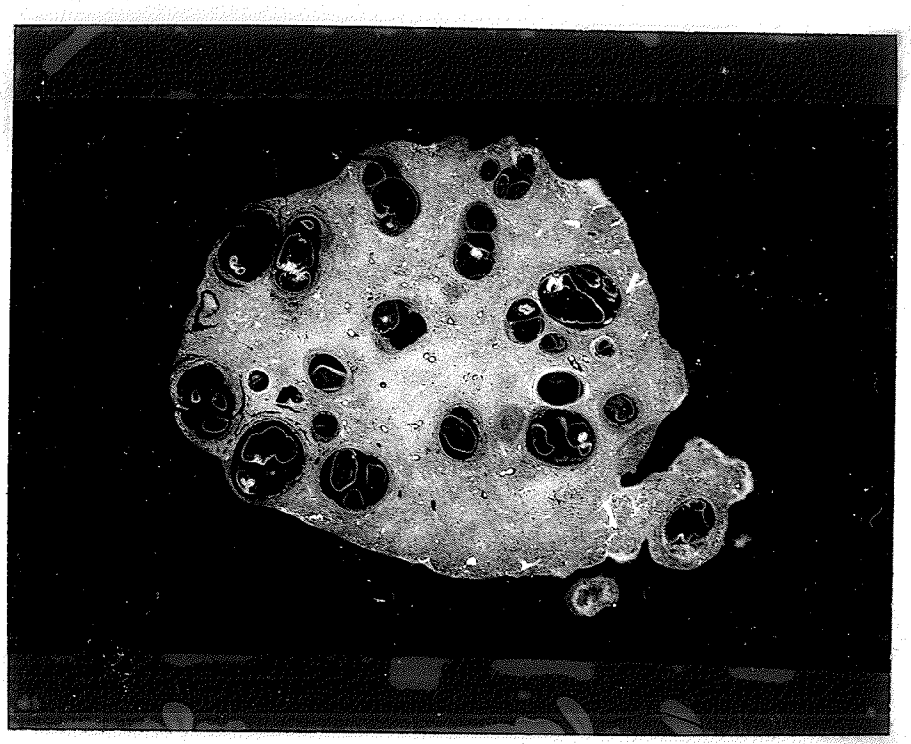


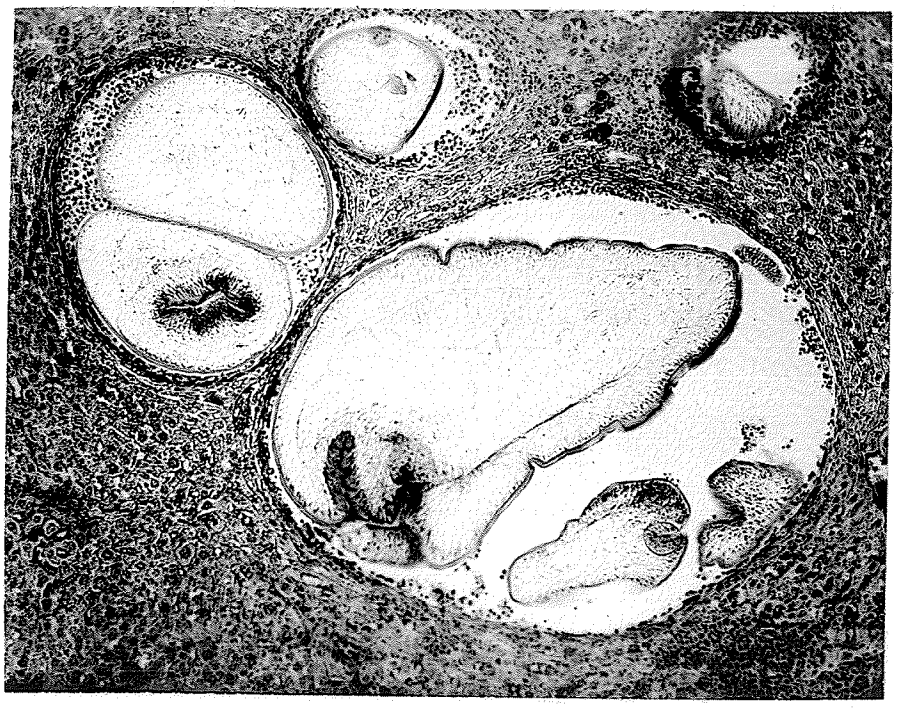
42

Fig. 43. Thick section of a liver lobe infected with tetrathyridia. Mallory Triple stain. Paper negative; x 12.

Fig. 44. Section of the liver of an SWR mouse, 50 DPI. Tetrathyridia are encysted in the liver tissue. Mallory Triple stain; x 85.



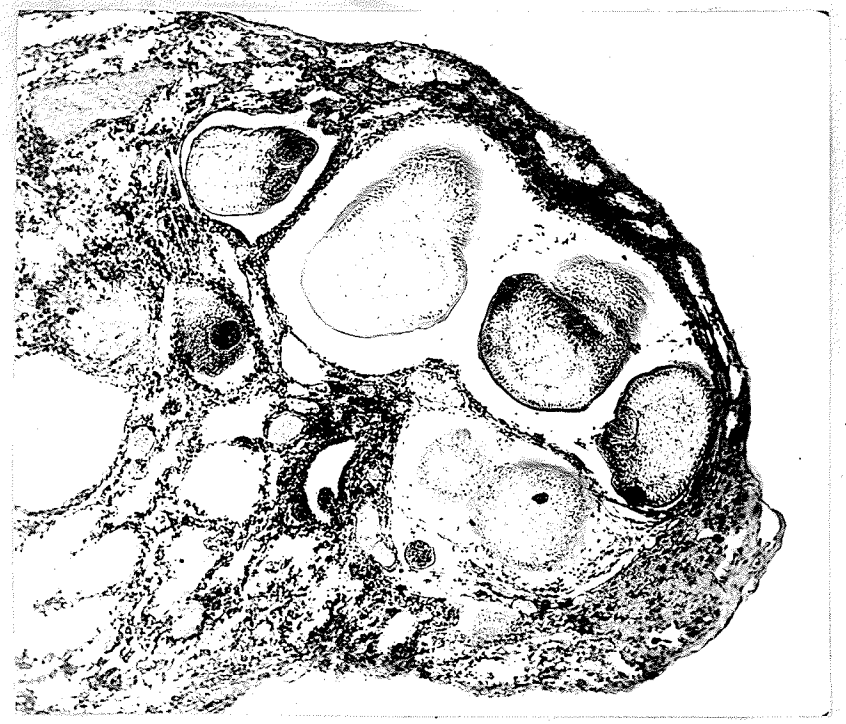
43



44

Fig. 45. Lung of a cotton rat infected with tetrathyridia, 162 DPI. H.E.; x 30.

Fig. 46. Another part of the same section. Note the two tetrathyridia; x 200.



