Studies on the biology of *Plagiorchis elegans* (Rudolphi, 1802), (Trematoda: Digenea) in its mammalian and molluscan hosts.

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To my Mother and Father.

ABSTRACT

The taxonomy of the genus Plagiorchis Lühe, 1899 is reviewed with particular reference to the species P. elegans (Rud. 1802) and a list of Plagiorchis species previously recorded in Britain is given.

The life cycle was initially established using Lymnaea stagnalis, Chironomus sp. larvae and LACA mice; morphology, intraspecific variation and host specificity at all levels of the life cycle have been examined and described.

Besides L. stagnalis, L. palustris is susceptible to infection with P. elegans while L. peregra is refractory. Infections cause extensive damage to the digestive gland of L. stagnalis although they do not curtail its life span. Immature and mature specimens of L. stagnalis were infected; the former were castrated and the latter continued to reproduce. A single mother sporocyst of P. elegans produces approximately 650 daughter sporocysts from which develop several hundred thousand xiphidiocercariae.

Upon release cercariae are negatively phototropic and negatively geotropic, with light and temperature affecting the rate of cercarial emmission. Cercariae encyst in aquatic arthropods (Chironomus sp., Asellus aquaticus and Gammarus pulex) and precociously in the snail host.

Adult P. elegans range in size from 1.04 to 3.89mm by 0.34 to 0.96mm. Both mammals (mice, rats, gerbils and hamsters) and birds (chicks, ducklings and pigeons) are susceptible to infection. Egg counts were performed to follow the course of the infection in mice and rats. Primary infections of LACA mice are of short duration as a result of a host immune response. By means of surgical transplantation the life span of P. elegans was increased from the expected 21 days to 63 days. Specimens recovered when the life cycle was completed using various combinations of intermediate and final hosts and those recovered from first and second challenge infections of LACA mice are compared statistically using canonical variate analysis.

The only reliable anatomical criteria for distinguishing species of Plagiorchis are:

1. the relative sizes of the suckers and pharynx.
2. the presence or absence of a seminal receptacle, vas deferens and common genital atrium.
3. to a limited extent egg size and host specificity.

According to these criteria many previously described species are considered to be synonymous with P. elegans and its geographical range is

extensive.

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CONTENTS

Title page i
ABSTRACT ii
ACKNOWLEDGEMENTS iv
CONTENTS v
INTRODUCTION 1
SECTION 1. A review of the taxonomy of the genus Plagiorchis Lühe, 1899. 3
SECTION 2. Establishment of the life cycle and morphology of the larval stages. 15
Introduction 16
Materials and Methods 16
Results 17
Establishment of the life cycle 17
Morphology of the larval stages 17
Egg and micacidium 17
Mother and daughter sporocysts 18
Cercaria 19
Metacercaria 21
Discussion 22
Figures 27
SECTION 3. The first intermediate host 34
Introduction 35
Materials and Methods 35
Results 36
Host specificity 36
Exposure of Lymnaea stagnalis to unknown number of .. P. elegans eggs 36
Exposure of L. stagnalis to 1, 10 or 50 P. elegans .. eggs 38
Exposure of L. peregra and L. palustris 39
Percentage hatch of P. elegans eggs 41
Length of life of embryonated eggs 41
Fecundity of P. elegans mother sporocyst 42
Effects of parasitism upon the snail 42
Growth and survival of infected L. stagnalis 42
Pathology of infections of P. elegans in L. stagnalis . 44
Effects on reproduction in the snail host 45

Infection of immature *L. stagnalis* 45
Infection of mature *L. stagnalis* 46
Discussion 47
Figures 56
SECTION 4. Cercaria and metacercaria 65
Introduction 66
Materials and Methods 66
Results 67
Cercarial release; monomiracidial infections 67
Factors influencing cercarial release 69
Temperature 69
Photoperiodicity 69
Cercarial behaviour and longevity 70
Metacercaria 71
Host specificity 72
Amphibians 73
Molluscs 73
Encystment without host penetration 74
Attempts to induce encystment 74
Discussion 75
Figures 80
SECTION 5. Adult stage 84
Introduction 85
Materials and Methods 86
Results 88
Anatomy of adult *P. elegans* 88
Morphological and anatomical variation due to age . .. 89
LACA mice 89
Laboratory rats 92
Effects of various hosts on morphology 92
Definitive hosts 92
Second intermediate hosts 94
First intermediate hosts 94
Anatomical variation of *P. elegans* 95
Oral and ventral suckers 95
Caeca 95
Gonads 96
Uterus 96
Cirrus sac 97

Vitellaria 97
Final host specificity 98
Amphibians 98
Birds 99
Mammals 99
Intestinal length 101
Egg counts 101
Discussion 106
Figures 115
SECTION 6. Immunity 132
Introduction 133
Materials and Methods 134
Results 135
Duration of the primary infection 135
Egg counts 135
Challenge infections 142
High initial dose; low challenge dose 142
Low initial dose; low challenge dose 143
Second challenge infections 143
Age resistance 143
Distribution 147
Primary infections 147
Challenge infections 148
Discussion 149
Figures 152
SECTION 7. Transplantation 159
Introduction 160
Materials and Methods 161
Results 162
Series I Preliminary experiment 162
Series II Successive transplants 162
Series III Resistant donor mice 165
Series IV Resistant recipient mice 165
Discussion 169
Figures 171
SECTION 8. Statistical analysis 176
Introduction 177
Materials and Methods 177

Results 179
Discussion 182
Figures 185
SECTION 9. Synonymy and geographical distribution 187
Synonymy 188
Geographical distribution 189
DISCUSSION. 197
APPENDIX I. Egg dimensions of some *Plagiorchis* species 200
APPENDIX II. Size relationships of the suckers, pharynx and gonads to one another. 202
BIBLIOGRAPHY 205

INTRODUCTION

The taxonomic classification of the Digenea (Trematoda: Platyhelminthes) depends at the present largely upon the morphological features of the adult parasite; although the importance of larval structures, life history, physiology and host relationships has been emphasized, this information is rare (Stunkard, 1957; Haley, 1962; Dawes, 1968; Yamaguti, 1971). Even when these data are available such diverse opinions exist that the systematics of the Digenea often becomes more, rather than less, confused (Stunkard, 1963). The position of the Digenea itself is the subject of considerable controversy, principally because of recently acquired informationcconcerning the larvae and life cycles of the monogeneans and digeneans (Stunkard, 1963; Llewellyn, 1965; Erasmus, 1972). But for the purpose of this study and in accord with Stunkard (1963), Dawes (1968) and Yamaguti (1971) the Digenea is considered to hold the rank of Order within the Class Trematoda.

Looss (1899) was the first to present a method for establishing relationships based on the comparative internal morphology of adult worms. However without supporting observations on developmental stages, it is not possible using this method to show whether structural similarities are the result of divergent or convergent evolution.

With additional information, taxonomists have included in their diagnoses larval morphology and life history data. Lühe (1909) classified cercariae on morphological features such as the absence or presence and location of the suckers, the stylet and the structure of the tail. Lebour (1912) emphasized developmental features and distinguished between cercariae which were produced in sporocysts and those which developed in rediae. Cort (1917), Faust (1919) and LaRue (1926) suggested that besides the larval stages the characteristics of the excretory system are important in formulating a natural scheme of classification. LaRue (1957) later reviewed the history of digenetic trematode classification and presented a new system based on life history data and embryonic development.

On the basis of adult morphology more than sixty families have been established within the Digenea. Many of these families, including the Plagiorchiidae, have not as yet been clearly defined; as a result agreement as to their interfamilial relationships is unresolved, as is the taxonomic position of many genera (Stunkard, 1963; Dawes, 1968).

While the general morphology and life cycle of the genera within the Plagiorchiinae are well established (Yamaguti, 1958;1971), considerable

disagreement remains at the species level. Of the type genus Plagiorchis Lühe, 1899 more than 140 species have been described, a number of them on the basis of very few specimens and many more without regard to the life cycle. The extent of morphological variation and host specificity of Plagiorchis species at all stages of the life cycle are incompletely known, with the result that the taxonomy of the genus is in a confused state, a fact which has been emphasized in the present account by the detailed literature review. Thus the establishment of a laboratory cultured population using the eggs from one worm, provides the only assurance that one is dealing with a single species.

The major aim of this study has been to determine the morphological modifications induced by the respective intermediate and final hosts of P. elegans and thereby establish its specific morphological variation and ultimately its synonymy and geographical distribution. To examine the complex interactions involved it was necessary to develop a laboratory-reared population of P. elegans; from this stock population intraspecific variation could be investigated and identified using different combinations of intermediate and final hosts. Furthermore, because the immunological state of the definitive host may produce phenotypic variations in the parasite (Jenkins, Ogilvie and McLaren, 1976), challenge infections using Laboratory Animal Centre Accredited (LACA) mice were carried out and age resistance in the mice was also investigated. The possible effect of the hosts on the anatomy of P. elegans was then examined statistically by means of canonical variate analysis.

Since adult morphology alone is insufficient either to describe or distinguish between species, the life history of P. elegans and morphology of the larval stages have been described and various aspects of the host-parasite relationship including behaviour, host-specificity, duration of infection and fecundity have been examined. It became apparent after initial investigations that the completion of the life cycle depends most acutely on the relationship between the parasite and its first intermediate gastropod host. Therefore an extensive treatment of this association was conducted.

Section 1

A review of the taxonomy of the genus *Plagiorchis* Lühe, 1899, with special reference to the species *Plagiorchis elegans* (Rudolphi, 1802).

The generic name Plagiorchis first appeared in the literature in 1899; at that time Lühe published an account of the genus in which he placed the species Distomum reniferum Looss, 1899 and D. horridum Leidy, 1850. Subsequently however the above species were referred to the genera Astiotrema Looss, 1900 and Styphlodora Looss, 1899 respectively. Two days after Lühe's article was published Looss (1899) published an article on the closely related genus Lepoderma which included descriptions of the type species L. ramlianum and the subfamily Lepodermatinae. There is some doubt among taxonomists concerning the inclusion of the species ramlianum in the genus Lepoderma because the cercariae develop in rediae and they have an I-shaped excretory bladder in contrast to all other members of the genus, whose cercariae develop in sporocysts and characteristically have a Y-shaped excretory bladder in both the cercarial and adult stages.

Braun (1901) noted a very close similarity between the genera Plagiorchis and Lepoderma and considered them to be synonymous giving the name Plagiorchis priority because of its earlier date of publication. However this criterion was not considered acceptable by all taxonomists; Odhner (1911) and Mehra (1931) accepted the name Lepoderma because it was tied to a type species and the subfamily Lepodermatinae was established in 1899, while Pratt described the subfamily Plagiorchiinae in 1902. The writer is inclined to agree with Odhner (1911) and Mehra (1931), but since the name Plagiorchis has become accepted over the years through common usage, to do so would only add to the state of confusion within the genus. Therefore the generic name Plagiorchis is accepted and used in this thesis.

Since the early studies by Looss (1899), Lühe (1909) and Pratt (1902), work on the subfamily Plagiorchiinae (syn. Lepodermatinae) has been conducted by Mehra (1931, 1937), McMullen (1937a) and Olsen (1937). The presence of a seminal receptacle in some species of the genus Plagiorchis and its apparent absence in the majority of the other species have caused considerable disagreement among taxonomists. In their independent generic diagnoses Mehra (1937) and Olsen (1937) both stated that a seminal receptacle is absent in the genus Plagiorchis and they erected the genera Neolepoderma and Plagiorchoides respectively to accommodate species possessing such a structure. While Tubangui (1946) and Skrjabin and Antipin (1958) accepted the establishment of the latter genus, Park (1939a) on the other hand doubted the validity of the presence or absence of a rudimentary seminal receptacle as a taxonomic character; he had observed it in young specimens of P. noblei Park, 1936, but found that in old worms it was either obliterated or void of

spermatazoa. However he did eventually accept it and suggested that as a consequence of removing from the genus Plagiorchis those species which possess a rudimentary seminal receptacle, the characteristics of the genus would be more uniform. Since then a number of species of Plagiorchis, each having a rudimentary seminal receptacle, have been described. They include:

P. goodmani Najarian, 1961
P. peterborensis Kavelaars & Bourns, 1968
P. neomidis Brendow, 1970
P. farnleyensis Diaz, 1976
P. kirkstallensis Diaz, 1976

In his generic diagnosis Yamaguti (1971) noted that a seminal receptacle may be rudimentary or lacking.

Besides the genera Lepoderma Looss, 1899, Neolepoderma Mehra, 1937 and Plagiorchoides Olsen, 1937, Yamaguti (1971) included Cercolecithos Perkins, 1928, Choristogonoporus Stunkard, 1938 and Reynoldstrema Cheng, 1959 as synonyms of Plagiorchis Lühe, 1899. Cercolecithos comprises a single species C. arrectus, which was based solely on the original description of Distoma arrectum Molin, 1859 from European lizards. However Lent and Freitas (1940) fully described and illustrated trematodes from Lacerta vivipara as being identical with D. arrectum Molin and redesignated the species as Plagiorchis molini n.sp.. Lopez-Neyra (1940) also assigned D. arrectum Molin to the genus Plagiorchis, but renamed specimens from Lacerta ocellata as P. mentulatum. The validity of the genus Choristogonoporus is doubtful since it is based on a single alcohol preserved specimen which Stunkard (1938) neither illustrated nor adequately described. Finally, Cheng (1959) erected the genus Reynoldstrema to accommodate Glypthelmins africana Dollfus, 1950. Vercammen-Grandjean (1960) transferred G. africana to the genus Plagiorchis, but Fischthal and Thomas (1968) accepted Reynoldstrema and its single species R. africana (Dollfus, 1950) Cheng, 1959 and assigned two other species to it: R. laurenti (Vercammen-Grandjean, 1960) n. comb. and R. berghei (Vercammen-Grandjean, 1960) n. comb.. It is of note that Beverley-Burton (1963) cautioned in her redescription of G. africana that some species placed in the genus Plagiorchis from amphibians and reptiles show more similarity to G. africana than do accepted species of Glypthelmins Stafford, 1905. While the adults of the two genera are similar, they may be readily distinguished by the existence of a Y-shaped excretory bladder in the former genus in contrast to an I-shaped bladder in the latter genus and by substantial differences in their life cycles (Cheng, 1961; Martin, 1969; Yamaguti, 1971).

At the present time four subgenera and more than 140 species have been included in the genus. Schulz and Skworzow (1931) divided the genus into two subgenera based on the distribution of the vitellaria between the oral and ventral suckers. In the subgenus Multiglandularis (type species P. (Multiglandularis) multiglandularis Semenow, 1922) the vitelline follicles from each side meet in the midline anterior to the ventral sucker to form a broad commissure, while in the subgenus Plagiorchis (type species P. (Plagiorchis) vespertilionis (Mueller, 1784)) the vitellaria are either not confluent in this location or only very few follicles are present. Timofeeva (1962) erected the genus Metaplagiorchis to accommodate those species in which the caeca and/or vitellaria do not extend to the posterior extremity of the body. However Yamaguti (1971) did not consider these characteristics of sufficient importance to warrant the establishment of a new genus and relegated Metaplagiorchis to subgeneric rank, with P. (Metaplagiorchis) ramlianus (Looss, 1899) as the type species. Yamaguti (1971) also erected the subgenus Pseudoplagiorchis (type species P. (Pseudoplagiorchis) erraticus (Rud. 1819)) to accommodate those species in which the uterus passes lateral to the testes and the preacetabular genital pore is median instead of submedian. In other respects the two subgenera Pseudoplagiorchis and Plagiorchis are very similar.

In 1959 Odening revised the genus Plagiorchis and divided it into species groups within the subgenera of Schulz and Skworzow (1931). The subgenus Multiglandularis comprised three groups:

i) cirratus-laricola group
ii) multiglandularis group
iii) notabilis-muris group

and the subgenus Plagiorchis comprised four groups:

i) vespertilionis group
ii) maculosus group
iii) elegans-triangularis group
iv) isolated species group.

The idea of these species groups was later abandoned by Odening (1961), when he became aware of evidence negating the validity of the subgenera Plagiorchis and Multiglandularis. Braun (1902), Skrjabin and Antipin (1958), Styczynska-Jurewicz (1962), and even Schulz and Skworzow (1931) noted that the presence or absence of an anterior vitellarian commissure is not a regular characteristic within a species.