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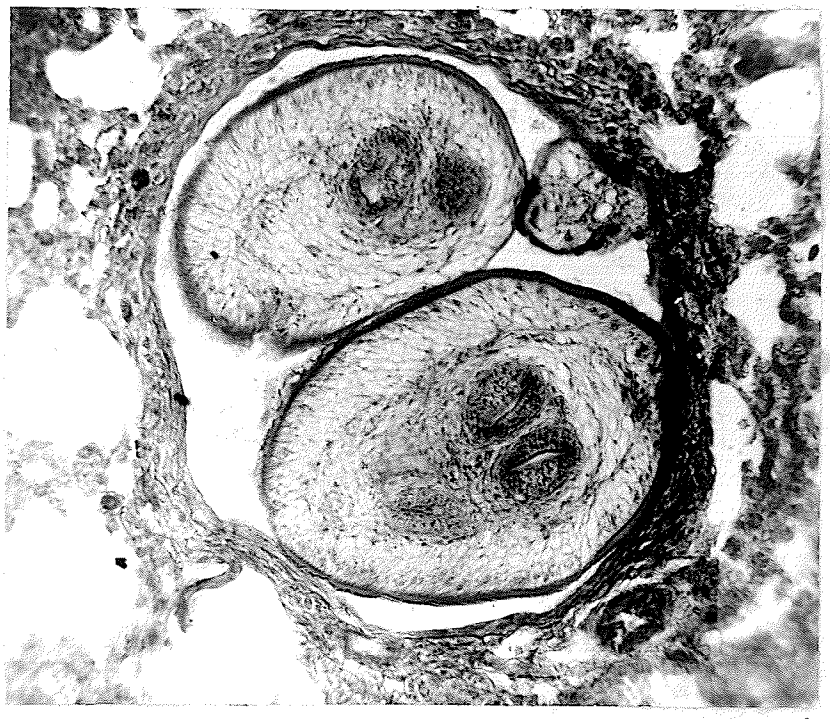
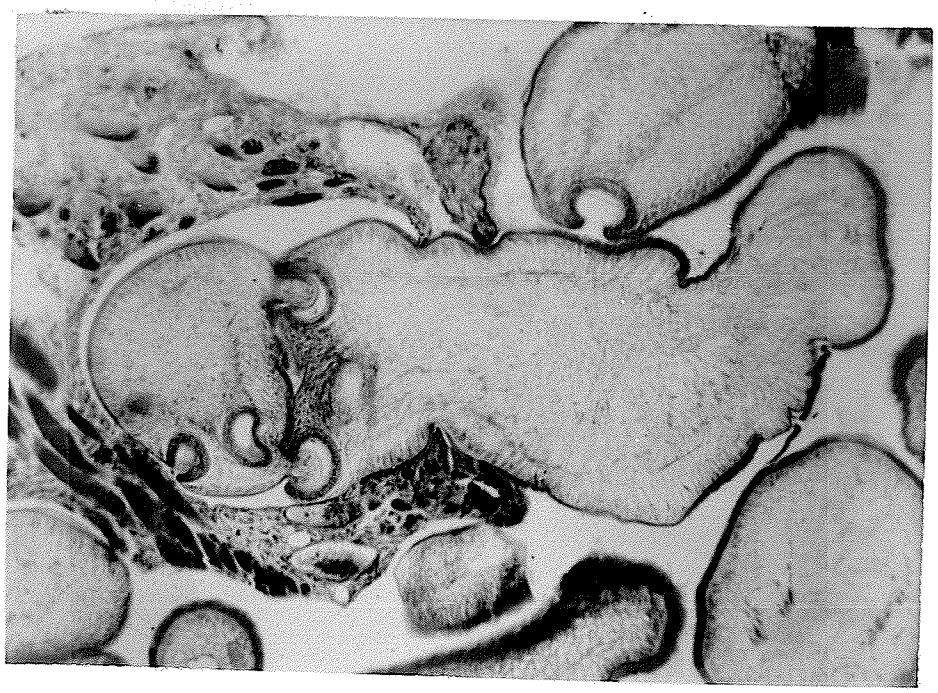


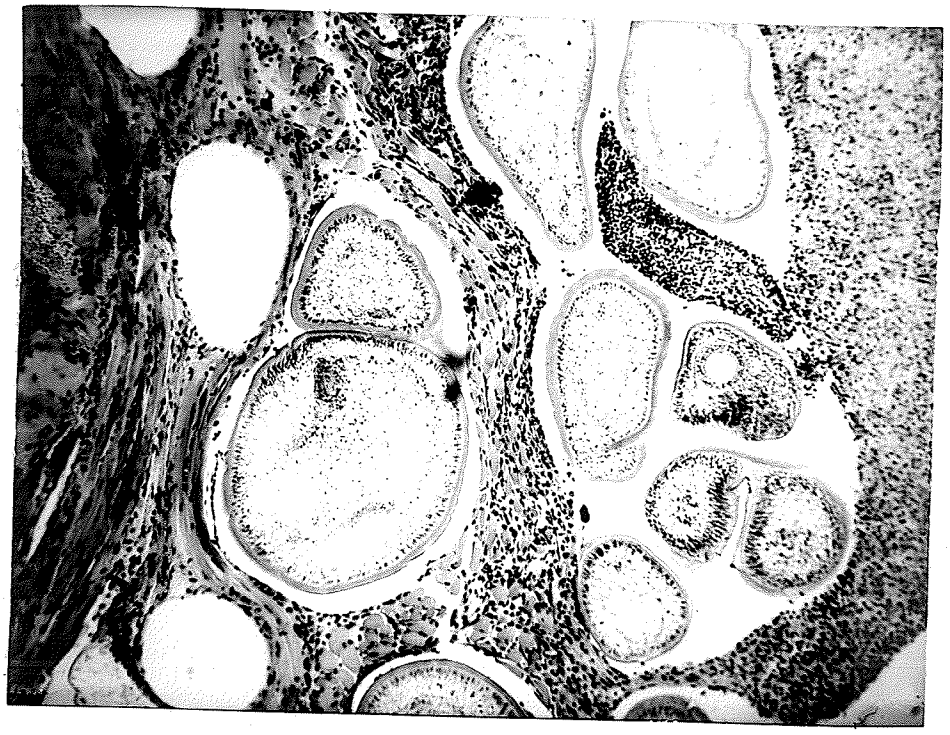
46

Fig. 47. Ventral abdominal wall of an LDF_1 female, infected intraperitoneally with tetrathyridia, 198 DPI. A large larva is attached by two of its suckers to another larva. Mallory Triple stain; x 95.

Fig. 48. Thickened and heavily infected peritoneum of an SWR mouse, 50 DPI. H.E.; x 95.



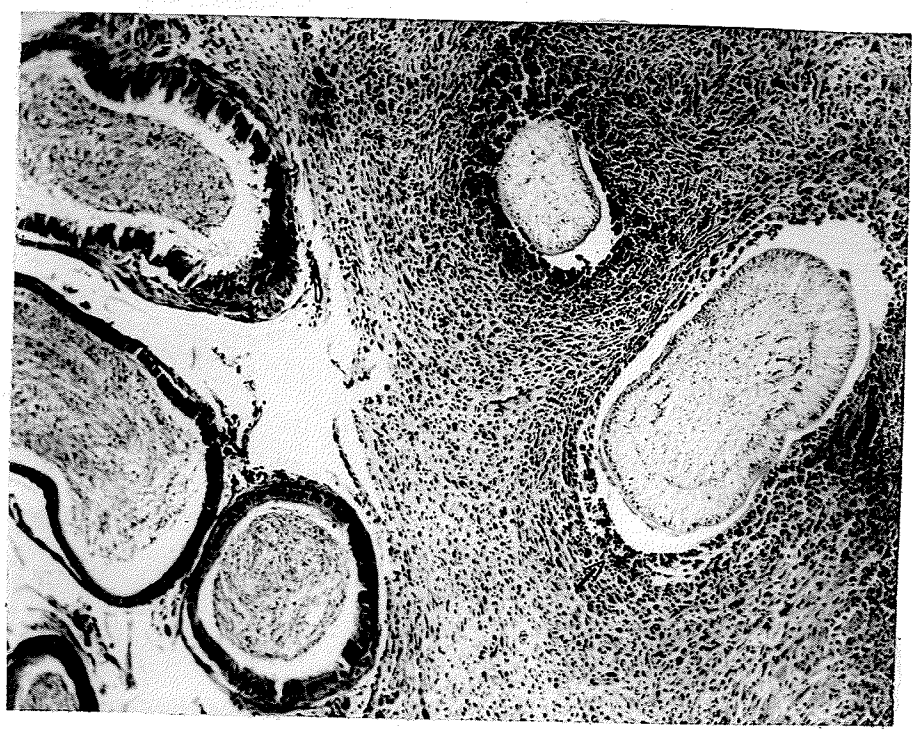
47



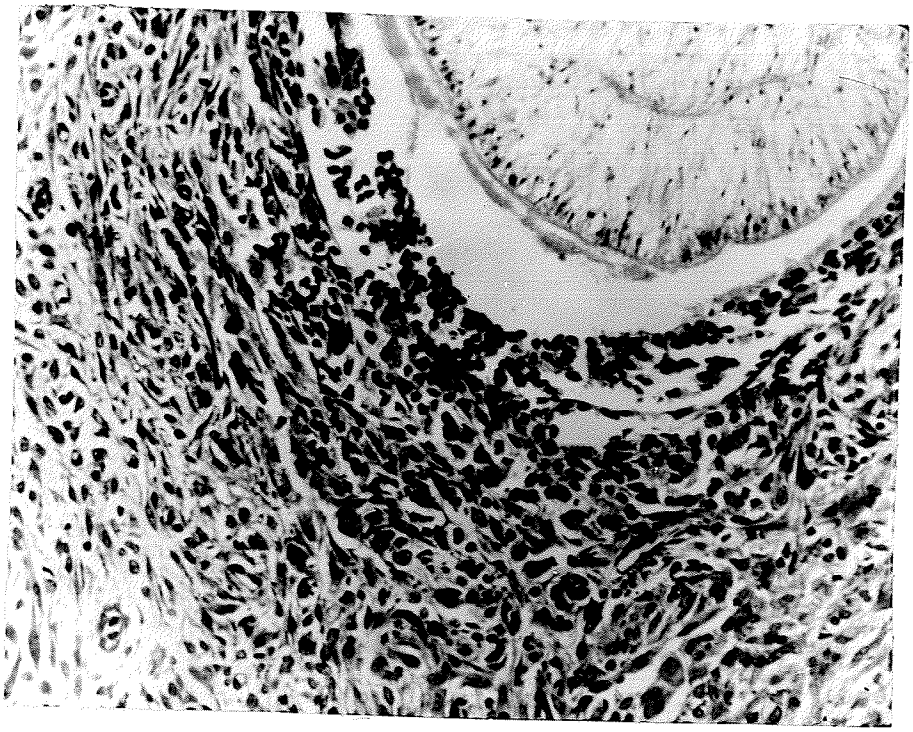
48

Fig. 49. Upper part of a testis with collecting epididymal ducts. Note the thickenning of the tunica albuginea as a result of inflammation caused by tetrathyridia. SWR mouse, 50 DPI. H.E.; x 95.

Fig. 50. A portion of the previous section marked by an arrow. Note the heavy accumulation of leucocytes around the parasite. H.E.; x 280.



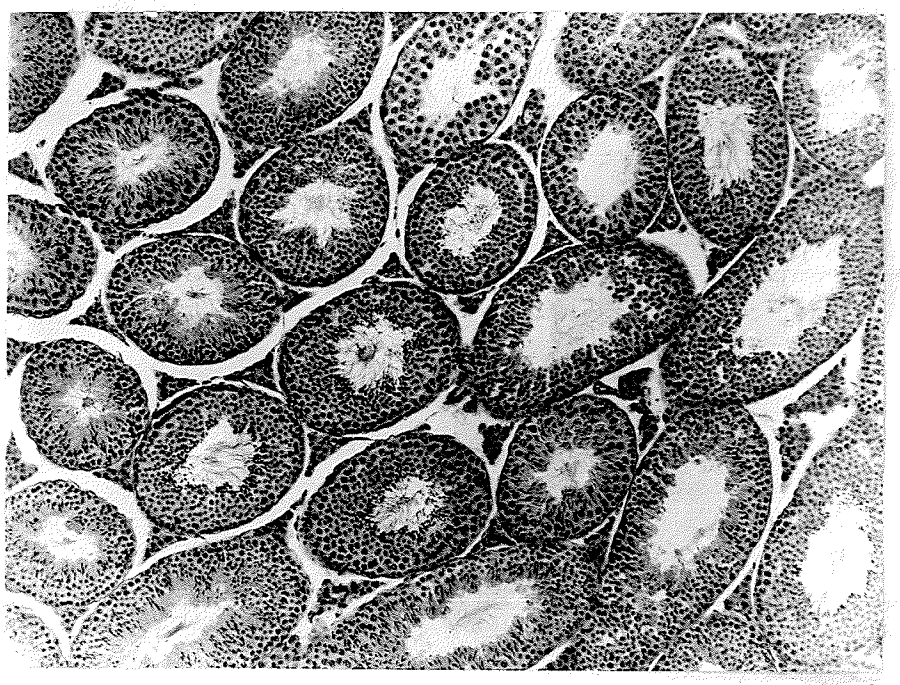
49



50

Fig. 51. Seminiferous tubules of a normal SWR male, 50 DPI. The tubules are filled with numerous spermatozoa - normal spermatogenesis. H.E.; x 100.

Fig. 52. One seminiferous tubule from Fig.51; x 400.

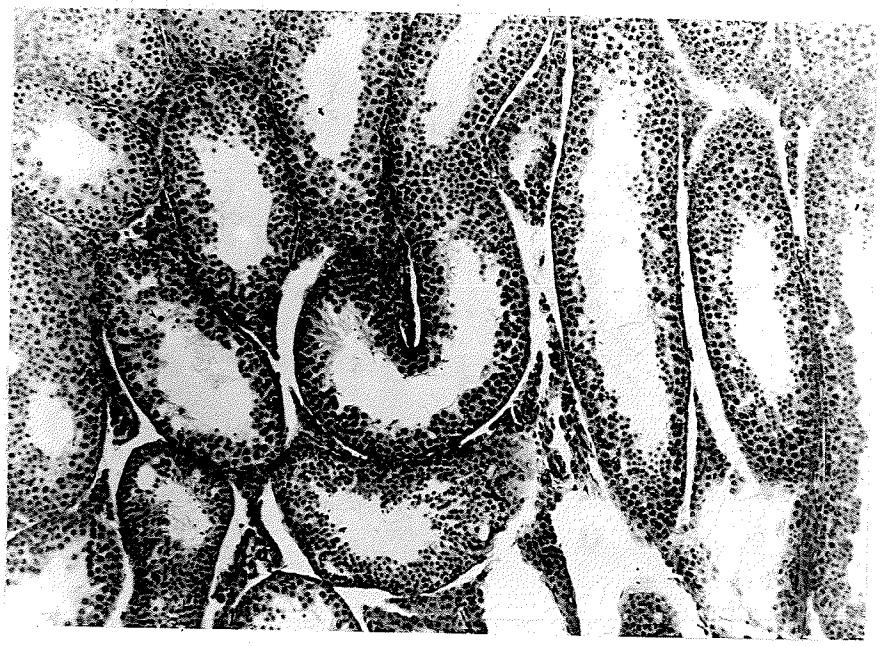


51

52

Fig. 53. Seminiferous tubules of an SWR mouse, 50 DPI, which has received 2 injections of cyclophosphamide, 200 mg/kg. Spermatogenesis inhibited by the cytostatic drug. H.E.; x 100.

Fig. 54. A seminiferous tubule from the previous section; x 400.



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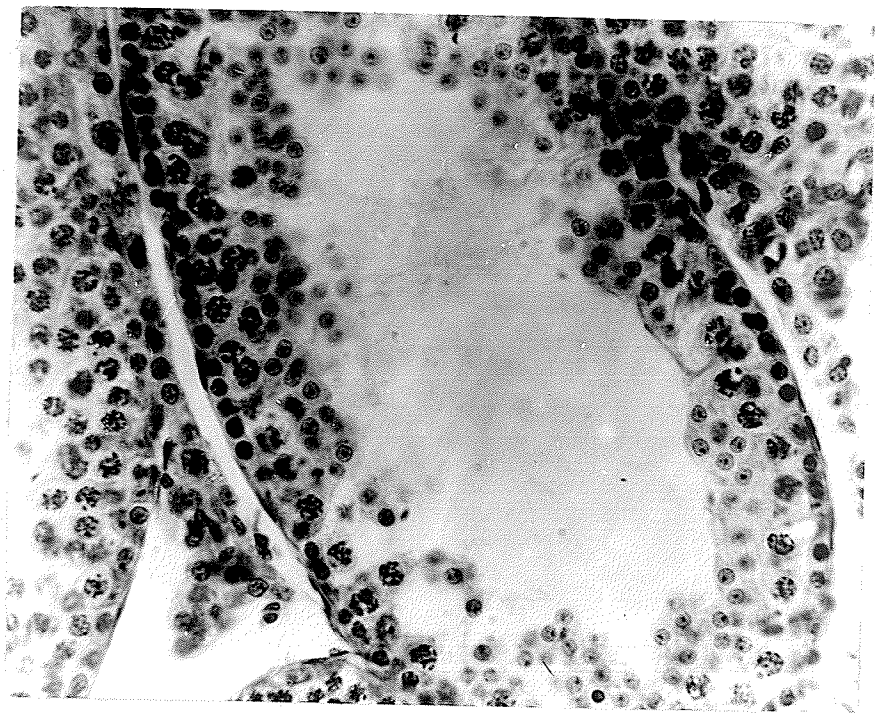
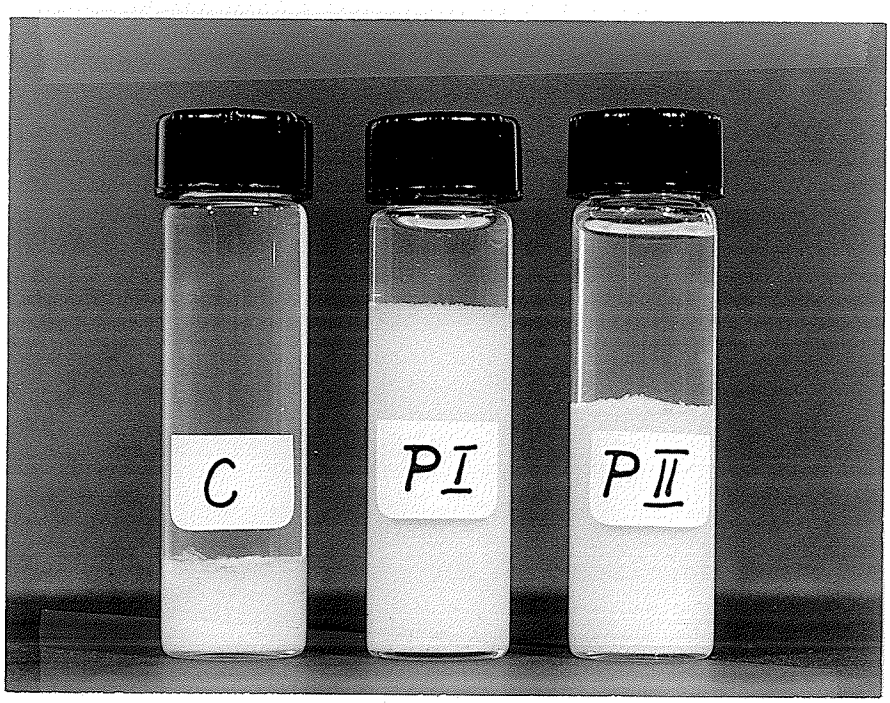