Odening (1959) removed from the genus Plagiorchis those species in which the uterus does not extend either beyond the posterior testis or into the fourth quarter of the body; these species he placed in the

subfamily Opisthioglyphinae:

P. arcuatus Strom, 1924

P. exasperatus (Rud. 1819) = P. microti Soltys, 1949

P. nanus (Rud. 1802) = P. fastuosus Szidat, 1924

=?P. morosovi Sobolev, 1946

P. opisthovitellinus Soltys, 1954.

It was further suggested by Odening (1959) that all species in which the uterus alone occupies the posterior end of the body be removed from the genus Plagiorchis and placed in a new genus, which he did not name, but which should be erected specifically to accommodate them, including:

P. didelphidis (Parona, 1894)

P. hepaticus Lutz, 1928

P. himalayii Jordan, 1930

P. lenti Freitas, 1941

P. molini Lent & Freitas, 1944/1945

P. momplei Dollfus, 1932

P. ptschelkini Sobolev, 1946

P. yosidensis Ogata, 1942

The criteria given by Odening for the establishment of a new genus were very similar to those of Timofeeva (1962) for the erection of the genus Metaplagiorchis; however they have not been widely accepted and the above species remain in the genus Plagiorchis.

Members of the genus Plagiorchis have been recorded from all classes of vertebrates, including fish. However, the only descriptions of Plagiorchis from fish known to the writer comprise P. corti from Schilbeodes gyrinus (Lamont, 1921), P. amerius from Ameriurus nebulosus (McCoy, 1928), and P. geminus from both of these species of fish (Mueller, 1930). The latter author (1930) noted a close similarity between P. amerius and P. corti and suggested that they should be regarded as synonyms. All three species were subsequently referred to the genus Alloglossidium Simer, 1929 by Van Cleave and Mueller (1934) who observed that the excretory bladder of these fish parasites is I-shaped in contrast to the Y-shaped excretory bladder characteristic of the genus Plagiorchis.

The species given in Table 1.1 at the end of this section have been recorded in Britain. It may be noted that P. elegans, the subject of the present thesis, has not previously been found in Britain. Further, the present British record refers to intra-molluscan and cercarial stages only.

According to Braun (1902) P. elegans was first described by Rudolphi in 1802 as Fasciola elegans from nestling house sparrows; Braun (1902) gives the following synonyms of P. elegans:

Distoma elegans Rudolphi, 1809

Distomum elegans Creplin, 1829(?); Diesing, 1850

Mühling, 1896 = D. cirratum Rudolphi, 1802

Distoma (Brachylaimus) elegans Dujardin, 1845; Stossich, 1892 = D. cirratus Rudolphi, 1802

Distomum erraticum v. Linstow, 1894.

Braun (1902) examined four of Rudolphi's specimens and his description of them is summarized here.

Body oblong, flattened; both ends rounded or hind end tapered. Two to 2.30 mm long; 0.66 mm wide. Spines visible in anterior of worm. (Because the tegument of the specimens examined by Braun had begun to decay the distribution of the spines given may not be correct.) Oral sucker 0.177 mm long, 0.156 mm wide; acetabulum 0.104 mm in diameter, approximately one third from anterior end. Pharynx immediately follows oral sucker, always possesses spherical lumen. Caeca originate close behind pharynx; reach to posterior end. Ovary globular, always larger than ventral sucker; lies to one side anterior to obliquely situated testes. Cirrus pouch long and slender; C-shaped; bends around ventral sucker; arises in front of it, where metraterm terminates. Vitellaria well developed, fill sides of body from level of pharynx to posterior extremity. In front of ventral sucker and behind testes vitellaria may extend dorsally from each side to midline. (The specimen of P. elegans illustrated by Braun does not possess an anterior vitellarian commissure.) Eggs 32 to 36 μm wide.

Rudolphi (Braun, 1902), when describing P. elegans and P. cirratus, noted a strong similarity between them. Braun (1902) was able to compare specimens of P. cirratus from the Vienna Collection withtthe type specimens of P. elegans. He postulated that they could be maintained as independent species based on the differences in body and egg size and the extent of the vitellaria. Although Braun (1902) did not illustrate P. cirratus he stated that an anterior vitellarian commissure is generally absent. Because the characters mentioned above may vary within a species, Braun (1902) suggested that the greatest emphasis should be placed on differences in the sucker ratios. In specimens of P. cirratus examined by Braun the suckers were nearly the same size while in P. elegans the oral sucker was considerably larger than the ventral sucker. Mühling (1896) on the other hand described specimens of P. cirratus in which the ventral sucker was smaller than the oral sucker. Braun (1902) stated that if they were to be considered synonymous, the name elegans

should take precedence, since it appeared first in the paper by Rudolphi (1802). It is indeed unfortunate that Rudolphi's preparations of P. elegans and P. cirratus have been destroyed (personal communication from Mr. S. Prudhoe). The absence of the type specimens has made the comparative study of these two species exceedingly difficult.

Recent studies on P. elegans have been carried out by Styczynska-Jurewicz (1962) and Sharpilo and Sharpilo (1972). Styczynska-Jurewicz (1962) investigated the life cycle using naturally infected snails and discussed the synonymy of P. elegans. Sharpilo and Sharpilo (1972) studied the relationships of various forms of Plagiorchis in reptiles and rodents and concluded that a number of species should be regarded as synonyms of P. elegans.

Despite the considerable volume of literature that exists concerning the taxonomy of Plagiorchis, it is evident that the question of the relationship of P. elegans to other members of the genus has not been resolved.

Table 1.1. Species of *Plagiorchis* Lühe, 1899 recorded in Britain.

Species	Author (year)	Host(s)	Common name	Site
*P. notabilis**	Nicoll (1909, 1923a)	*Anthus petrosus* = *A. spinoletta*	rock pipit	Scotland
		Motacilla flava	blue-headed wagtail	"
	Baylis (1939)	*Motacilla yarrelli* = *M. alba yarrelli*	pied wagtail	Cumberland
	Horton-Smith & Long (1954)	*Gallus gallus*	domestic fowl	Montgomeryshire
P. cirratus (Rud. 1802)	Lewis (1926, 1927)	*Turdus merula*	blackbird	Wales
	Baylis (1939)	*Larus ridibundus*	black-headed gull	London
	Jennings & Soulsby (1957)	*L. canus*	common gull	not given
P. maculosus (Rud. 1802)	Baylis (1939)	*Micropus apus* = *Apus apus*	swift	Kent
P. muris	Baylis (1928)	*Apodemus sylvaticus*	wood-mouse	near Oxford
	(1939)	*Rattus norvegicus*	brown rat	Cambridgeshire
	Elton, Ford & Baker (1931)	*Apodemus sylvaticus*	long-tailed field mouse	near Oxford
	Fahmy & Rayski (1963)	*Ovis aries*	sheep	Scotland

Table 1.1. continued.

P. laricola Skrjabin, 1924	Foggie (1937)	Meleagris sp.	turkey (poults)	Northern Ireland
P. mentulatus (Rud. 1819)	Baylis (1939)	Rana temporaria	common frog	Cambridgeshire
P. megalorchis*	Rees (1952)	Meleagris sp.+	turkey (poults)	Radnorshire, Wales
	Jordan (1953)	"	"	"
	Rayski (1964)	Phaisianus sp.	pheasant	Peebleshire, Scotland
P. lutrae*	Fahmy (1954)	Lutra lutra	otter	Edinburgh, Scotland
P. agrestis*	Hecht (1961)	Microtus agrestis	field vole	not given
P. vitellatus (v. Linstow, 1875)	Fraser (1974)	Larus argentatus	herring gull	Scotland
		L. ridibundus	black-headed gull	"
		L. fuscus	lesser black-back gull	"
P. farnleyensis*	Diaz (1976)	Mus musculus+	laboratory mouse	Leeds
		Rattus norvegicus+	brown rat	"
		Gallus gallus+	domestic foul (chicks)	"
		Columba livia+	ferral pigeon	"

Table 1.1. continued.

P. kirkstallensis*	Diaz (1976)	Rattus norvegicus+	brown rat	Leeds
		Mus musculus+	laboratory mouse	"

The following species were recorded by Nicoll as parasites of British birds (1923a) and mammals (1923b). However he was citing continental observations and they are included here for the sake of completeness.

Species	Host (s)	Common name
P. cirratus (Rud. 1802)	Corvus corone	Carrion crow
	C. monedula	Jackdaw
	C. frugilegus	Rook
	Pica pica	Magpie
	Sturnus vulgaris	Starling
	Passer montanus	Tree swallow
	Larus marinus	Greater black-back gull
	Motacilla alba	White wagtail
P. elegans (Rud. 1802)	Passer domesticus	House sparrow
	Motacilla alba	White wagtail
	Parus major	Great tit
	P. palustris	Marsh tit
	P. caeruleus	Blue tit

Table 1.1. continued.

	Otus scops	Scops owl
	Falco subbuteo	Hobby falcon
P. maculosus (Rud. 1802)	Hirundo rustica	Swallow
	Delichon urbica	House Martin
	Riparia riparia	Sand Martin
	Micropus apus = Apus apus	Swift
	Caprimulgus europaeus	Nightjar
P. nanus (Rud. 1802)	Limnocryptes gallinula = Lymnocryptes minimus	Jacksnipe
P. permixtus Braun, 1901	Hirundo rustica	Swallow
P. triangularis (Diesing, 1850)	Merops apiaster	Bee-eater
P. vitellatus (v. Linstow, 1875)	Totanus hypoleucus = Tringa hypoleucus	Common sandpiper
P. aspersus Stossich, 1904	Plecotus auritus	Long-eared bat
P. vespertilionis (Mueller, 1784)	Rhinolophus ferrum-equinum	Greater horseshoe bat
	R. hipposideros	Lesser horseshoe bat
	Plecotus auritus	Long-eared bat
	Nyctalus noctula	Noctule

Table 1.1. continued.

Pipistrellus pipistrellus	Pipistrelle
Leuconoe daubentoni = *Myotis daubentoni*	Daubenton's bat
Myotis nattereri	Natterer's bat
M. mystacinus	Whiskered bat

* indicates description of new species.

+ indicates experimental host.

Section 2

Establishment of the life cycle and morphology of the larval stages

INTRODUCTION.

As noted above (p. 7), Plagiorchis elegans has not previously been found in Britain. Although the life cycle and morphology of the life history stages have been described (Styczynska-Jurewicz, 1962), they have not up till now been examined using a pure laboratory strain of parasite. The results of such an investigation and a comparison of P. elegans with other members of the genus based on larval morphology are given below.

MATERIALS and METHODS.

Laboratory-reared Lymnaea stagnalis were used as the first intermediate host and were maintained on a diet of lettuce, dandelion leaves and trout pellets (Cooper Nutrition Products Ltd., Stepfield, Witham, Essex). Chironomid larvae were purchased commercially and sampled on a regular basis. Examination of approximately 20% of the larvae used revealed only two metacercarial cysts, one from each of two larvae, which when fed by stomach tube to Laboratory Animal Centre Accredited (LACA) mice failed to produce an infection. Because the incidence of natural infections of Chironomus sp. were not only rare but the two cysts recovered did not infect LACA mice, the commercially obtained Chironomus sp. were considered to be a suitable second intermediate host for experimental purposes. Adult P. elegans were maintained in laboratory-reared LACA mice; the mice were fed Oxoid Pasteurized Breeding Diet and water ad libitum.

Living eggs, daughter sporocysts, cercariae and encysted metacercariae were examined unstained. Cercariae were also stained intravitally with neutral red (Humason, 1967), while additional studies of the tail and stylet of cercariae were conducted using interference microscopy. Measurements were made under high power magnification (oil immersion; x 900). Eggs, daughter sporocysts and metacercarial cysts were measured alive, while cercariae were heat killed in hot 10% formol saline (Lillie, 1965) prior to being measured. All specimens were flattened under the slight pressure of a cover-slip, but eggs were measured in utero. For the purpose of more closely examining the structure of the daughter sporocysts and cercariae of experimentally infected L. stagnalis, paraffin wax (m.p. 58°C) serial sections 5 and 7 µm were cut and stained with either Mallory's or Masson's Triple stain (Humason, 1967).

RESULTS.

Establishment of the life cycle of Plagiorchis elegans experimentally.

The life cycle of *P. elegans* was initially established in the laboratory as follows. Chironomid larvae in small volumes of water were exposed to cercariae released from a single naturally infected *Lymnaea stagnalis*. Metacercariae were teased from the larvae after eight days and fed by stomach tube to LACA mice; ten days later a single adult specimen recovered from the mice was selected and torn apart in a small petri dish containing 0.9% normal saline. The debris and saline were carefully pipetted off, replaced with distilled water and the eggs were allowed to incubate at room temperature for eight days. The period of eight days was chosen because, although miracidia are evident within some eggs after four days, it takes approximately a week for the majority of miracidia to develop. If left much longer than this under the experimental conditions employed, the miracidia apparently become susceptible to invasion by bacteria. Fifty laboratory-reared *L. stagnalis* 3.0 to 5.0 mm in length were then exposed to a total of several hundred fully embryonated eggs for 24 hours. The snails were subsequently maintained in an aerated aquarium at 22°C. Fifty-six days later 32 of the 36 surviving snails (89%) were found to be releasing cercariae, while the remaining 4 were uninfected. (Subsequent experiments demonstrated that when snails are maintained at 22°C cercarial release commences 38 to 40 days post-infection.)

Morphology of the larval stages.

Egg and miracidium:

The eggs of *P. elegans* are tanned and operculate possessing a slight protuberance at the posterior end (Fig. 2.1). They are released by the adults in the small intestine of the definitive host from whose body they are expelled in the faeces. Embryonated eggs must be eaten by the lymnaeid first intermediate host before hatching.

Measurements were made of 20 eggs in the ascending limb of the uterus in each of two living 7-day old worms harvested from mice. Table 2.1 illustrates that considerable variation occurs in the dimensions of the eggs; the combined range of lengths from both worms was greater than that for either individual worm. Further it may be noted that the mean lengths of eggs from the two adult specimens were significantly different.

Table 2.1. Measurements (in μm) of eggs in the ascending limb of the uterus of two 7-day old *P. elegans* from mice. Standard error is given in parentheses.

	number measured	mean length	mean width	range length	range width
Adult 1	20	38.5 (0.33)	23.2 (0.14)	36 - 42	22 - 24
Adult 2	20	42.3 (0.44)*	22.3 (0.11)	40 - 45	22 - 23
Total	40	40.2 (0.41)	22.8 (0.11)	36 - 45	22 - 24

* Student's *t* test $p < 0.001$

Although the anatomy of the miracidium has not been elucidated it is ciliated with an undetermined number of epidermal plates and two flame cells.

Mother and daughter sporocysts:

After ingestion the eggs hatch probably in the stomach or intestine of the snail host; the miracidium penetrates the epithelium of the snail alimentary tract before developing into a mother sporocyst between the base of this epithelium and the underlying basement membrane. The exact site of penetration has not been ascertained, but it appears that further development is limited to those miracidia that penetrate the intestine, since mother sporocysts have only been observed along this organ. When immature snails (3 to 5 mm) are used as hosts, it is possible to see the developing sporocysts throughtthe semi-transparent shell after about four weeks at 22°C. The mother sporocysts can in some instances be seen almost to encircle the intestine. Each contains many daughter sporocysts.

The daughter sporocysts are found primarily in the digestive gland and are of various shapes and sizes, depending not only on their degree of development, but on the number of germ balls and/or cercarial embryos present. Young sporocysts may be sausage shaped, have one rather pointed and one blunt end, be dumbell shaped, or have a constriction at only one end; they may be even more contorted than this (Fig. 2.5). They are motile and capable of independent migration. Older sporocysts may be cigar shaped, oval to elliptical, or bent in an L or an S shape (Fig. 2.6). In infections maintained for nine months at 17°C the daughter sporocysts range in length from 0.60 to 2.36 mm and in

width from 0.21 to 0.68 mm; they contain from 5 to 36 cercarial embryos.

The daughter sporocyst wall is composed of a single layer of cells; the wall is flatter in young sporocysts than in old sporocysts. It is ensheathed in a thick and warty membrane, which later in the infection becomes pigmented giving the snail digestive gland an orange appearance (Fig 2.7). The outer membrane has staining properties more similar to the host connective tissue than to the sporocyst wall (Fig 2.9) and as can be seen from Figure 2.8 it is not attached to the daughter sporocysts. No birth pore has been observed.

Cercaria: (Table 2.2):

The xiphidiocercaria of P. elegans (Figs. 2.2 A, B; 2.10) is a member of the Polyadena group, Cort (1915) of the Cercariae Armatae Lühe (1909). The characteristics of this group are:

1. development in elongate sporocysts within gastropods.
2. tail slender without finfolds; except when much extended tail less than body length.
3. acetabulum posterior to mid body and smaller than oral sucker.
4. stylet about 30µm in length, six times as long as broad, shoulders about two thirds of distance from base to point.
5. penetration gland cells 6 or more on each side between the acetabulum and pharynx.
6. excretory bladder bicornuate.

Body oval to elliptical in outline, covered by transverse rows of minute spines. More prominent spines present in caudal pocket; tail simple, aspinous. Hairlike processes probably tactile in function, projecting from cercarial body; their distribution not established. Small refractile granules approximately 1.4 to 5.0 µm in diameter scattered throughout body. Cystogenous gland cells numerous, obscuring much of internal structure, extending from level of pharynx to posterior extremity of the body, less pronounced in vertical midline.

Stylet (Table 2.3; Figs 2.3 A, B; 2.11) javelin shaped, dorsal to mouth; acidophilic. Point of nib curves dorsally. Thickened shoulders about two thirds of distance along shaft. Base of stylet reinforced. Invagination in centre of stylet base present in 96% of stylets examined. Abnormally developed stylets rarely observed.

Two groups of eight nucleated penetration gland cells located on either side of midline from bifurcation of caeca to near posterior limit of ventral sucker, five outer, three inner cells. Smallest of

three inner cells often obscured by cystogenous gland cells; three outer and two inner anterior gland cells basophilic. Three posterior gland cells acidophilic. Penetration gland ducts following a slightly sinuous course to open at stylet nib.

Table 2.2. Measurements of cercaria of P. elegans (n = 40; measurements in mm).

	mean		range	
	length (SE)	width (SE)	length	width
body	0.240(0.006)	0.100(0.003)	0.160–0.330	0.080–0.140
tail	0.180(0.006)	0.020(at base)	0.110–0.220	0.020
oral sucker	0.048(0.001)	0.053(0.002)	0.038–0.059	0.040–0.072
ventral sucker	0.035(0.001)	0.033(0.001)	0.023–0.043	0.023–0.045
pharynx	0.019(0.003)	0.020(0.001)	0.013–0.025	0.014–0.027

Table 2.3. Cercaria of P. elegans, stylet measurements (n = 40; measurements in μm)

	mean (SE)	range
length	28.4(0.18)	27 - 31
width at shaft	4.1(0.05)	4 - 5
shoulders: length	4.05(0.03)	4 - 5
width	7.10(0.08)	6 - 8
base: length	5.40(0.11)	4 - 6
width	6.00(0.05)	5 - 7
invagination: depth in base	2.75(0.17)	absent to 4

Two cerebral ganglia connected by transverse commissure dorsal to pharynx; three nerves observed arising from each ganglion - dorsal, ventral and lateral.

Mouth aperture on ventral surface approximately in centre of oral sucker. Prepharynx short followed by trilobed muscular pharynx.

Oesophagus short, bifurcating into intestinal caeca about midway between oral sucker and acetabulum. Caeca extending nearly to posterior extremity of body.

Excretory bladder situated within posterior third of body and with shape characteristic of genus Plagiorchis Lühe, 1899; when distended has form of anterior and posterior chambers linked by narrow waist, chambers approximately equal in length, anterior ⌣-shaped, posterior spherical, lined with epithelial cells. Whole bladder extremely contractile. Excretory pore ventral, near base of tail.

Pattern of protonephridial system mesostomate. Main excretory duct slightly convoluted and subterminal. Secondary anterior and posterior collecting ducts join main duct at approximately posterior limit of ventral sucker. Flame cell pattern difficult to establish but apparently similar to that described by McMullen (1937a) for genus Plagiorchis - $2[(3 + 3 + 3) + (3 + 3 + 3)]$.

In some specimens an apparent longitudinal duct present in tail; when present not seen to connect with excretory bladder anteriorly nor to tip of tail (Fig. 2.12, 2.13).

Genital anlage dorsal to ventral sucker, consisting of C - shaped mass of cells extending approximately from slightly beyond anterior margin of acetabulum to slightly beyond its posterior border. In dorsal plan view of specimens flattened under slight pressure of cover slip anlage displaced to right side of ventral sucker.

Metacercaria (Fig. 2.4; Table 2.4).

Cyst elliptical to round in outline with wall thin and pliable when first formed. Stylet released within cyst, eventually disintegrating, but both stylet and penetration glands occasionally remain visible after 40 days. Excretory bladder of various shapes most often ⌣ or ⋈ - shaped. Several days after encystment bladder filled with dark concretions. Caeca more distinct than in cercaria , probably because cystogenous gland cells have discharged their contents. Genital anlage as in cercaria.

Table 2.4. Measurements (in mm) of metacercarial cysts of P. elegans from chironomid larvae.

age	no. measured	mean dimensions (SE)
8 hours	20	0.168(0.004) x 0.153(0.002)
24 hours	20	0.138(0.003) x 0.125(0.002)
7 days	20	0.132(0.002) x 0.125(0.002)
40 days	20	0.128(0.002) x 0.122(0.001)

DISCUSSION.

The life cycles of only a small proportion of Plagiorchis species (about 18 out of 140) have been completed using naturally infected snail first intermediate hosts. Among the life cycles investigated but without the establishment of a laboratory strain are:

species of Plagiorchis	reference
P. muris (Tanabe, 1922)	Dollfus (1925)
P. ramlianum (Azim, 1935)	Azim (1935)
P. muris (Tanabe, 1922)	McMullen (1937 b)
P. proximus Barker, 1915	McMullen (1937 b)
P. micracanthos Macy, 1931	McMullen (1937 b)
P. megalorchis Rees, 1952	Rees (1952)
P. cirratus (Rud. 1802)	Buttner & Vacher (1959)
P. vespertilionis parorchis Macy, 1960	Macy (1960)
P. elegans (Rud. 1802)	Styczynska-Jurewicz (1962)
P. noblei Park, 1936	Williams (1963)
P. dilimanensis Velasquez, 1964	Velasquez (1964)
P. laricola Skrjabin, 1924	Zdarska (1966)
P. momplei Dollfus, 1932	Richard et al. (1968)
P. peterborensis Kavelaars & Bourns, 1968	Kavelaars & Bourns (1968)
P. neomidis Brendow, 1970	Brendow (1970)
P. farnleyensis Diaz, 1976	Diaz (1976)
P. kirkstallensis Diaz, 1976	Diaz (1976)
P. neomidis Brendow, 1970	Theron (1976)

The four species listed below are the only ones where laboratory infections of the first intermediate host have been described:

species of Plagiorchis	reference
P. jaenschi Johnston & Angel, 1951	Johnston & Angel (1951)
P. maculosus Angel, 1959	Angel (1959)
P. goodmani Najarian, 1961	Najarian (1961)
P. noblei Park, 1936	Blankespoor (1974; 1977)

Blankespoor (1974, 1977) infected laboratory-reared snails with the eggs of one worm to obtain a pure strain, but unfortunately did not describe the larval stages. Johnston and Angel (1951), Angel (1959) and Najarian (1961) established laboratory infections of P. jaenschi, P. maculosus and P. goodmani respectively, although in each case they

exposed snails to the eggs of more than one worm; as a result their experimental strains were subject to variation due to their mixed genetic backgrounds.

By infecting snails with the eggs of a single individual the writer was enabled to study the structure and morphological variation of the larval stages of a pure strain of P. elegans. The eggs of P. elegans are 40.2 µm (36-45 µm) long and 22.8 µm (22-24 µm) wide. While egg size has been considered to be of diagnostic significance by some taxonomists, notably Blankespoor (1974), the dimensions of many members of the genus fall within or overlap the range of those of P. elegans measured during the present study (Appendix 1). The size of the egg is considered by the writer to be of some diagnostic significance and should certainly be included in the species descriptions, but experimental evidence obtained by the author has demonstrated that the length of the egg may vary significantly even between samples of a clone, the same age and in the same definitive host species. In the literature many measurements of eggs are given without reference to the age of the adult specimen, number of eggs measured, species of host or whether the eggs are measured in utero or teased from the worm. Such data should always be included when egg dimensions are used in specific descriptions.

Daughter sporocysts vary considerably in size, depending not only on their degree of development, but on the number of cercarial embryos and germ cells present. Plagiorchis ramlianum (Azim, 1935) can be distinguished from P. elegans and from all other members of the genus, because it develops in rediae and its cercaria possesses an I-shaped excretory bladder. For these reasons this species should probably be removed from the genus Plagiorchis.

Cort and Ameel (1944) described the outer epithelium or paletot of the daughter sporocysts as being derived from the mother sporocysts and noted that in mature daughter sporocysts the paletot acquires an orange pigment. A contrasting view was expressed by Schell (1961); after studying the development of another plagiorchiid, Haplometrana intestinalis Lucker, 1931, he described the paletot as being of host origin. Schell suggested that the connective tissue cells of the mother sporocyst membrane, originally derived from the basement membrane of the snail's alimentary tract, proliferate and invade the mother sporocyst, eventually partitioning off the developing daughter sporocysts. The writer agrees with Schell that the paletot is of host origin for three reasons:

firstly, the paletot is not attached to the daughter sporocyst wall;

secondly, its staining properties are similar to those of the host connective tissue rather than to the parasite wall,

finally, the acquisition of an orange pigment by the paletot is consistent with the occurrence of a host response (Erasmus, 1972).

Many descriptions concerned with cercarial morphology are incomplete, making an accurate comparison of *Plagiorchis* species difficult and in some cases impossible. Rees (1952) however has provided an excellent account of the life history and larval stages of *P. megalorchis*. The larval stages of *P. megalorchis* can be distinguished from those of *P. elegans* by the small size of the metacercaria and egg in the former species and also by the presence of a caudal excretory duct in the tail of the cercaria of *P. megalorchis* which connects with the bladder. Although a structure resembling a duct in the tail of *P. elegans* has been seen (p. 21), it was not present in all cercariae examined and in no instance has it been observed either to connect with the excretory bladder or to extend to the tip of the tail. Among other cercariae possessing an apparent caudal duct are *P. neomidis* Brendow (1970), *P. cirratus* Buttner & Vacher (1959) and *P. kirkstallensis* Diaz (1976).

The cystogenous gland cells frequently obscure the internal structures of the cercariae, in particular the penetration gland cells, flame cells, caeca and the genital anlage so that one must accept that descriptions will agree only within limits. The cercariae of *P. muris* McMullen (1937b), *P. laricola* Zdarska (1966) and *P. elegans* Styczynska-Jurewicz (1962) are very similar to those of *P. elegans* described in the present account, but each of them has fewer than 8 pairs of penetration gland cells; *P. jaenschi* Johnston & Angel (1951) has 10 pairs, while no record of the number of penetration gland cells is available for *P. vespertilionis parorchis* Macy (1960), *P. peterborensis* Kavelaars & Bourns (1968), *P. momplei* Richard *et al.* (1968), or *P. proximus* McMullen (1937b).

In the present investigation 15 flame cells were found on either side of the cercarial body, although the established flame cell formula for the genus is $2\ [(3 + 3 + 3) + (3 + 3 + 3)] = 36$ McMullen (1937a). However in the original description of the excretory system of *Cercaria polyadena* Cort (1915) found only two flame cells in the posterior group on each side, $2\ [(3 + 3 + 3) + (3 + 3 + 2)] = 34$.

In contrast to the cercaria of *P. elegans* in which the caeca extend to the posterior of the body, the caeca are very short in the cercariae of *P. goodmani* Najarian (1961) and *P. momplei* Richard *et al.*

(1968), while the caeca of P. farnleyensis Diaz (1976) are of intermediate length, being 2/3 the length of the body. The lengths of the caeca were not given for the cercariae of P. maculosus Angel (1959), P. vespertilionis parorchis Macy (1960), or P. neomidis Theron (1976).

The stylets of P. laricola Zdarska (1966), P. peterborensis Kavelaars & Bourns (1968) and P. elegans as noted in the present work are closely similar in that in all three species a slight invagination is present in the middle of the stylet base. Yet, as stated previously, this invagination was not present in all of the stylets examined by the writer and therefore should not be used as a diagnostic character. The size of the stylets may vary directly with the size of the cercariae and since the cercariae vary substantially in size even within a pure strain, the size of the stylet is considered to be a questionable criterion for distinguishing between species. The writer has noted only a single case in which a stylet was not evident; refractile globules could be seen in the position where the stylet should have been present.

Infections of P. kirkstallensis were obtained by Diaz (1976) from Lymnaea stagnalis collected from the same site at which the present author initially obtained infections of P. elegans. The cercariae of the two species are very similar and in both species abnormally developed stylets are known to occur. However in contrast to P. elegans in which small refractile granules are scattered throughout the cercarial body and a few are present in the tail, no refractile granules were present in the cercaria of P. kirkstallensis. The main excretory ducts of P. kirkstallensis are terminal rather than subterminal as in P. elegans. Furthermore, Diaz (1976) stated that 8 or 9 hairlike structures are present on both sides of the cercarial body. Although such structures are known to occur in P. elegans they are very difficult to see; they were more obvious in some cercariae than others and as a result their distribution was not ascertained.

It may be noted here that the cercaria of P. noblei Williams (1963) very closely resembles both the cercaria of P. kirkstallensis and that of P. elegans, apart from the presence of refractile granules in the latter species. Based on the adult morphology (p.111) the three species are considered by the author to be synonymous and the presence of refractile granules in the cercaria of P. elegans and their absence in the cercariae of P. kirkstallensis and P. noblei are believed to be a manifestation of intraspecific variation.

A number of xiphidiocercariae are morphologically very similar to the cercaria of P. elegans. They are the cercariae of:-

species	reference
P. micracanthos Macy, 1931	McMullen (1937 b)
P. muris (Tanabe, 1922)	McMullen (1937 b)
P. cirratus (Rud. 1802)	Buttner & Vacher (1959)
P. vespertilionis parorchis Macy, 1960	Macy (1960)
P. elegans (Rud. 1802)	Styczynska-Jurewicz (1962)
P. noblei Park, 1936	Williams (1963)
P. laricola Skrjabin, 1924	Zdarska (1966)
P. peterborensis Kavelaars & Bourns, 1968	Kavelaars & Bourns (1968)
P. kirkstallensis Diaz, 1976	Diaz (1976)

The metacercariae of P. cirratus Buttner & Vacher (1959), P. neomidis Brendow (1970), P. fastuosus Krasnolobova (1973) and P. neomidis Theron (1976) can be distinguished from the metacercaria of P. elegans because in the three former species the genital anlage develops into gonads while the organism remains within the metacercarial cyst; sperm are found in the seminal vesicle, but no eggs are present in utero, while in the latter species no further development of the genital anlage occurs. It is not clear from the above articles whether or not the development of the gonads is a necessary prerequisite for the onset of infectivity towards the final host.

In an attempt to distinguish between different species of Plagiorchis many taxonomists use criteria which do not allow for the existence of morphological variation in the larval stages, in particular that of the cercaria; consequently a number of species have been erected on the basis of inadequate evidence. Although the cercaria of those species listed above are morphologically similar to P. elegans, a discussion of the question of synonymy is deferred until data concerning various other aspects of the life cycle of P. elegans are presented.

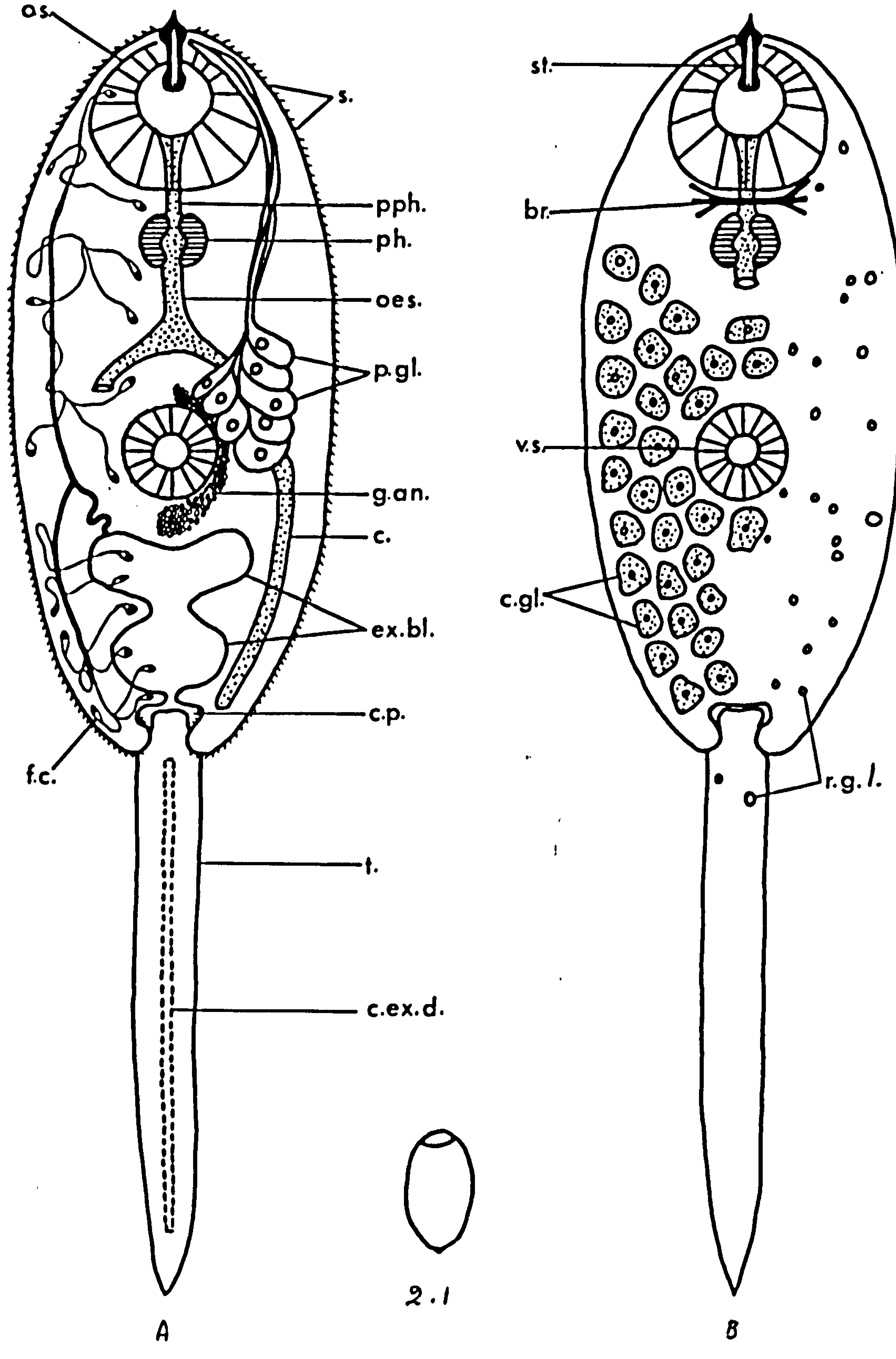
Fig. 2.1. Egg of <u>P</u>. <u>elegans</u>.