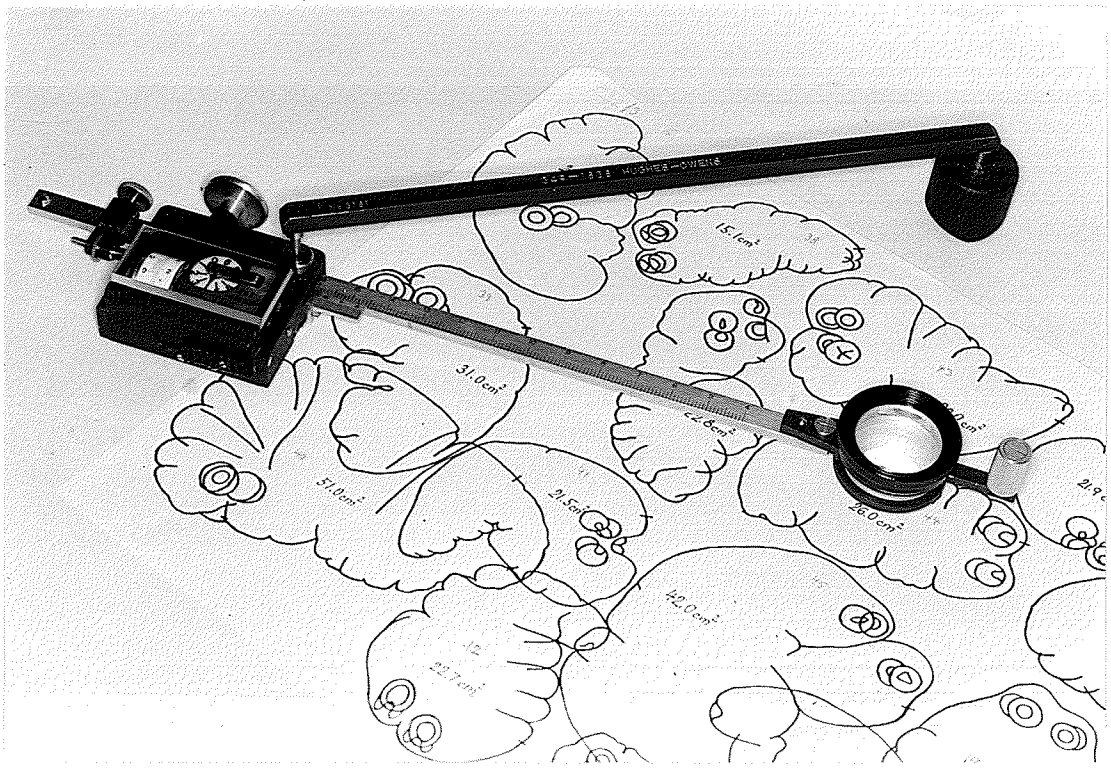
54

Fig. 55. Tetrathyridia collected from the peritoneal cavities of SWR mice controls (C), and those injected by cyclophosphamide once (PI) and twice (PII); experiment 2.

Fig. 56. Using polar compensating planimeter for measurements of the areas of the larvae.



55



56

Fig. 57. Probable life cycle of Mesocestoides spp.

1-4 Development in the definitive host: 1. Constriction of tetrathyridium at the neck region. 2. Discarded portion of the body. 3. Scolex. 4. Strobilation of the neck region and production of adult worm. 5-11. In vitro development (after Voge, 1967), probably resembling that in the first intermediate host. 5. Hatched oncosphere. 6. Early hrowth stage. 7. Tail differentiation. 8. Differentiation of anterior end. 9. Constriction at juncture of body and tail withdrawn anterior end. 10. Loss of tail, appearance of excretory pore. 11. Early tetrathyridial stage. 12-13. Development in the second intermediate host. 12. Tetrathyridium - nondividing. 13. Tetrathyridium - dividing. Asexual multiplication of tetrathyridia occurs probably only in some mammals.