Fig. 2.2 A,B. Semidiagrammatic illustrations of the cercaria of <u>P</u>. <u>elegans</u>, dorsal view. For the sake of clarity bilateral systems are drawn of only one side of the body.

A. To show digestive system, penetration gland cells, genital anlage, excretory bladder, flame cells (from Rees (1952)) and caudal "duct".

B. To show distribution of cystogenous gland cells, refractile granules and brain.

br.	brain
c.	caeca
c. gl.	cystogenous gland cells
c.p.	caudal pocket
ex.bl.	excretory bladder
f.c.	flame cells
g.an.	genital anlage
oes.	oesophagus
o.s.	oral sucker
p.gl.	penetration gland cells
ph.	pharynx
pph.	prepharynx
r.gl.	refractile granules
s.	spines
st.	stylet
t.	tail
v.s.	ventral sucker
c. ex. d.	caudal excretory duct.



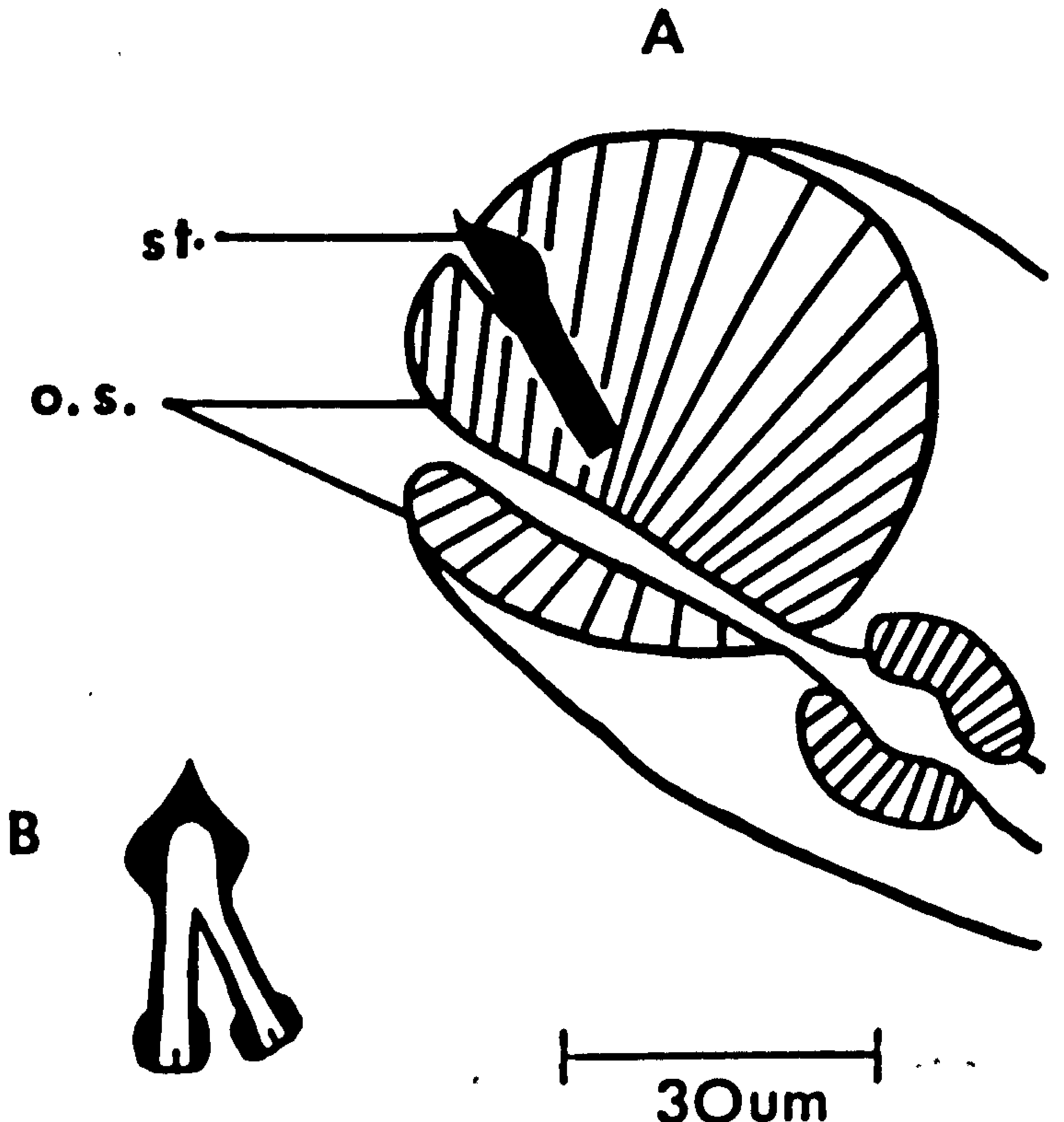
os.
s.
pph.
ph.
oes.
p.gl.
g.an.
c.
ex.bl.
c.p.
f.c.
t.
c.ex.d.
st.
br.
v.s.
c.gl.
r.g.l.
2.1
A
B
50μm

Fig. 2.3 A. Diagram of sagittal section through oral sucker of cercaria illustrating the orientation of the stylet.

B. Abnormally developed stylet.

Fig. 2.4. Mature metacercarial cyst.

c.w.	cyst wall
ex.bl.	excretory bladder
o.s.	oral sucker
ph.	pharynx
st.	stylet
v.s.	ventral sucker


A
st.
o.s.
B
30um

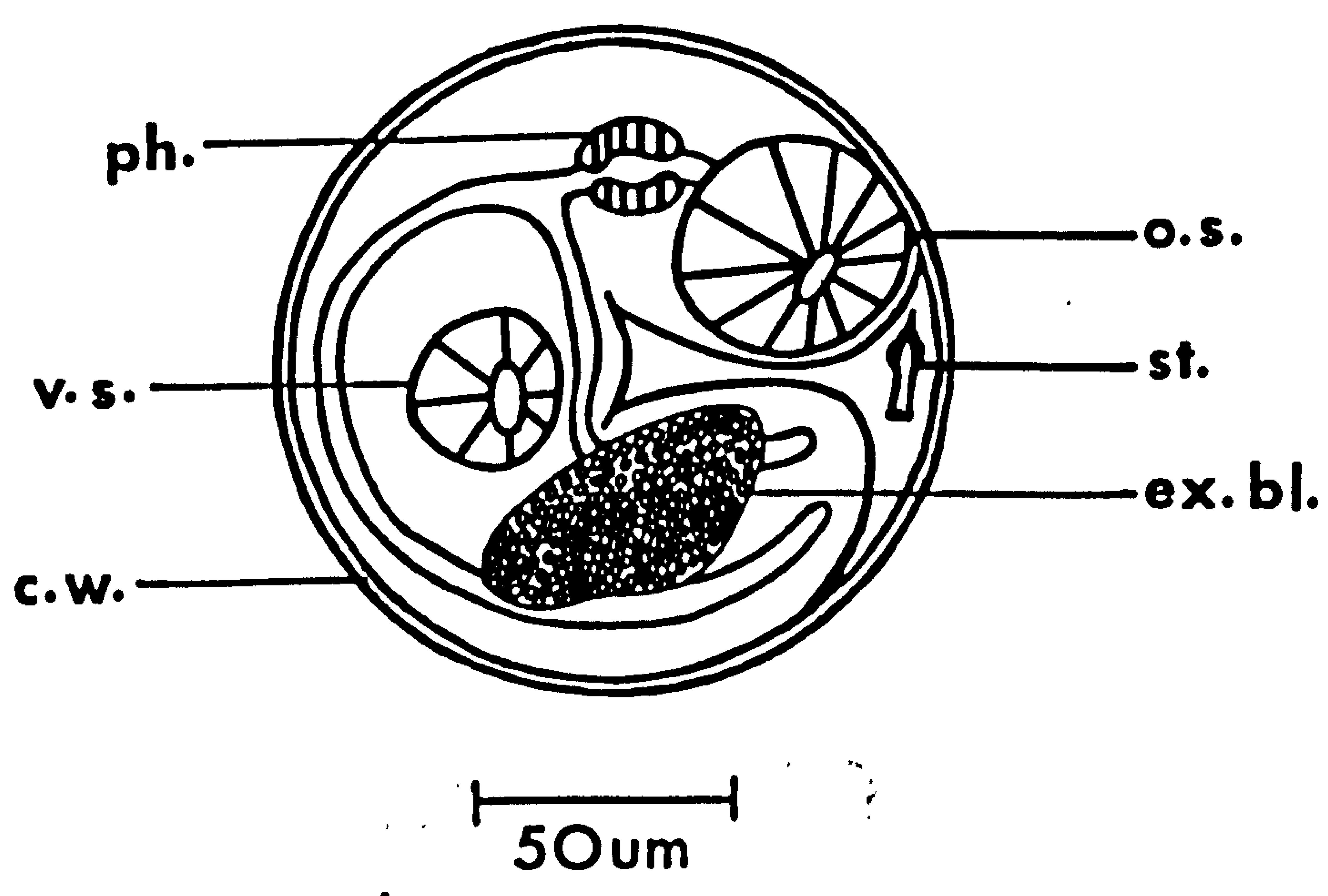
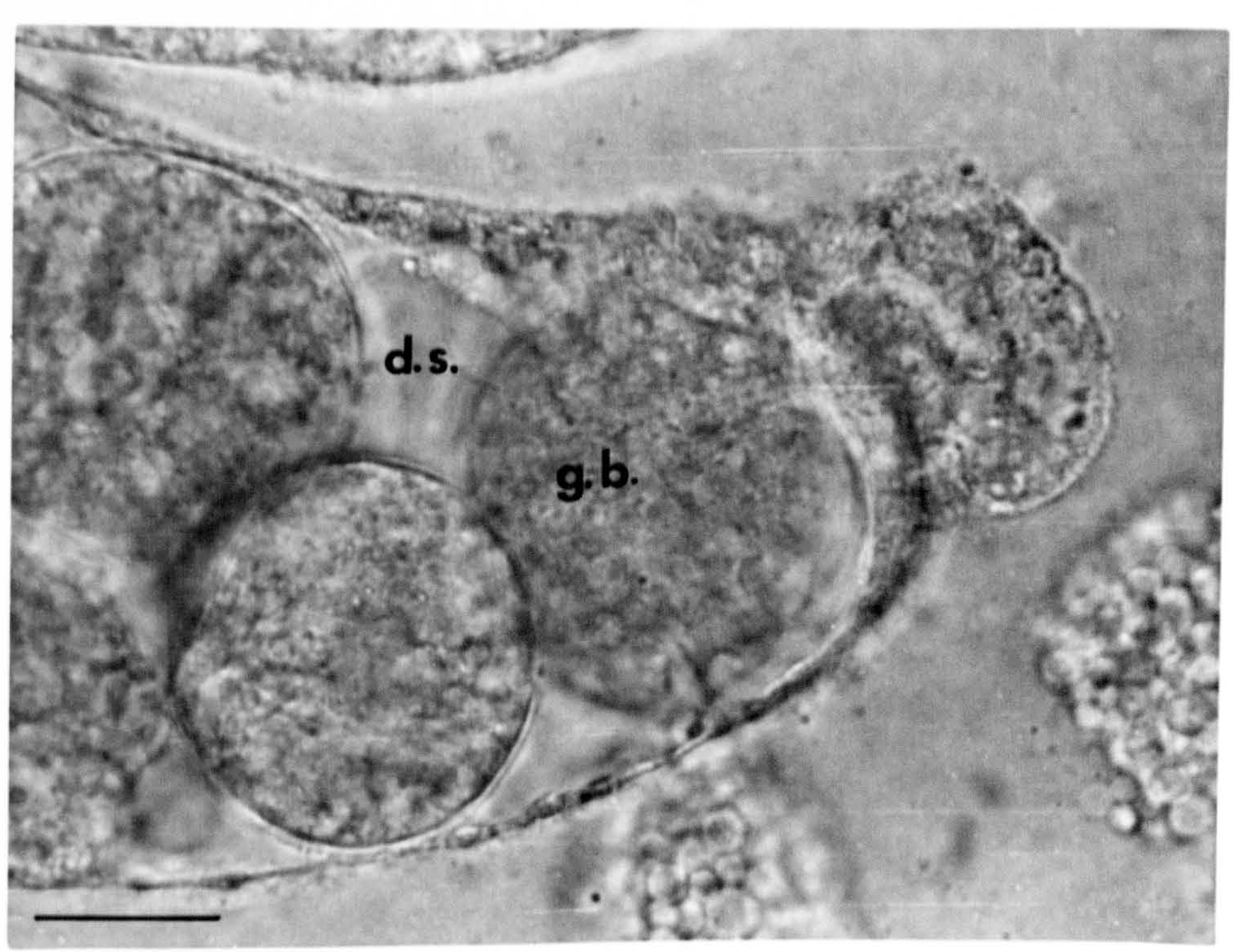

ph.
o.s.
st.
v.s.
ex.bl.
c.w.
50um

Fig. 2.5. Part of an immature daughter sporocyst in migratory phase containing germ balls. Scale 50µm.

Fig. 2.6. Mature daughter sporocysts (d.s.) illustrating the variation in size and shape of the sporocysts and in the number of germ balls (g.b.) and cercariae (c.) present. Scale 200µm.


d.s.
g.b.

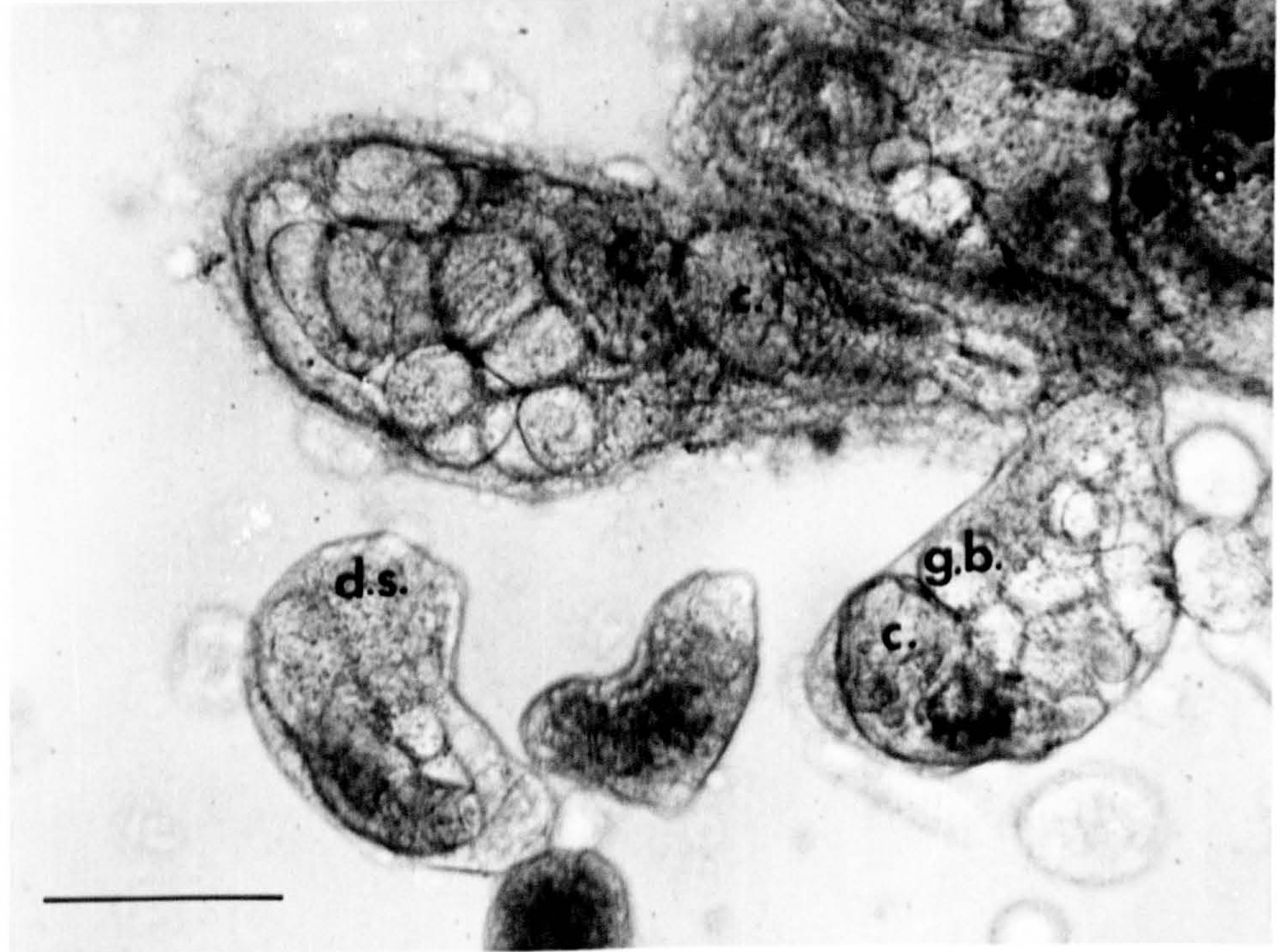

c.
d.s.
g.b.
c.

Fig. 2.7. In mature infections of L. stagnalis the daughter sporocysts become pigmented giving the digestive gland an orange appearance. Scale 3.0mm.

d.gl.	digestive gland
d.sp.	daughter sporocysts
st.	stomach

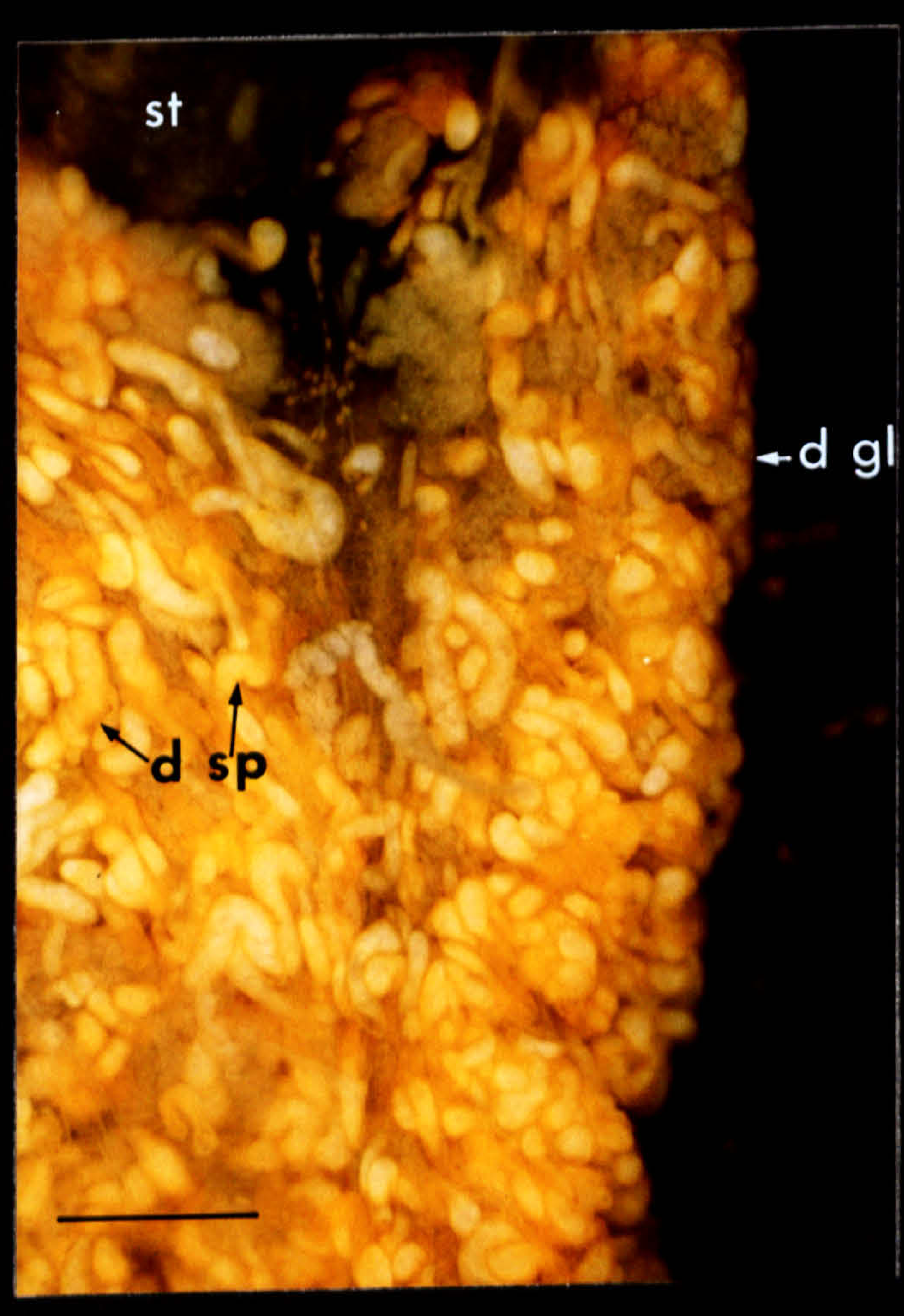
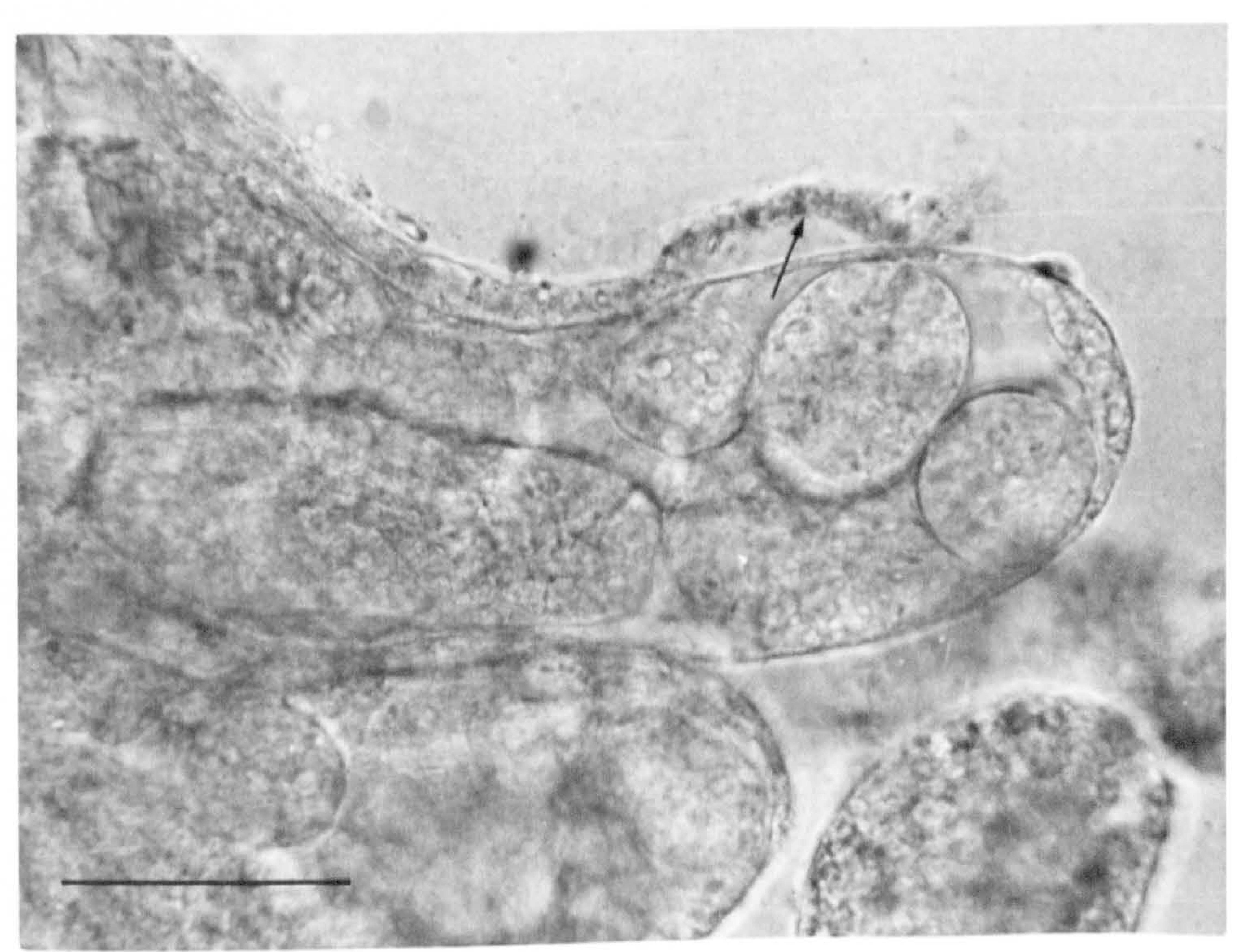

st
d gl
d
sp

Fig. 2.8. Living daughter sporocyst of P. elegans from freshly killed L. stagnalis. Note that thickened outer epithelium or paletot (arrow) has become separated from smooth epithelium of daughter sporocyst.

Fig. 2.9. Section through digestive gland of infected L. stagnalis demonstrating different staining intensities of the paletot and daughter sporocyst epithelium. Scale 50µm.

c.	anterior region of cercarial embryo
d.g.	digestive gland cells
d.e.	daughter sporocyst epithelium
h.t.	host connective tissue
p.	paletot (thickened outer epithelium of daughter sporocyst).



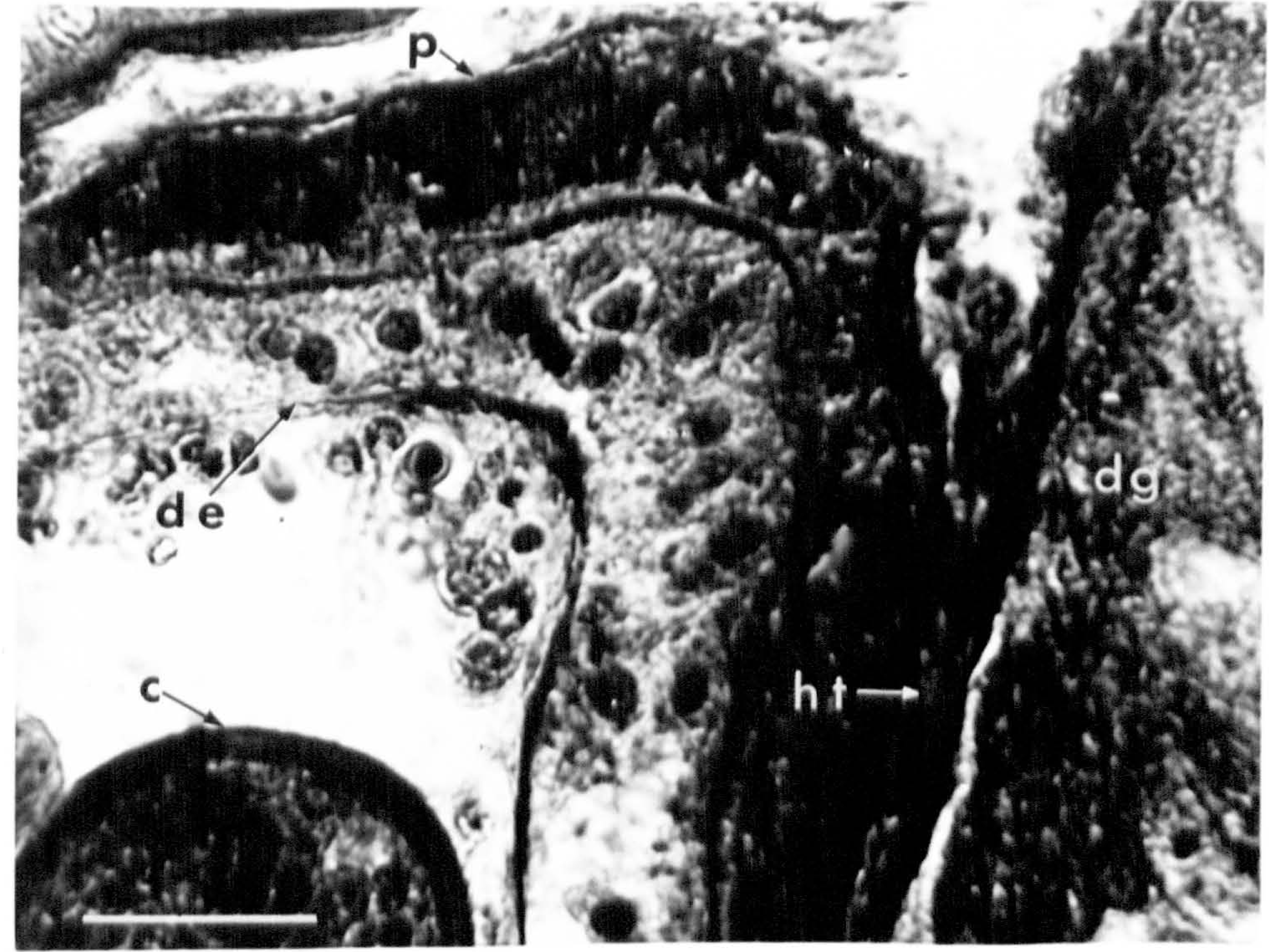
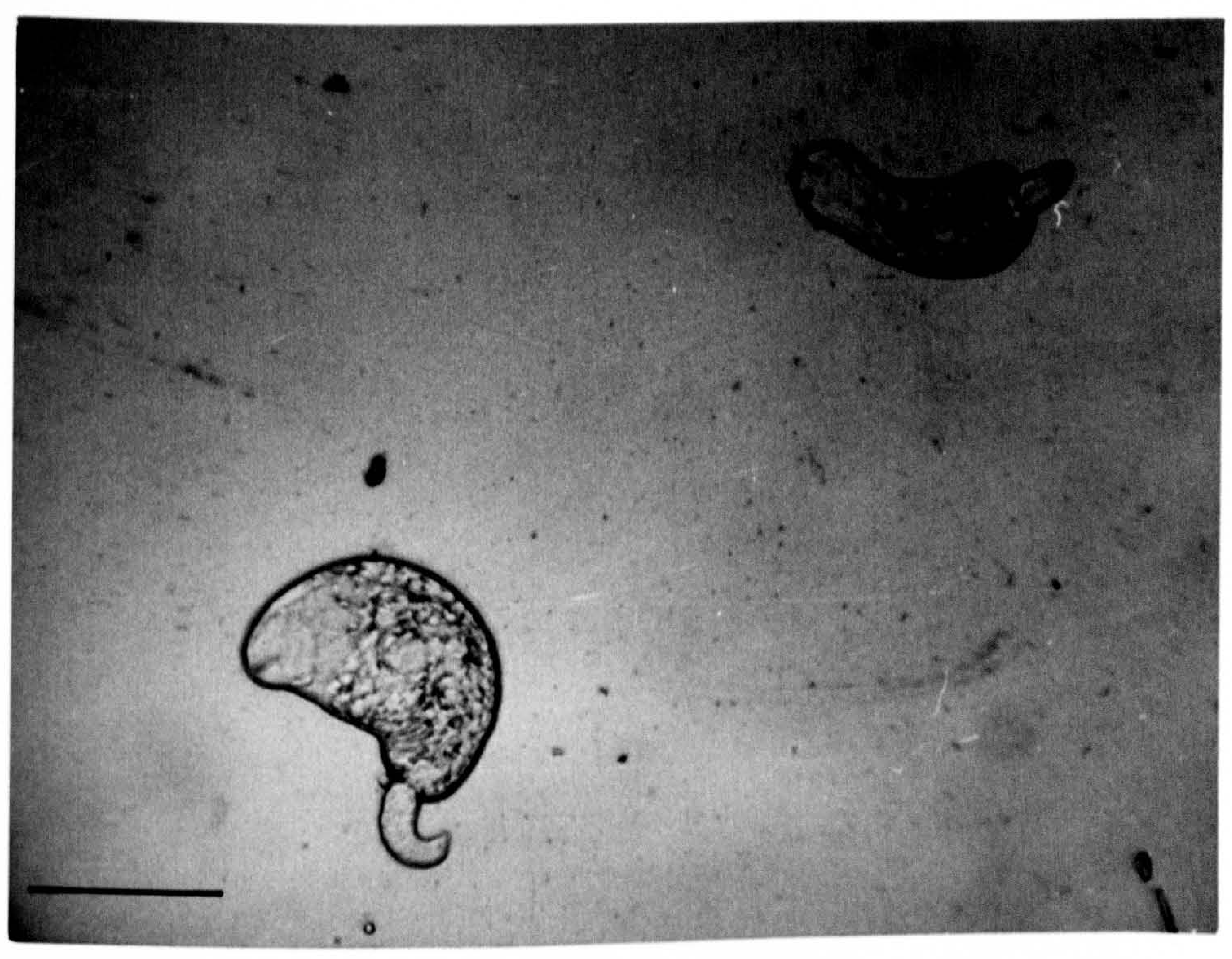

p
de
c
ht
dg

Fig. 2.10. Cercariae released from a single L. stagnalis infected with eggs from one P. elegans reflecting the variation in the size of the specimens recovered. Scale 150μm.