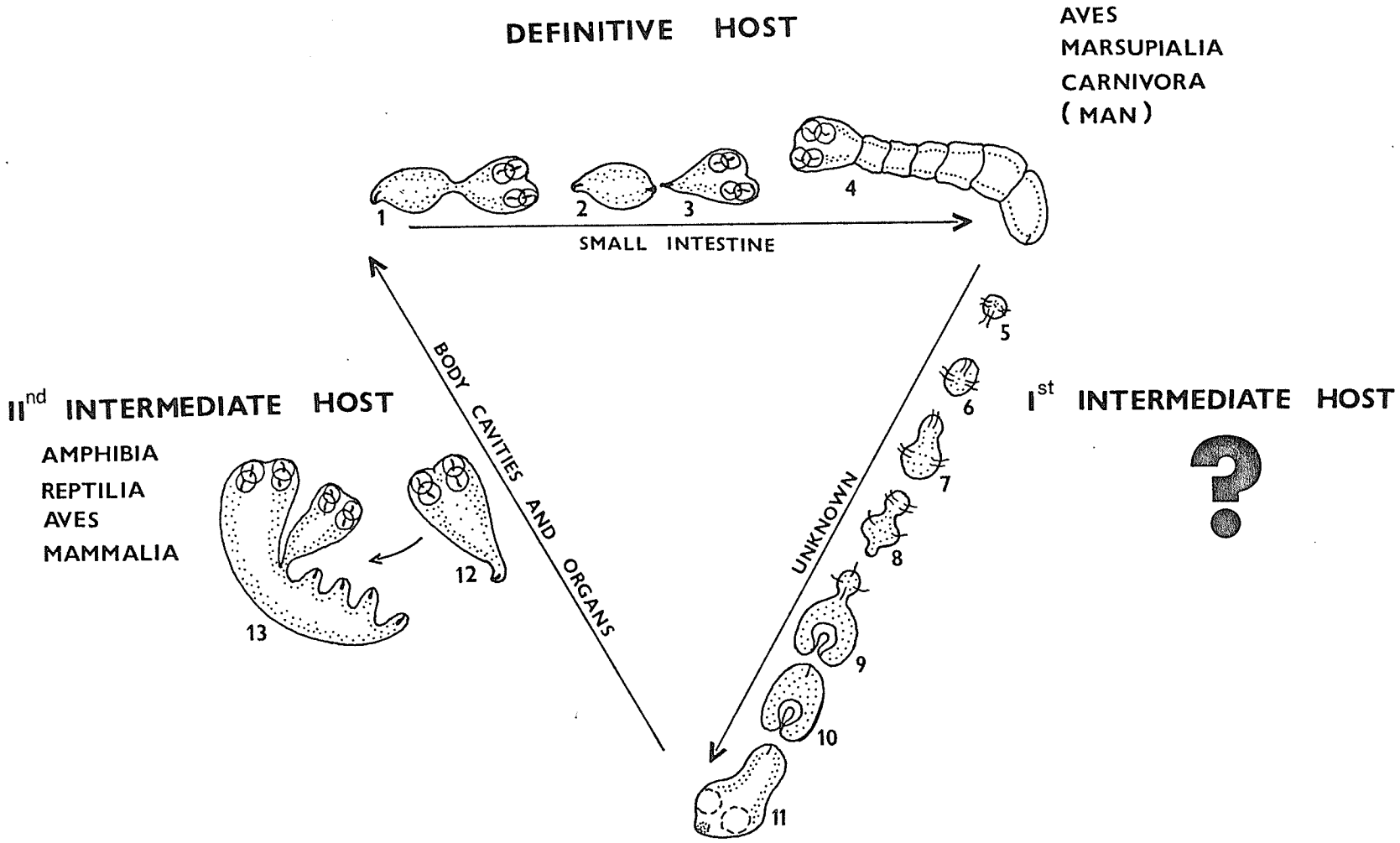

DEFINITIVE HOST
AVES
MARSUPIALIA
CARNIVORA
(MAN)
1
2
3
4
SMALL INTESTINE
BODY CAVITIES AND ORGANS
UNKNOWN
5
6
7
8
9
10
11
12
13
II nd INTERMEDIATE HOST
AMPHIBIA
REPTILIA
AVES
MAMMALIA
I st INTERMEDIATE HOST
?

Fig. 57

Fig. 58. Acceleration of the growth of tetrathyridial populations of Mesocestoides corti in SWR and LDF_1 mice by quinacrine and demecolcine. Numbers of animals in each group are given above the corresponding columns.

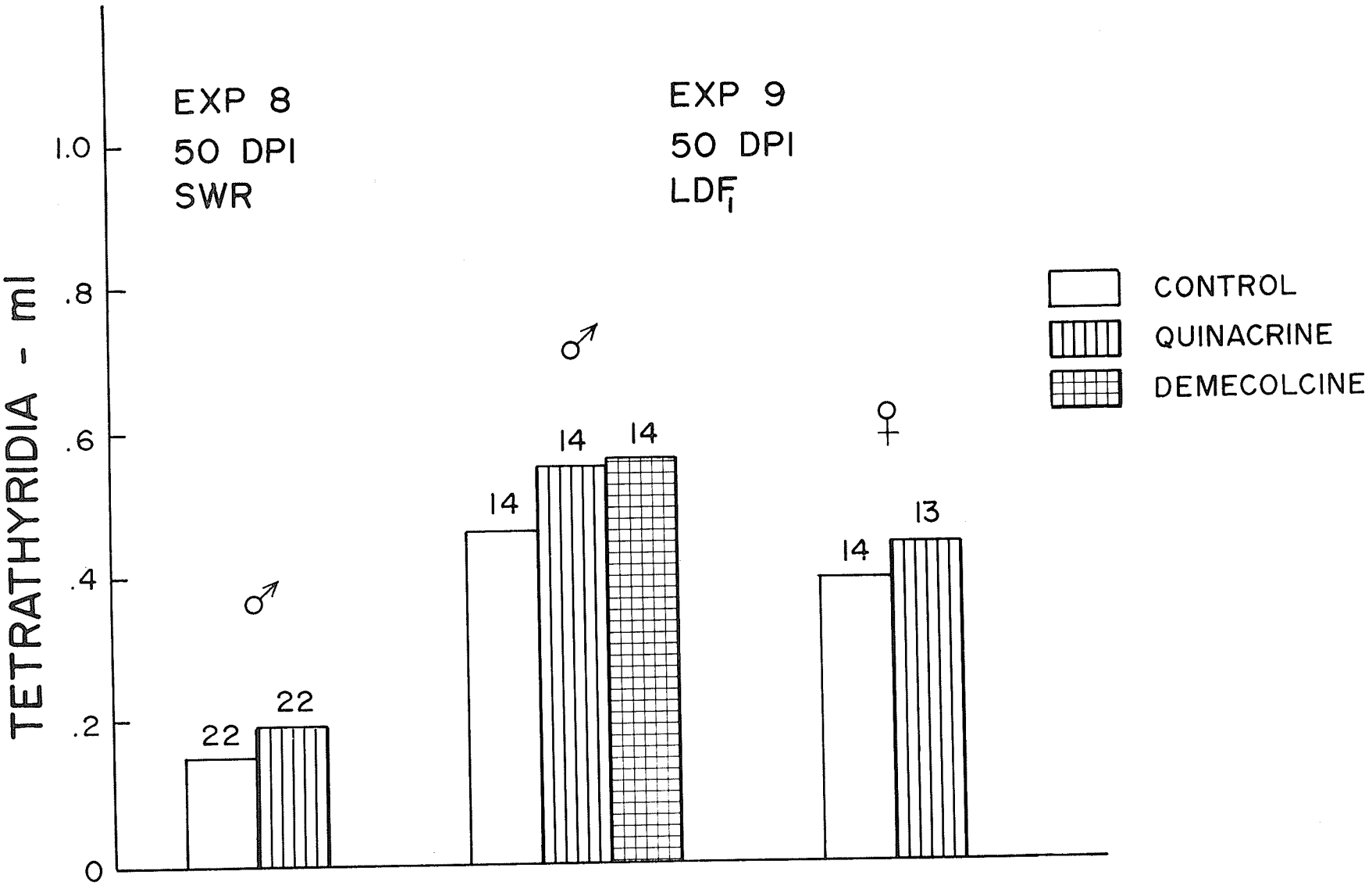

EXP 8
50 DPI
SWR
EXP 9
50 DPI
LDF1
TETRATHYRIDIA - ml
1.0
.8
.6
.4
.2
0
♂
♂
♀
22
22
14
14
14
14
13
CONTROL
QUINACRINE
DEMECOLCINE

Fig. 59. The variability of the body areas and of the number of suckers of two hundred tetrathyridia from male jirds ("gerbils"), 26 DPI, injected cyclophosphamide.

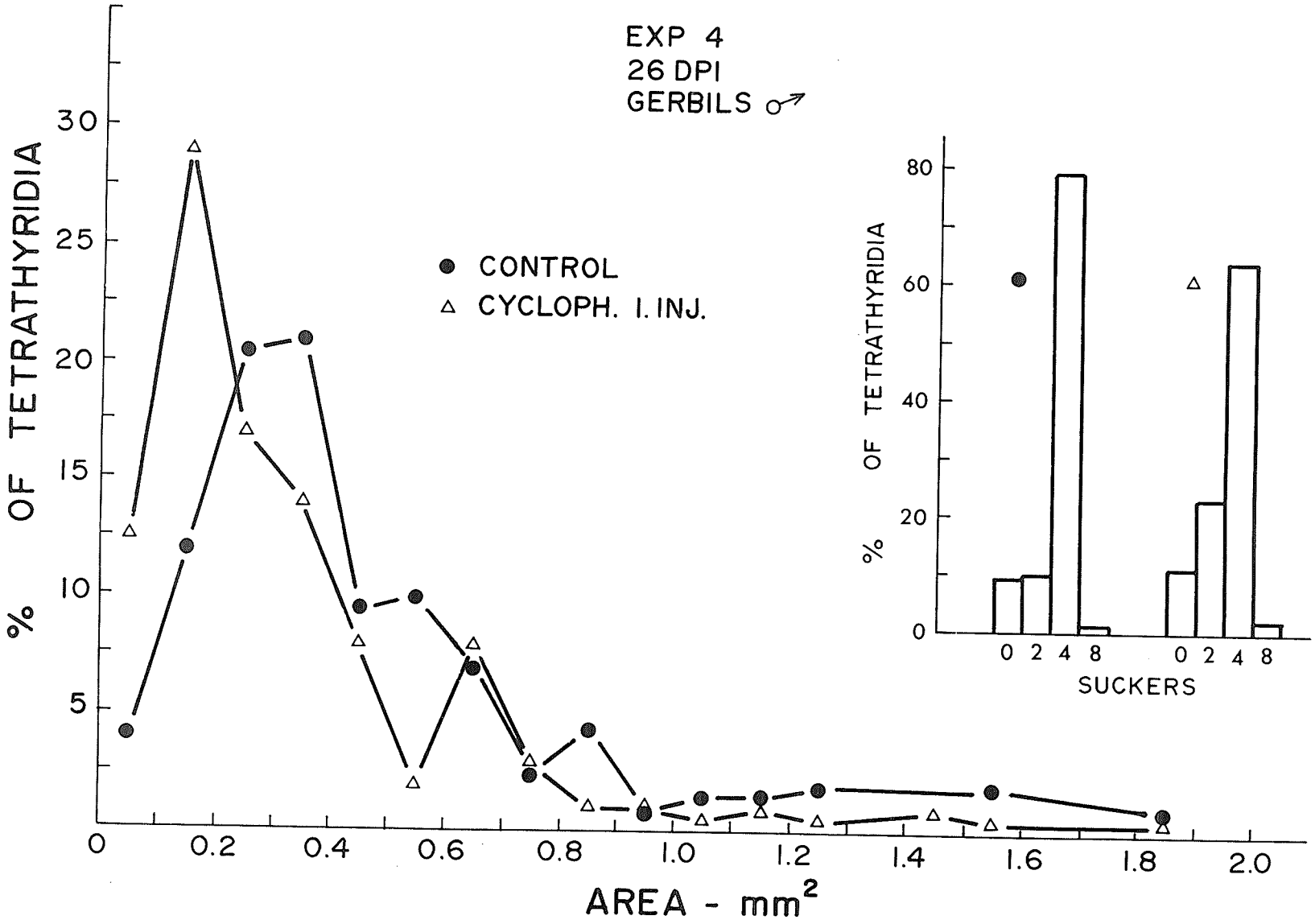
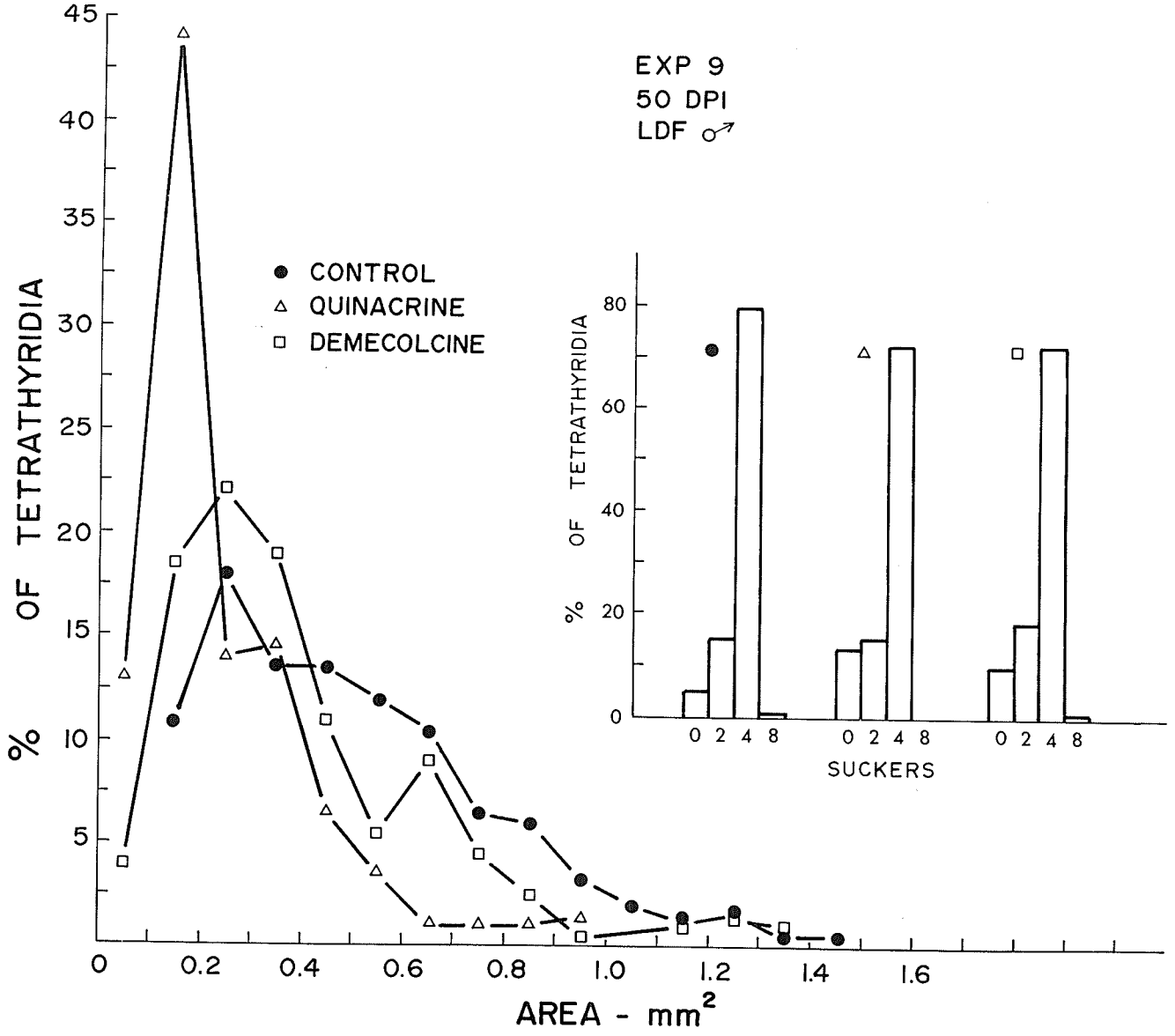

EXP 4
26 DPI
GERBILS ♂
CONTROL
CYCLOPH. I. INJ.
% OF TETRATHYRIDIA
AREA - mm²
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
5
10
15
20
25
30
% OF TETRATHYRIDIA
0
20
40
60
80
0 2 4 8
0 2 4 8
SUCKERS

Fig. 60. The variability of the body areas and of the number of suckers of two hundred tetrathyridia from LDF_1 male mice, 50 DPI, injected quinacrine and demecolcine.


EXP 9
50 DPI
LDF ♂
CONTROL
QUINACRINE
DEMECOLCINE
% OF TETRATHYRIDIA
AREA - mm²
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
5
10
15
20
25
30
35
40
45
% OF TETRATHYRIDIA
0
20
40
60
80
0 2 4 8
0 2 4 8
0 2 4 8
SUCKERS

APPENDIX B. Tables

TABLE 1.

Acephalic fragments from SWR mice, 31 DPI

Sex	Fragments alive
M	3
M	5
M	2
M	3
M	12
F	4
F	2
F	8
F	3
F	-

10 SWR mice were used, each received 20 fragments intraperitoneally.

TABLE 2.

Growth and development of polycephalic larvae[1] in SWR mice

Exp.	Sex	DPI	No. of polycephalic larvae per mouse	Other larvae ml
1	F	200	died	-
	F		died	-
	F		10	0.6
	F		8	0.4
	F		1	2.6
2	M	170	died	-
	M		22	1.0
	M		11	0.5
	M		2	0.4
	M		2	2.0

Larvae had at least 3 scolices on one body.