Fig. 2.11. Stylet of P. elegans cercaria as seen using interference microscopy. Note the small invagination in the centre of the stylet base(arrow). Scale 25μm.



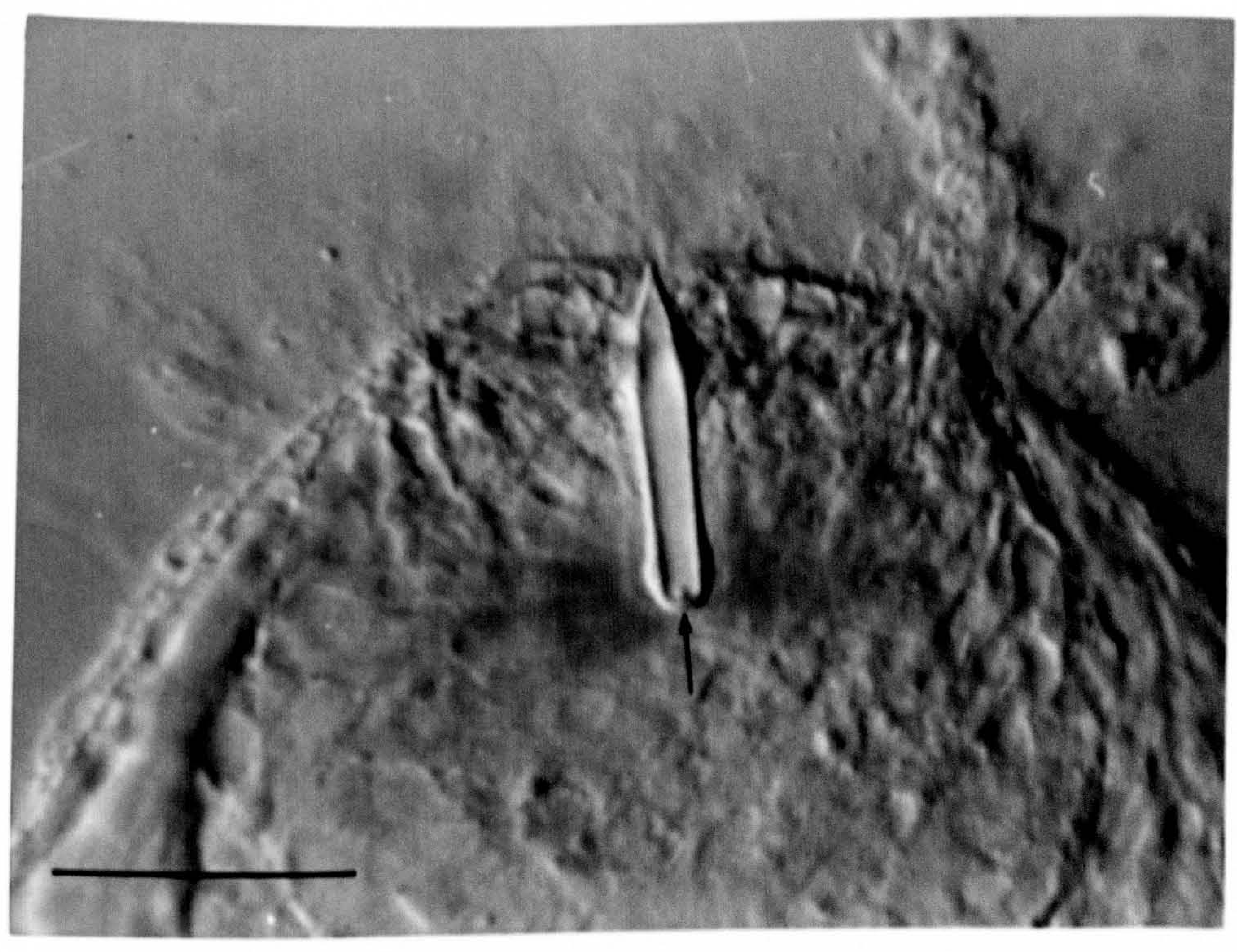
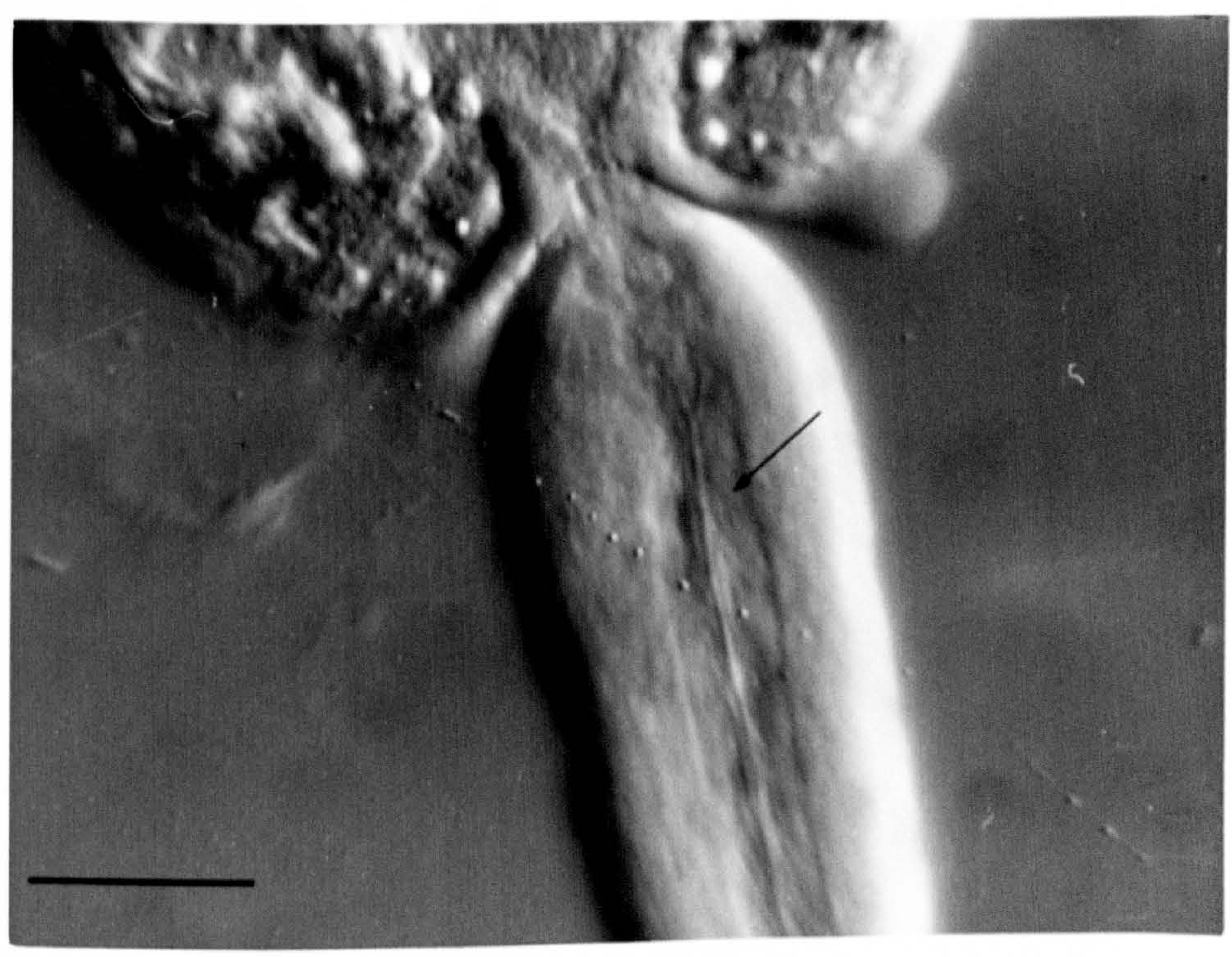
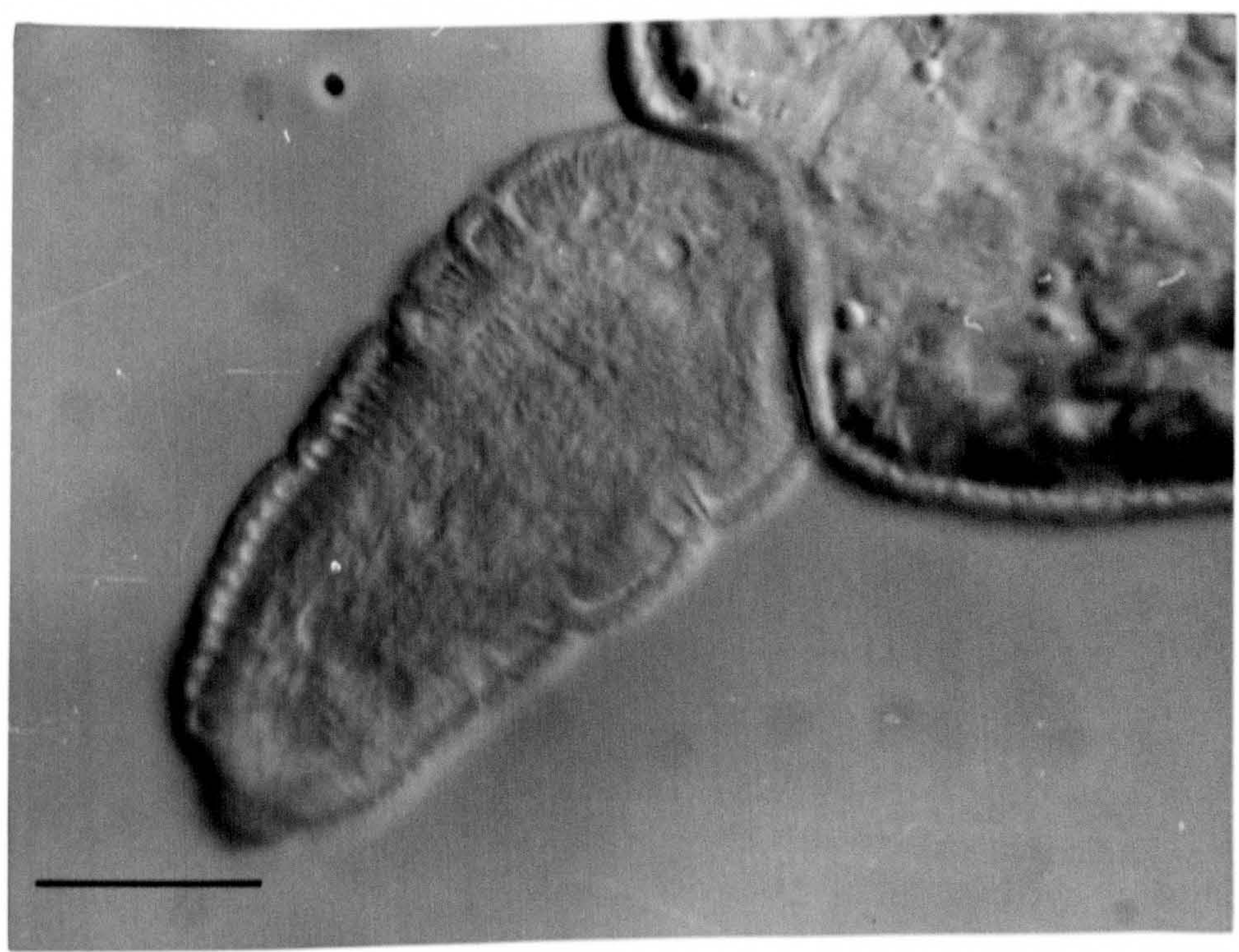


Fig. 2.12. Tail of cercaria of P. elegans as shown by interference microscopy; apparent caudal duct present. Scale 15μm.

Fig. 2.13. Tail of cercaria of P. elegans (specimen different from Fig. 2.12) also as seen using interference microscopy; caudal duct absent. Scale 15μm.





Section 3

The first intermediate host.

INTRODUCTION.

The vast majority of studies concerning the host-parasite relationship of larval digeneans and molluscs deal with the Fasciolidae and Schistosomatidae because of their importance economically and in the field of public health (Kendall, 1965; Boray, 1969; Meuleman, 1972). Although the life cycles of many *Plagiorchis* species have been described, relatively little is known either about their development in, or relationship to, their first intermediate gastropod hosts. Cort and Olivier (1943) investigated the larval development of *P. muris* Tanabe, 1922 in naturally occurring infections of *Stagnicola emarginata*; later, Cort and Ameel (1944) continued these investigations and expanded them to include *P. micracanthos* Macy, 1931 and *P. proximus* Barker, 1915.

An excellent review of the pathology of helminths in molluscs has been written by Wright (1966); more specifically, contributions to the knowledge of pathological effects of *Plagiorchis* species have been made by Pratt and Barton (1941) and Rees (1952). The former authors studied the damage inflicted by four species of larval digeneans, among them *P. muris* McMullen, (1937, upon the hepatopancreas and ovotestis of the pulmonate snail *Stagnicola emarginata*. In particular they noted that in serial histological sections of one specimen infected by *P. muris* the ovotestis and reproductive ducts were apparently absent. They also reported that only exceptionally was there a breakdown of the digestive gland tubules. In contrast to these observations Rees (1952), studying the effects of *P. megalorchis* on *Lymnaea peregra*, noted extensive damage to the digestive gland tubules in some instances, but she did not refer to any changes in the state of the host reproductive system.

The present investigation was undertaken to elucidate the relationship, including specificity, rate of development, and pathology of the infections, between an experimentally established strain of *P. elegans* and its lymnaeid first intermediate hosts.

MATERIALS and METHODS.

Adult *Lymnaea stagnalis*, *L. peregra* and *L. palustris* were bred in the laboratory in order to obtain cultures of uninfected snails for the purpose of examining the specificity of *P. elegans* towards the first intermediate host. The parent stocks of both *L. stagnalis* and

L. peregra were collected from the Leeds-Liverpool Canal, with additional specimens of L. peregra obtained from the River Wharfe at Otley. Lymnaea palustris was collected from Golden Acre Park and Market Weighton.

The laboratory-reared snails were exposed, unless otherwise stated, to known numbers of fully embryonated eggs of the experimentally established strain of P. elegans (p. 17). Subsequently the molluscs were maintained in aerated aquaria at either 18° or 22°C, under continuous fluorescent light, fed lettuce and dandelion leaves ad libitum, with commercial trout pellets (Cooper Nutrition Products Ltd,, Stepfield, Witham, Essex) to supplement the diet. The water was changed three times per week.

For histological sections, specimens of both uninfected and infected L. stagnalis were removed from their shells, fixed in warm Bouin's solution, embedded in paraffin wax (m.p. 58°C) and sectioned at 8µm. The sections were stained with Mallory's triple stain (Humason, 1967).

RESULTS.

Host specificity.

The exposure of L. stagnalis (3 to 5mm long) to undetermined numbers of P. elegans eggs.

In the first experiment (Table 3.1) 50 L. stagnalis (3 to 5mm long) were placed in each of four (5cm) petri dishes together with several hundred eggs teased from one ten-day old worm and incubated for eight days; this period was sufficient for the majority of the eggs to become embryonated. After several days' incubation the eggs were normally found to adhere to the bottom of the petri dish because of a growth of algae. Just before placing the snails in the dishes, most of the distilled water which had covered the eggs was removed leaving only a fine film of moisture to protect the eggs from dehydration. The snails were introduced and then approximately 15cm^3 of dechlorinated water was pipetted gently into each dish so as not to disturb the carpet of eggs on the dish bottoms. The petri dish covers were replaced and the pulmonates left at room temperature for 24h. No additional food was provided for the snails during this time in an attempt to induce them to eat the algal covered eggs and not float on the surface of the water while eating lettuce. Three of the petri dishes still

containing the snails, faeces and any uningested eggs were uncovered and placed in separate aerated aquaria at 18° C, in addition a control group of 50 uninfected L. stagnalis (3 to 5mm long) was maintained under the same conditions. In order to determine the effect of temperature on the rate of development of P. elegans the fourth group exposed to eggs was kept at 22°C as well as another control group of 50 uninfected L. stagnalis (3 to 5mm long).