TABLE 3

Growth and Multiplication of Tetrathyridia of *Mesocestoides corti* in Different Types of Mice. 49 DPI

Mice		No. Mice	Body Weight at infection gm.	Body Weight at Autopsy gm.	Weight Change gm.	Liver Infect.	Liver Weight gm.	Spleen Weight gm.	Tetrathyridia ml	t	p
LDF_1 ♂	7 mos.	10/10	34.40±0.80	35.74±0.94	+1.34	M	4.30±0.25	0.35±0.03	0.66±0.18	0.47	-
♀		7/10	25.31±0.51	27.23±0.72	+1.92	M	3.06±0.17	0.30±0.03	0.56±0.10	-	-
SEC ♂	8 mos.	10/10	22.40±1.80	25.69±0.87	+3.29	M	3.57±0.16	0.40±0.03	0.47±0.08	0.31	-
♀		7/10	19.30±0.30	21.94±0.92	+2.64	M	3.38±0.25	0.39±0.03	0.42±0.09	-	-
SWR ♂	9 mos.	10/10	26.73±0.53	27.94±0.92	+1.21	M	2.99±0.15	0.38±0.03	0.27±0.04	3.21	0.01
♀		10/10	24.51±0.76	25.65±0.71	+1.14	M	2.88±0.09	0.35±0.02	0.14±0.02	-	-
P.man.	12 mos. ♂	4/5	22.75±0.20	23.45±1.30	+0.70	L	1.70±0.20	0.10±0.02	0.15±0.04	-	-
	♀	3/5	22.33±0.21	23.45±1.30	+1.12	L	1.70±0.20	0.10±0.02	0.15±0.04	-	-

Peromyscus maniculatus = *P.man.*

TABLE 4

Host Reactions of Infected Controls and Cyclophosphamide Treated Animals

Exp.	No. Animals at Autopsy	DPI	Group	Body Weight at Infection gm.	Body Weight at Autopsy gm.	Weight Change gm.	Liver Weight gm.	Spleen Weight gm.
3	10	49	SWR♂ controls	22.18±0.60	28.24±0.72	+6.06	3.45±0.19	0.37±0.03
	9		cycloph.1.inj.	23.00±0.67	28.70±0.68	+5.70	2.52±0.12	0.29±0.01
	9		cycloph.2.inj.	23.20±0.68	24.57±1.51	+1.37	3.11±0.13	0.31±0.01
4	12	26	Jirds♂ controls	58.90±1.33	70.46±1.72	+11.56	7.21±0.30	1.35±0.11
	11		cycloph.1.inj.	59.00±1.32	62.17±3.36	+3.17	6.10±0.38	1.26±0.14
	9		♀ controls	54.12±1.86	73.04±4.06	+18.92	8.32±0.52	1.61±0.10
	16		cycloph.1.inj.	56.10±1.33	59.56±1.95	+3.46	6.86±0.27	1.41±0.08
5	19	50	SWR ♀ controls	19.40±0.14	21.42±0.68	+2.02	2.54±0.71	0.34±0.03
	16		cycloph.1.inj.	20.88±0.19	21.19±0.57	+0.31	3.10±0.16	0.27±0.02
	16		cycloph.2.inj.	20.93±0.13	21.55±0.63	+0.62	3.10±0.14	0.31±0.03

TABLE 5

Host Reactions of Infected Controls and Dactinomycin and Lucanthone Treated LDF_1 Mice, 50 DPI

Exp.	No. Animals at Autopsy	Sex	Group	Body Weight at Infection gm.	Body Weight at Autopsy gm.	Weight Change gm.	Liver Weight gm.	Spleen Weight gm.
7	25	M	controls	25.80±0.08	31.33±0.30	+2.53	3.88±0.12	0.31±0.01
	25	M	dactinomycin	30.32±0.20	32.76±0-42	+2.44	5.12±0.15	0.29±0.01
	23	M	lucanthone	30.28±0.40	32.60±0.42	+2.32	4.34±0.11	0.36±0.01
	25	F	controls	19.88±0.22	26.60±0.36	+6.72	2.93±0.11	0.27±0.01
	21	F	dactinomycin	20.92±0.08	27.02±0.33	+6.10	3.50±0.15	0.28±0.02
	26	F	lucanthone	21.00±0.02	25.83±0.34	+4.83	3.29±0.11	0.33±0.02

TABLE 6

Host Reactions of Infected Controls and Quinacrine and Demecolcine Treated Mice, 50 DPI

Exp.	No. Mice at Autopsy	Sex	Group		Body Weight at Infection gm.	Body Weight at Autopsy gm.	Weight Change gm.	Liver Weight gm.	Spleen Weight gm.
8	22	M	SWR	controls	22.40±0.60	24.66±0.61	+2.26	2.40±0.06	0.31±0.01
	22	M		quinacrine	25.50±0.39	27.36±0.56	+1.86	2.63±0.09	0.34±0.01
9	14	M	LDF_1	controls	25.65±0.33	30.88±0.56	+5.23	3.72±0.12	0.32±0.01
	14	M		quinacrine	26.76±0.28	31.30±0.45	+4.54	4.70±0.14	0.35±0.01
	14	M		demecolcine	26.75±0.15	31.49±0.57	+4.74	3.85±0.12	0.33±0.01
	14	F		controls	20.53±0.33	25.36±0.45	+4.83	2.99±0.08	0.33±0.02
	13	F		quinacrine	21.86±0.13	27.10±0.46	+5.24	3.88±0.21	0.36±0.02

TABLE 7

Number of Suckers of Measured Tetrathyridia[1)]

Exp.	Host	DPI	Group	SUCKERS Number 0	2	4	8	Percentage 0	2	4	8
2	SWR	50	controls	23	50	121	6	11.5	25.0	60.5	3.0
			cyclophosph. 1 inj.	23	85	92	-	11.5	42.5	46.0	-
			cyclophosph. 2 inj.	16	51	129	4	8.0	25.5	64.5	2.0
4	Jirds	26	controls	19	20	158	3	8.5	10.0	79.0	1.5
			cyclophosph. 1 inj.	22	46	128	4	11.0	23.0	64.0	2.0
6	SEC	55	controls	24	8	164	4	12.0	4.0	82.0	2.0
			dactinomycin	36	37	126	1	18.0	18.5	63.0	0.5
			lucanthone	32	42	125	1	16.0	21.0	62.5	0.5
9	LDF_1	50	controls	10	30	159	1	5.0	15.0	79.5	0.5
			quinacrine	26	30	144	-	13.0	15.0	72.0	-
			demecolcine	19	36	144	1	9.5	18.0	72.0	0.5

1) 200 larvae were measured and the number of suckers counted per each group.

TABLE 8

Areas of tetrathyridia in the male hosts[1)]

Exp.	Host	DPI	Group	Area of Tetrathyridia (mm^2) 0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.1	1.2	1.3	1.4	1.5	1.6	1.7	Group Area mm^2	Mean Area mm^2
2	SWR	50	controls	12	39	45	34	37	10	10	6	4	-	1	-	1	-	1				69.90	0.35±0.02
			cycloph.1 inj.	14	70	64	25	14	5	3	2	2	-									51.30	0.25±0.01
			cycloph.2 inj.	5	45	54	47	25	16	5	2	-	1	-								62.70	0.31±0.01
4	Jirds	26	controls	8	24	41	42	19	20	14	5	9	2	3	3	4	-	-	4	2		92.00	0.46±0.02
			cycloph.1 inj.	25	58	34	28	16	4	16	6	2	2	1	2	1	-	2	1	1		67.05	0.34±0.01
6	SEC	55	controls	13	36	45	29	18	9	12	11	5	2	2	6	5	2	1	-	4		89.00	0.44±0.02
			dactinomycin	14	49	49	30	26	8	11	4	5	1	-	3	-						65.70	0.33±0.01
			lucanthone	28	70	47	24	18	3	5	1	2	-	-	-	1	1	-				50.10	0.26±0.01
9	IDF_1	50	controls	-	22	36	27	27	24	21	13	12	7	4	2	3	1	1	-			100.20	0.50±0.02
			quinacrine	26	88	28	29	13	7	2	2	22	3	-								48.70	0.24±0.01
			demecolcine	8	37	44	38	22	11	18	9	5	1	-	2	3	2	-				78.60	0.39±0.02

1) 200 larvae were measured per each group

APPENDIX C. Additional References

ADDITIONAL REFERENCES

Berrill, N.J. 1952. Regeneration and budding in worms. Biol. Rev. 27: 401.

Dogiel, V.A. 1964. General Parasitology. Oliver & Boyd, Edinburgh and London.

Eckert, J. et al. 1969. Asexual multiplication of Mesocestoides corti (Cestoda) in the intestine of dogs and skunks. J. Parasitol. 55: 241-249.

Eigsti, O.J. and Dustin, P. 1955. Colchicine in agriculture, medicine, biology and chemistry. The Iowa State College Press, Ames, Iowa.

Faust, E.C. and Russell, P.F. 1957. Clinical Parasitology. Lea & Febiger, Philadelphia.

Freeman, R.S. 1957. Life cycle and morphology of Paruterina rauschi n. sp. and Paruterina candelabraria (Goeze, 1782) (Cestoda) from owls, and significance of plerocercoids in the order Cyclophyllidea. Can. J. Zool. 35: 349-370.