After 56 days two groups were examined for the release of cercariae - group 2 (18°C) and group 4 (22°C). Twenty-five (50%) of group 2 were releasing cercariae. A further 14 (28%) were known to be infected because sporocysts could be seen through their semitransparent shells. As explained on p.18 these are daughter sporocysts. The remaining 11 snails showed no signs of infection at this time. Thirty-two (89%) of the surviving 36 snails of group 4 were releasing cercariae and no sporocysts were observed through the shells of the other 4 snails, nor did they at any time release cercariae.

Sixty-two days later (a total of 118 days post-infection; Table 3.1) all the groups were examined. It is of note that in addition to the 39 snails of group 2 known to be infected 56 days after exposure to the eggs of P. elegans, 3 more snails were releasing cercariae 118 days after exposure. Table 3.1 illustrates that the majority of immature L.stagnalis became infected with P. elegans and that temperature, at least between 18 and 22°C, is apparently not a factor in the number of snails becoming infected although as shown above it does influence the rate of larval development.

Table 3.1. Percentage infection of immature L. stagnalis 118 days after exposure to P. elegans eggs. (Fifty snails per group initially.)

	number infected (%)	number uninfected (%)	total surviving (%)
		18°C	
Group 1	27 (68)	13 (32)	40 (80)
Group 2	42 (88)	6 (12)	48 (96)
Group 3	24 (80)	6 (20)	30 (60)
Control	0 (00)	39 (100)	39 (78)
		22°C	
Group 4	26 (87)	4 (13)	30 (60)
Control	0 (00)	32 (100)	32 (64)

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Exposure of immature L. stagnalis to 1, 10 or 50 P. elegans eggs.

Specimens of L. stagnalis 4 to 8mm in length were exposed to 1, 10 or 50 mature eggs of P. elegans and the rate of infection determined (Table 3.2). In each experiment eggs were teased from P. elegans in small petri dishes and incubated at room temperature for varying lengths of time. At first eggs appearing to be embryonated when observed under a dissecting microscope (x 70) were more closely examined using a high power microscope (x 400) for the presence of a fully developed miracidium before they were fed to snails. Eventually it was possible, with an accuracy of 99%, to distinguish fully embryonated eggs from immature or non-viable eggs using a dissecting microscope by the clear central area and refractile granules seen at the periphery of the former eggs as opposed to the reticular appearance of the latter eggs (Fig. 3.1). The 1% of eggs erroneously diagnosed as fertile under the dissecting microscope were either non-embryonated or had been invaded by bacteria and killed. Because it was no longer necessary to use a high power microscope less manipulation of the eggs was required and therefore fewer of them were lost.

Eggs of P. elegans were gently detached from the bottom of the petri dish with a dissecting probe, so that when floating they could be counted as they were drawn into a fine (approximately 1.0mm diameter) glass tube by capillary action, and immediately expelled into a 5.0 cm^3 well of an immunological tray. A single L. stagnalis was carefully placed directly on top of the drop containing the desired number of eggs, then approximately 3cm^3 of dechlorinated water was gently pipetted into each well. To prevent evaporation and the snails from escaping the tray was covered with a glass plate and incubated at 18°C for 3 days. In an effort to give the snails the greatest opportunity of encountering and eating the eggs, lettuce was not provided until the second day and the water was not changed (although water lost through evaporation was replaced) until the fourth day post exposure, at which time the snails were placed together, according to the dosage, in aerated aquaria at 18°C. Seventeen days later all the aquaria were placed in a 22°C constant temperature room and after a further 21 days the snails were examined under a dissecting microscope for the presence of sporocysts, which as explained above can be seen through their semitransparent shells. Infected and uninfected specimens were thereafter maintained in separate aquaria until they died; all the snails initially observed to be uninfected remained uninfected.

As demonstrated in Table 3.2 substantially greater percentages

of L. stagnalis were infected when the snails were exposed to 10 and 50 than to single eggs of P. elegans. It is also of note that, regardless of the dosage, between 76 and 90% of the snails survived at least 42 days following exposure.

Table 3.2. Rate of infection of immature L. stagnalis after exposure to 1, 10 or 50 embryonated eggs of P. elegans. (* eggs from self-fertilized worm).

number of eggs	1	1*	10	50
number of snails	57	57	38	20
size (mm)	4-6	4-8	4-6	4-6
incubation of eggs (days)	13	45	24	12
snails surviving after 42 days	45	48	29	18
number infected	6	3	18	14
% of surviving snails infected	13.3	6.2	62.0	76.6

Exposure of L. peregra and L. palustris to eggs of P. elegans.

1st experiment: Twenty-one L. peregra (2 to 3mm long; parent stock from Kirkstall Power Station, Leeds) and 24 L. palustris (2 to 6mm long; parent stock from Golden Acre and Market Weighton) were each exposed to 50 embryonated eggs of P. elegans as described above. They were subsequently maintained at 18°C in separate aquaria according to species (Table 3.3). Controls consisting of 20 L. peregra (2 to 3mm) and 20 L. palustris (2 to 6mm) were also maintained at 18°C in separate aquaria.

In no instance were cercariae released by L. peregra nor were any sporocysts visible through their shells when the snails themselves were examined microscopically 68 days after exposure. Dissection 14 days later of both the exposed and control snails revealed no trace of infection.

Lymnaea palustris possesses a thicker and more heavily pigmented shell than either L. stagnalis or L. peregra and it was not possible to ascertain without dissection whether any of the 6 snails not releasing cercariae 83 days after exposure harboured immature infections.

After a further 39 days 19 of the surviving 22 (86.8%) snails were releasing cercariae; the remaining 3 snails and the controls were dissected and found to be free of infection.

Table 3.3. Exposure of immature L. peregra and L. palustris to 50 embryonated eggs of P. elegans.

species	L. peregra	controls	L. palustris	controls
size mm	2-3	2-3	2-6	2-6
number of snails	21	20	24	20
eggs incubated days	37	-	24	-
number of snails surviving (days post infection)	17 (68)	19 (68)	23 (83) 22 (122)	20 (83) 18 (122)
number infected	0	0	17+ 19*	0+ 0*
% surviving snails infected	0	0	74+ 87*	0+ 0*

+83 days post infection.
*122 days post infection.

2nd experiment: Another attempt was made to infect individuals of L. peregra, altering the experiment as described below:

1. using larger snails (3-5mm), because such specimens of L. stagnalis and L. palüstris became infected.
2. using younger eggs, since the eggs (approximately 37 days old) used in the 1st experiment were older than those fed to either L. stagnalis (approximately 12 days old) or L. palustris (approximately 24 days old).
3. using more eggs.

Thirty 3-5mm L. peregra reared in the laboratory from a parent stock collected from the R. Wharfe at Otley were exposed for 24 h in a small petri-dish (5cm diameter) to the eggs teased from 5 7-day old P. elegans and incubated at room temperature for 8 days. The snails were then maintained in an aerated aquarium at 18°C. After 74 days all 24 surviving specimens were dissected and examined but no trace of infection was found.

The percentage hatch of embryonated eggs of P. elegans following ingestion by L. stagnalis, L. peregra and L. palustris.

Fully embryonated eggs of P. elegans must be ingested by the molluscan first intermediate host before hatching. The following experiment was performed to determine whether or not there are differences in the percentages of viable eggs which hatch in susceptible and refractory lymnaeid species.

Five 7-day old P. elegans were teased apart in each of three small petri dishes; the eggs were covered with distilled water and allowed to incubate for 28 days at room temperature. Five 4-6mm specimens of L. stagnalis, L. palustris or L. peregra were placed in dishes 1, 2 or 3 respectively. After 8h the faeces in each dish were removed and examined microscopically for the presence of hatched and apparently viable unhatched eggs. Non-embryonated eggs were not included in the determination of the total number of eggs present since such eggs do not hatch upon ingestion by the snail.

As shown in Table 3.4 approximately the same percentage of eggs hatch when ingested by L. stagnalis and L. palustris, while a considerably smaller proportion hatch when eaten by L. peregra.

Table 3.4. Hatching of embryonated eggs of P. elegans following ingestion by three species of lymnaeid snails.

snail species	total no. eggs present in faeces	no. eggs hatched (ave./snail)	% hatched
L. stagnalis	435	150 (30.0)	34.5
L. palustris	193	58 (11.6)	30.1
L. peregra	358	38 (7.6)	10.6

From the above data it is clear that under the experimental conditions employed, not only were large numbers of eggs ingested by the snails, but a considerable proportion of viable eggs hatched.

The length of life of embryonated eggs.

Flame cells and movement of the miracidium within eggs of P. elegans maintained at room temperature in small petri dishes containing distill-

ed water are visible within four days after having been teased from adult worms, although the majority of eggs require 8 to 12 days to become fully embryonated and infective. Successful laboratory infections have been obtained using eggs incubated for as long as 45 days (p. 39).

Fecundity of the mother sporocyst of P. elegans.

The mother sporocyst of P. elegans is highly prolific. The numbers of daughter sporocysts counted in two approximately 10-month old monomiracidial infections of L. stagnalis were 667 and 643. In a single L. palustris dissected nine months after infection, although it was difficult to count the sporocysts accurately because they were tightly packed, the number was estimated to be 3550. Thus if each miracidium produces one mother sporocyst from which nearly 650 daughter sporocysts develop, then the L. palustris dissected had been infected by 5 or 6 miracidia of the 50 to which it had been exposed (p. 40).

The careful examination of a large number of living sporocysts teased from infected snails and of microscopic sections of snail digestive glands has failed to reveal any evidence of a second generation of daughter sporocysts.

Effects of parasitism upon the snail host.

Growth and survival of infected L. stagnalis.

There were several reasons for attempting to infect snails with 1, 10 or 50 eggs.

a. To ascertain the number of eggs required to establish an infection of P. elegans in L. stagnalis.
b. To establish a clone.
c. To follow the course of infections resulting from ingestion of a single egg.

Once these data were obtained the snails were not discarded but were maintained to provide material for further laboratory infections. Although the conditions for maintaining them were not ideal for determining the effect on growth and survival of the snails the following observations were made (see Table 3.5). Firstly, in no instance did gigantism occur. The largest snails observed were 4.0cm by 2.0cm when infected and 3.6 by 1.9cm when uninfected; this is well within the range for adult L. stagnalis either infected or uninfected collected at

Table 3.5. Shell dimensions (in cm) and age (in days) of L. stagnalis surviving 229 or more days after exposure to 1, 10 or 50 eggs of P. elegans. Days post exposure are given in parentheses.

Group A infected; 1 egg/snail			Group B infected; 10 eggs/snail			Group C infected; 50 eggs/snail		
length	width	age	length	width	age	length	width	age
3.9	1.9	355 (261)	3.3	1.5	463 (368)	3.2	1.6	454 (333)
3.7	2.0	357 (263)	3.4	1.7	468 (373)	3.4	1.7	454 (333)
3.8	1.9	365 (271)	4.0	2.0	471 (376)	3.7	1.9	486 (365)
3.6	1.7	366 (272)	3.3	1.7	471 (376)			
			3.6	1.8	489 (394)			
			3.5	1.9	520 (425)			
			3.4	1.9	538 (443)			
			3.4	1.7	547 (452)			
			3.7	2.1	552 (457)			
noninfected			noninfected			noninfected		
2.7	1.4	323*(229)	3.0	1.5	375 (280)	3.1	1.6	442 (321)
2.9	1.5	323*(229)	2.9	1.5	442 (347)	3.6	2.0	468 (347)
2.9	1.6	323*(229)	3.3	1.7	458 (363)			
2.7	1.4	325 (231)	3.4	1.9	467 (372)			
2.8	1.4	325 (231)						
2.7	1.4	325 (231)						

Kirkstall Power Station (range 3.0 by 1.5 to 4.5 by 2.3cm). Secondly, infected L. stagnalis have survived under experimental conditions for as long as 552 days (457 days post-infection), while a noninfected specimen survived for 468 days.

Precisely why the noninfected snails of Group A* succumbed at approximately the same time is not known. However fouling of the water due to the deaths of two snails during days 321 and 322 was probably a significant factor in their nearly simultaneous deaths.

Pathology of infections of Plagiorchis elegans in Lymnaea stagnalis.

The digestive gland (the terms digestive gland, hepatopancreas and liver are used synonymously by different authors) of L. stagnalis is composed of two lobes; each lobe comprises many blindly ending tubules which are separated from each other by a connective tissue network. There are only two recognized types of cells making up the liver tubules, the columnar digestive gland cells and the calcareous cells. The former cells are more numerous, taller and thinner than the latter cells (Figs. 3.2, 3.3). The nucleus is situated in the proximal end of the cell, while the mid-region is occupied by food vacuoles and excretory vacuoles. The cytoplasm is densest at the distal end of the cells and the contents of the excretory vacuoles are emptied directly into the lumen of each tubule from the distal end of the cell. Calcareous cells are most often triangular in shape, being broadest at their basal end; although normally they occur singly sometimes two or three are found together.

Daughter sporocysts of P. elegans migrate from the mother sporocyst, situated on the intestine, anteriorly into the head and foot and posteriorly through the connective tissue network of the digestive gland but never through the lumen of the hepatic tubules.

In a specimen of L. stagnalis infected for four months by an unknown number of P. elegans miracidia, the connective tissue was partially destroyed and in some regions there was marked cytolysis of the digestive cells (Figs. 3.4, 3.5); the cell contents appear to be released into the tubule lumen. Additionally distal migration of the nucleus occurs even when the cells remain intact. The calcareous cells do not appear to lyse.

Damage to the digestive gland is progressive. Within 8 months of infection even in a case of a monomiracidial imfection cytolysis resulted in almost complete breakdown of the cells comprising the liver tubules (Fig. 3.6) leaving little apparently functional tissue. The distal

and lateral membranes degenerate and the cell contents, including the nucleus, escape into the lumen. Such destruction is not dependent upon the proximity of the sporocysts to the tubules (Fig. 3.7).

Although L. stagnalis is a suitable host for P. elegans, migrating cercariae may be encapsulated by molluscan amoebocytes (Fig. 3.8).

Effects on reproduction in the snail host.

Infection of immature L. stagnalis.

When the infection of P. elegans established in immature L. stagnalis is fully developed the daughter sporocysts occupy a considerable volume of the visceral sac. Figures 3.9, 3.10 and 3.13 show sections of infected and noninfected L. stagnalis when approximately 7-months old. The ovotestis of L. stagnalis is an irregularly shaped organ surrounded on all but the inner columella side by the digestive gland; it is composed of numerous, blindly-ending sacs or acini. During the breeding season the gonad of noninfected snails is extremely well developed and confines the digestive gland tubules to the periphery of the visceral sac. (Noninfected L. stagnalis bred regularly in the laboratory throughout the year in the present investigation, in some instances egg deposition occurred as often as every other day.) The gonad of infected specimens is rudimentary; furthermore the liver tubules and daughter sporocysts are fairly equally distributed throughout the visceral sac. The digestive gland tubules of infected and noninfected L. stagnalis appear to be approximately equal in size and number, although in the former hosts they are composed of substantially less functional tissue. There was no evidence that the presence of the sporocysts caused any more mechanical pressure on the tubules than would be exerted by the gonad of a mature noninfected L. stagnalis.

In the present investigation when either immature L. stagnalis (3 to 8mm long) or L. palustris (2 to 6mm long) were infected with P. elegans the development of the reproductive system was inhibited. In the majority of specimens dissected 8 or more months after infection the ovotestis and accessory reproductive organs were reduced or as in the case of the albumen gland and muciparous gland notably absent, while the terminal genitalia were rudimentary (Figs. 3.11, 3.12 and 3.13). Occasionally however the reproductive system continues to develop; as shown in Fig. 3.14 the penial complex and prostate glands have attained approximately the normal size, while both the muciparous gland and oöthecal gland have become slightly enlarged, although the albumen gland remains apparently absent.

Infected snails were maintained separately from noninfected specimens and their tanks were carefully checked for the presence of egg masses. In no instance did either L. stagnalis or L. palustris, infected when immature, produce any egg masses. In addition, despite the fact that one specimen survived 457 days after infection and a number of others lived for more than a year beyond the date of infection, they did not outlive their infections.

Infection of mature L. stagnalis.

In order to ascertain the effects of P. elegans on egg production during the prepatent period, an attempt was made to infect sexually mature L. stagnalis. The snails used were judged to be mature because they produced egg masses when individually isolated prior to exposure to eggs of P. elegans. Thirty-two L. stagnalis were each exposed to 50 fully embryonated eggs in 15cm^3 of dechlorinated water (a greater volume of water was used because the snails employed were larger than those previously infected, being approximately 1.5 to 2.0cm in length and were therefore not small enough to fit into the wells of the immunological tray.) After being isolated with the eggs for 24h at room temperature, the pulmonates were kept individually in small plastic containers which were immersed in two aerated aquaria at 18°C. The containers were covered with cheese cloth to allow the oxygenated water to circulate freely and the water was changed three times per week; lettuce leaves were provided ad libitum.