Freeman, R.S. 1964. Studies on responses of intermediate hosts to infection with Taenia crassiceps (Zeder, 1800) (Cestoda). Can. J. Zool. 42: 367-385.

Gleason, N.N. and Healy, G.R. 1967. Report of a case of *Mesocestoides* in a child in Missouri. J. Parasitol. 53: 83-84.

Goodman, L.S. and Gilman, A. 1965. The pharmacological basis of therapeutics. The MacMillan Company, LTD. New York.

Green, E.L. 1966. Biology of the Laboratory Mouse. 2nd Ed. Mc Graw-Hill LTD.

Hart, J.L. 1968. A study of some aspects of the structure of the tetrathyridia of *Mesocestoides* (Cestoda: Cyclophyllidea) and its ability to regenerate *in vivo* and *in vitro*. Ph.D. Thesis, University of California, Los Angeles.

Hoeppli, R.J.C. 1925. *Mesocestoides corti*, a new species of cestode from the mouse. J. Parasitol. 12: 91-96.

James, H.A. and M.J. Ulmer, 1967. New amphibian host records for *Mesocestoides* sp. (Cestoda: Cyclophyllidea). J. Parasitol. 53: 59.

James, H.A. 1968. Studies on the genus *Mesocestoides* (Cestoda: Cyclophyllidea). Ph.D. Thesis, Iowa State University.

Lubinsky, G. 1960. The maintenance of *Echinococcus multilocularis* s. without the definitive host. Can. J. Zool. 38: 149-151.

Lubinsky, G. 1964. Growth of the vegetatively propag-

ated strain of larval Echinococcus multilocularis in some strains of Jackson mice and thier hybrids. Can. J. Zool. 42: 1099-1103.

Lubinsky, G. 1967. Accelerating growth of a vegetatively propagated strain of larval Echinococcus multilocularis, Leuck. 1863. Can. J. Zool. 45: 175-180.

MacInnis, A.J. and M. Voge, 1970. Experiments and Techniques in Parasitology. W.H. Freeman and Company San Francisko.

Mönning, H.O. 1950. Veterinary Helmintology and Entomology. Bailliere, Tindall and Cox, London.

Nordlinder, H. 1969. Malformations after Treatment of New-Born Mice with a single Dose of Cyclophosphamide. Experimentia 25: 1296.

Soldatova, A.P. 1944. A contribution to the study of the development cycle in the cestode Mesocestoides lineatus (Goeze, 1782), parasitic of carnivorous mammals. C.R. (Doklady) Acad. Sci. U.R.S.S. 45: 310-312.

Southwell, T. and Prashad, B. 1918. Methods of asexual and parthenogenetic reproduction in cestodes. J. Parasitol. 4: 122-129.

Ssolonitzin, I.A. 1933. Mehrfacher Tetrathyridios der serösen Höhlen des Hundes. Ztschr. Infekti-

onskr. Haustiere. 45: 144-156.

Stecher, P.G. et al. (editors). 1968. The Merck index 8th ed. Merck and Company, Inc. Rahway, N.J. U.S.A.

Voge, M. 1953. New host records for Mesocestoides (Cestoda: Cyclophyllidea) in California. Amer. Midland Natur. 49: 249-251.

Voge, M. 1954. Exogenous proliferation in a larval taenid. (Cestoda: Cyclophyllidea) obtained from the body cavity of Peruvian rodents. J. Parasitol. 40: 411-413.

Voge, M. 1955. A list of cestode parasites from California mammals. Amer. Midland Natur. 54: 413-417.

Voge, M. 1955. North American cestodes of the genus Mesocestoides. U. Calif. Publ. Zool. 59: 125-156.

Voge, M. and Berntzen, A.K. 1963. Asexual multiplication of larval tapeworms as the cause of fatal parasitic asites in dogs. J. Parasitol. 49: 983-988.

Voge, M. 1967. The Post-embryonic Developmental Stages od Cestodes. Advances in Parasitol. 5: 247-289.

Voge, M. 1967. Development in vitro of Mesocestoides

(Cestoda) from oncosphere to young tetrathyridium. J. Parasitol. 53: 78-82.

Voge, M. and Seidel, J.S. 1968. Continnuous growth in vitro of Mesocestoides (Cestoda) from oncosphere to fully developed tetrathyridium. J. Parasitol. 54: 269-271.

Wardle and McLeod 1952. The Zoology of Tapeworms. Univ. of Minnesota, Press.

Webster, J.D. 1949. Fragmentary studies on the life history of the cestode Mesocestoides latus. J. Parasitol. 35: 83-90.

APPENDIX D. Taxonomy

Taxonomic position of the genus Mesocestoides

The genus Mesocestoides was established in 1863 by Vaillant for a cestode with "characteristics of both Taenias and of Bothriocephales" which he found in the small intestine of Genetta genetta L. (his Viverra genetta), and named Mesocestoides ambiguus.

Into this genus were later transferred the following species described prior to 1863:

M. angustatus (Rudolphi, 1819) from Meles taxus
M. canis lagopodis (Rudolphi, 1810) from Alopex lagopus
M. cateniformis (Gmelin, 1790) from foxes (Vulpes)
M. chrysaeti (Viborg, 1795) from Aquila chrysaeti
M. imbutiformis (Polonio, 1860) from Anser ferus
M. lineatus (Goeze, 1782) from Felis sylvestris
M. litteratus (Batsch, 1786) from fox (Vulpes)
M. margaritifera (Creplin, 1829) from birds of prey
M. perlatus (Goeze, 1782) from Buteo buteo L.
M. pseudocucumerina (Bailliet, 1863) from dog (Canis)
M. tenuis (Creplin, 1829) from Falco subbuteo

Several other species of this genus were described between 1863 and 1897, when Perrier has established the

family Mesocestoididae. Well over 40 species of this genus were described up to 1972, and most of them are regarded as junior synonyms of a few valid species.

Witenberg in his revision of the genus Mesocestoides (1934) recognized only the folloving species of this genus:

M. charadrii Fuhrmann, 1909 from Tringa (Limonites) minuta
M. lineatus (Goeze, 1782) from Felis sylvestris
M. perlatus (Goeze, 1782) from Buteo buteo L.

Wardle and McLeod (1952) in their "Zoology of Tapeworms" recognized the following species:

M. ambiguus Vaillant, 1863 from Viverra genetta
M. bassarisci MacCallum, 1921 from Bassariscus astutus
M. caestus Cameron, 1925 from Mellivora ratel
M. charadrii Fuhrmann, 1909 from Tringa (Limonites) minuta
M. corti Hoeppli, 1925 from Mus musculus
M. kirbyi Chandler 1944 from Canis latrans
M. latus Mueller, 1927 from Mephitis minnesotae
M. lineatus (Goeze, 1782) from Felis sylvestris
M. litteratus (Batsch, 1786) from fox (Vulpes)
M. materni Chandler, 1942 from Lynx rufus
M. perlatus (Goeze, 1782) from Buteo buteo L.
M. tenuis Meggitt, 1931 from dog (Canis)
M. variabilis Mueller, 1927 from Urocyon cinereoargenteus

However, the recent trend based on the realisation of the very high individual variability of *Mesocestoides* species is to reduce further the number of recognized species. Thus Voge (1955) in her revision of the genus *Mesocestoides* recognized only 4 species:

M. corti Hoeppli, 1925 from *Mus musculus*
M. kirbyi Chandler, 1944 from *Canis latrans*
M. latus Mueller, 1927 from *Mephitis minnesotae*
M. lineatus (Goeze, 1782) from *Felis sylvestris*

The species with which the present work was done, *Mesocestoides corti*, was described by Hoeppli in 1925 on the basis of material collected by W.W. Cort in 1909 at Colorado Springs from *Mus musculus*. This consisted of about one hundred adult speciments from the intestine of the host. According to Wardle and McLeod (1952), the systematic position of the *Mesocestoides* with which the present work deals is:

Phylum	Platyhelminthes	Carus, 1863
Class	Cestoda	Monticelli, 1892
Subclass	Eucestoda	Southwell, 1930
Order	Cyclophyllidea	Braun, 1900
Family	Mesocestoididae	Perrier, 1897
Genus	*Mesocestoides*	Vaillant, 1863
Species	*corti*	Hoeppli, 1925