After several weeks it was evident that the snails were not thriving under these conditions; eight of them died within 6 weeks. The snails were then removed from the plastic containers and replaced in the aquaria, 12 per tank; this allowed them to obtain oxygen from the surface because it was considered probable that they were not obtaining a sufficient amount of oxygen from the circulating water.

Only 10 snails survived more than 75 days postinfection; of these 1,found to be noninfected, lived for 125 days, while 2 infected specimens lived for 125 and 127 days respectively beyond the date of infection.

When the 10 snails were dissected no trace of infection was found in 8 of them, but the gonad and reproductive structures were apparent in the 2 infected L. stagnalis. Therefore the effect of P. elegans upon host reproduction varies depending upon the state of maturity of the snails infected (Figs. 3.15, 3.16). It is also worth noting that both egg masses and cercariae were produced by the infected snails until within a week of their deaths.

DISCUSSION.

The host-parasite relationship between species of larval digeneans and molluscs is extremely complex and may vary depending on the strain of parasite and snail involved. Wright (1966) suggested that the term "compatibility" be used to describe the overall relationship including the susceptibility of molluscs and the infectivity of flukes, noting that some snail populations may be highly susceptible to infection while others, even within the same species, may be less susceptible or completely resistant.

According to Basch (1975) the hatching of eggs in the snail's digestive tract is a manifestation of compatibility, while the inability to hatch is a measure of incompatibility. Factors which affect hatching and subsequent penetration are probably the physical and biochemical characteristics of the molluscan gut. The gut wall itself may act as a barrier to the miracidium as it tries to penetrate. Cheng (1968) posed the question ". . . whether the miracidial cytolytic enzymes must be chemically specific for the integument of specific species of molluscs" and further stated that " if this is the case, compatibility of enzymes to substrate could serve as a factor governing successful penetration, hence host-compatibility." Basch (1975) considered it likely that compatibility is tested independently for each miracidium attempting to penetrate a snail. Possibly invading miracidia enhance their infectivity by camouflaging themselves by adsorbing host material onto their surfaces or protect themselves in some other way, thus evading the host response (Wright, 1974).

It has been experimentally demonstrated by the writer that both L. stagnalis and L. palustris are susceptible to infection by P. elegans while L. peregra is refractory. The experiment designed specifically to determine the percentage of fully embryonated eggs which hatch when ingested by L. stagnalis, L. palustris and L. peregra revealed that significantly more eggs hatch when eaten by the compatible species L. stagnalis and L. palustris than by the incompatible species L. peregra. But the numbers that hatch in L. peregra are sufficient to infect L. stagnalis. As a result it is considered that either the gut wall of L. peregra impedes penetration by P. elegans miracidia or if penetration does occur then additional factors are involved in preventing the successful establishment of infection, such as an internal cellular response resulting in the encapsulation of the miracidia by molluscan leucocytes and fibrous material (Cheng, 1968).

Host specificity displayed in digenean infections is usually much greater at the first intermediate host level than for either the second or final hosts, although young snails of abnormal host species often exhibit less resistance than old snails of the same species. Kendall (1965) successfully infected 5 of the 6 British lymnaeid species with Fasciola hepatica. When infected within the first few days of hatching infections developed to maturity, that is to cercarial release, in L. stagnalis, L. palustris and L. glabra; to the redial stage in L. peregra, while L. truncatula, the natural molluscan host, was susceptible at any age. Only L. auricularia was completely resistant. Boray (1967) experimentally infected L. peregra with F. hepatica and found that if the infections were achieved within 7 days of hatching some snails eventually produced cercariae.

Despite the fact that there is a host response to infection by P. elegans in the form of the paletot and encapsulation of migrating cercariae, the development of infection is not inhibited nor does the infected host die prematurely. Furthermore incidences of infection of 62 and 77% were seen in immature L. stagnalis 42 days after exposure to 10 and 50 eggs respectively. Similarly, 74% of the surviving L. palustris, which had each been exposed to 50 eggs, were releasing cercariae 83 days post-infection, yet in 2 experiments only about 13 and 6% of the immature L. stagnalis became infected upon exposure to single eggs. In the last 2 experiments several factors may be responsible for the low rate of infection:

1. The presence of some non-viable eggs.
2. The failure to ingest eggs, although eggs were available to grazing snails for 3 days in only 3 to 4cm^3 of water.
3. The incompatibility of individual snails towards individual miracidia (Basch, 1975).

When exposed to greater numbers of eggs (10 or 50 per snail) the molluscs would certainly have had more opportunity to ingest viable, compatible eggs. It is emphasized however that when 4 groups of 50 L. stagnalis were placed with an undetermined number of eggs (1 group / eggs of 1 worm) between 12 and 32% of each group surviving 118 days beyond the date of exposure did not develop infections. Thus some snails, even within the same laboratory population, may be less susceptible than others to infection by P. elegans.

A number of L. stagnalis have survived more than one year in the laboratory; the longest surviving specimen was one which had been infected by exposure to 10 eggs and lived to be 552 days old (approximately 18.4 months). Under natural conditions the life span of L. stag-

nalis in Great Britain has been estimated to be anywhere from one to two years. Boycott (1936)observed freshwater pulmonates mainly in the south of England and stated that they were all annuals with a single exception, Planorbarius corneus, which may live longer. Hunter (1957) considered only L. stagnalis, L. auricularia and P. corneus capable of living beyond a year. Berrie (1965) conducted a more recent study on a population of L. stagnalis in a small pond at Bellshill, Lanarkshire and found that some of the snails survived for two years, breeding during both years. He was uncertain however as to whether snails which reach their maximum size by the end of the first year die and furthermore felt that if this is the case then under favourable conditions, such as a calcareous habitat (Campion, 1956), the life cycle may be completed within a single year, while under unfavourable conditions it may not be concluded until the second year. In the present instance it is believed that the population from which the parent stock of L. stagnalis was first obtained lives beyond one year mainly because adult L. stagnalis were present in the canal at Kirkstall Power Station throughout the year while adults of L. peregra, a known annual, disappeared from the canal during the summer months.

Species of Plagiorchis have been recorded from various snail hosts as listed below in chronological order.

Plagiorchis sp.	snail host	reference
P. muris	Lymnaea pervia	Dollfus (1925)
P. ramlianum	Bulinus contortus	Azim (1935)
P. muris	Stagnicola emarginata angulata	McMullen (1937)
P. jaenschi	L. lessoni	Johnston & Angel (1951)
P. goodmani	L. palustris	Najarian (1952)
P. megalorchis	L. peregra	Rees (1952)
P. maculosus	L. lessoni	Angel (1959)
P. cirratus	L. (Radix) limosa	Buttner & Vacher (1959)
"	L. stagnalis	" "
P. vespertilionis parorchis	L. stagnalis	Macy (1960)
P. elegans	L. stagnalis	Styczynska-Jurewicz (1962)
P. noblei	L. (Stagnicola) reflexa	Williams (1963)
P. noblei	S. reflexa	Daniell & Ulmer (1964)
P. dilimanensis	L. philippinensis	Velasquez (1964)
P. laricola	L. stagnalis	Zdarska (1966)

P. peterborensis	L. stagnalis	Kavelaars & Bourns (1968)
P. momplei	L. hovarum	Richard et al. (1968)
P. neomidis	Radix peregra	Brendow (1970)
P. laricola	L. ovata	Krasnolobova (1971)
P. noblei	L. stagnalis	Blankespoor (1974)
"	S. reflexa	" "
P. farnleyensis	L. stagnalis	Diaz (1976)
P. kirkstallensis	L. stagnalis	Diaz (1976)
P. neomidis	R. limosa	Theron (1976)

It is evident from this list that confusion exists concerning the taxonomy of some of the molluscan hosts, since the names Radix and Stagnicola refer to both genera and subgenera. Hubendick (1951) and Hyman (1967) considered Lymnaea, Radix, Stagnicola and Galba on the basis of their morphology to be subgenera of Lymnaea. In contrast, Burch and Lindsay (1973) have provided experimental evidence which demonstrates that Lymnaea, Stagnicola, Fossaria (=Galba) and Radix each forms a distinct immunological group when their foot muscle proteins are compared. In addition these authors noted that classical taxonomic divisions within the family which are based on shell characteristics are more reliable in general than either radular teeth or the structure of the reproductive tract. Whether differences in foot muscle proteins are sufficient to distinguish between genera or subgenera depends on the weight that individual molluscan taxonomists attribute to such criteria. It is clear however that the above genera or subgenera form a closely related group, the individual members of which are subject to considerable morphological variation depending on the environmental conditions in which they live, making their natural relationships difficult to establish. Moreover it is evident that the taxonomy of the group remains unresolved. In the present work Macan's (1969) key to British gastropods was used to identify the snails collected; he agrees with Hubendick (1951) and Hyman (1967) in that he considers the genus Lymnaea to comprise several subgenera.

If one accepts that subgenera exist within the genus Lymnaea then members of the genus Plagiorchis are highly specific with regard to their first intermediate host. If on the other hand one accepts the conclusions of Burch and Lindsay (1973) then species of Plagiorchis are at the very least confined as the host records indicate to snails of the family Lymnaeidae. The fact that P. ramlianum (Azim, 1935) was recorded from Bulinus contortus (family Bulinidae) is taken as further evidence (see p. 23) that this trematode species should be

removed from the genus Plagiorchis.

Kendall (1965) suggested that while a few lymnaeid species are capable of transmitting Fasciola hepatica in any one locality only a single species is involved. Although it has been demonstrated by the present experiments that P. elegans may utilize either L. stagnalis (immature or mature) or L. palustris (at least when immature) as first intermediate host with apparently equal success, it is of interest that the author did not find these species inhabiting the same bodies of water. Lymnaea stagnalis is confined to hard water habitats while L. palustris may survive in either hard or soft water (Macan, 1969). However the possibility of infecting both species would probably extend the range of P. elegans infections, depending on the range of the second intermediate and definitive hosts.

The mother sporocyst of P. elegans is extremely prolific, producing under experimental conditions a single generation of approximately 650 daughter sporocysts. Cort and Olivier (1943) have provided the only other estimate of mother sporocyst fecundity of a Plagiorchis species - 300 to 500 daughter sporocysts for each mother sporocyst of P. muris.

Occasionally rediae of F. hepatica produce either daughter rediae or cercariae (Kendall, 1965) and in the molluscan stages prior to the formation of Cercaria X Baylis a second generation of daughter sporocysts is produced (Erasmus, 1958). In the former example the variation in the life cycle was believed to be due to fluctuation in maintenance temperature, while in the latter instance no explanation was offered for the occurrence of two daughter sporocyst generations. However snails were kept at a constant temperature in the present experiment; perhaps if the temperature were varied a second generation of daughter sporocysts would be obtained.

Temperature may also be a factor in the loss of larval infections. Stirewalt (1954) found that when Australorbis glabratus infected with Schistosoma mansoni was maintained below the optimal temperature, snails had a tendency to lose their infections, yet when the temperature was optimal the snails remained infected until their deaths. She suggested that the loss of infection was caused by an inhibition of larval development. On no occasion has the writer noted a loss of P. elegans infections in either L. stagnalis or L. palustris despite the fact that some parasitized snails have survived more than a year after infection. In all the experiments conducted the maintenance temperature was either 18° or 22°C.

Studies of the damage inflicted upon the gastropod first interme-

diate host by larval Plagiorchis species are rare, consisting of the investigations by Pratt and Barton (1941) and Rees (1952) concerning P. muris and P. megalorchis respectively. In the one specimen of Stagnicola emarginata sectioned by the former authors hystolysis of only a single liver tubule of the thousands examined was noted, the damage was attributed to the sporocysts present on either side of the tubule. Although Pratt and Barton did not give the approximate age or size of the snail, they did report that it was killed in August and heavily parasitized; the sporocysts were found between the liver tubules even into the apex of the spire.

In the present investigation cytolysis of the cells comprising the digestive gland tubules has been shown to be progressive; it is much less frequently encountered in young (4-month old) than in old (8-month old) infections of L. stagnalis by P. elegans. Towards the end of the snail's life, it is doubtful that very much functional liver tissue remains, since few if any of the cells of the tubule walls remain intact. Rees (1952) obtained similar results during her study of P. megalorchis infections in L. peregra; extensive cytolysis occurred and the digestive gland was almost entirely replaced by sporocysts and escaping cercariae. She suggested that toxic substances released by the sporocysts may be responsible for the breakdown of the tubule epithelium.

James (1965) compared the effects of starvation and parasitization by larval digeneans on the digestive gland cells of Littorina saxatilis and found that the same type of cytolysis occurred under both circumstances. Contrary to Rees (1952) he felt that pressure exerted by the parasites on the tubules is responsible for their breakdown rather than the release and accumulation of toxic waste material. Further James believed that the large number of developing larvae may close the lumen of the tubules and as a result prevent the passage of food material into the more distal parts of the digestive gland.

It is difficult to state conclusively whether the pathological effects on the digestive gland tubules noted during the present study are due to the presence of excretory products or mechanical pressure. What can be said however is that the burden of larval P. elegans in the infections observed does not appear either to occupy any more of the available space or to compress the liver tubules to a greater extent than a normal mature ovotestis during the breeding season. Furthermore, experimentally established infections of P. elegans have not been observed to shorten the life of L. stagnalis in the laboratory. It is quite probable that Sinitsin (1931) is correct in his suggestion

that the digestive gland of gastropods is more than sufficient for their needs and losing even a large proportion of it does not affect them adversely.

A general account of host castration by parasites has been written by Baudoin (1975). He suggests that such castration is adaptive and results in the increased fitness of the parasite. By inhibiting the development of the reproductive system the parasite may benefit by:

1. an increase in available energy.
2. an increase in host viability.
3. an increase in host growth.

In the case of P. elegans, infections of immature L. stagnalis inhibit the further development of the molluscan reproductive tract, essentially castrating the snails. Although the reproductive system in some instances continues to develop slowly, the ability to produce egg masses is never attained. Pratt and Barton (1941) reported the only previous occurrence of host castration by a larval Plagiorchis species. During the month of August they collected specimens of Stagnicola emarginata angulata; they sectioned one snail which was shedding cercariae of P. muris McMullen, 1937 and for comparison one which was not shedding cercariae. Although only ova were observed within the ovotestis of the latter snail they stated that this was due to the fact that during August the male reproductive phase had been completed, consequently the snail was functionally a female. When they examined sections of the parasitized snail they found no structure which could be unmistakably identified as the ovotestis. In addition Pratt and Barton noted that the sporocysts were most numerous in the region where the ovotestis is normally present.

McClelland and Bourns (1969) observed that when immature L. stagnalis are infected with Trichobilharzia ocellata they fail to reproduce for the duration of the infection, but two 10-month old snails outlived their infections and produced 1 or 2 egg masses per week. It is possible that a similar phenomenon would occur if L. stagnalis survived longer than sporocysts of P. elegans, since as stated above further development of the snail reproductive system has occasionally been observed in infected L. stagnalis.

Several proposals have been put forward to explain host castration by larval digeneans -

1. mechanical pressure exerted directly on the gonad by the larvae (Rees, W., 1936).
2. starvation of the ovotestis (James, 1965).
3. release of a toxic substance by the daughter sporocysts

(Cheng & Snyder, 1962; Rees, F., 1952).

4, hormonal effect (McClelland & Bourns, 1969).

It may be that castration is due to a combination of these factors in the present series of experiments. As seen in Fig. 3.16 daughter sporocysts migrate along the reproductive tract, possibly pressing directly against the ovotestis. Figure 3.13 shows that the ovotestis is bordered on three sides by daughter sporocysts, yet it appears to be less closely apposed to the surrounding tissue than in the section of an uninfected snail (Fig. 3.10).

If either the release of toxic substances or starvation of the ovotestis is responsible for castration, then one would expect that the state of maturity of the snails at the time of infection would not matter; reproduction would be prevented when immature snails were infected and gamete production would decrease in the case of mature snails. The author was not able to determine whether the rate of egg production declined in L. stagnalis infected when mature, because the snails were not maintained satisfactorily in solitary conditions. It was noted however that they continued to produce egg masses for up to 125 days post-infection.

Perhaps it is most likely that the sporocysts affect the molluscan endocrine system, possibly by producing a substance that mimics a host hormone as suggested by McClelland and Bourns (1969) or by direct utilization of host hormones required for the development of the snail reproductive system.

Although the data presented in Table 3.5 give the impression that L. stagnalis generally lives longer and in many cases grows to be larger when infected by exposure to 10 or 50 eggs than when either noninfected or exposed to 1 egg, the results have been influenced by two factors. Firstly, the number of snails maintained per aquarium tank was not constant, because the numbers of infected and noninfected snails was not equal at any one dose. Secondly, snails harbouring monomiracidial infections were isolated in small finger bowls for 24h at weekly intervals to obtain cercarial counts (p.67).

In one case of attempted monomiracidial infections 86.7% of the snails were not infected 42 days post-infection; thus their aquarium tank initially contained 39 snails while there were only 6 snails which did become infected. On the other hand the numbers of snails infected and not infected by exposure to 10 eggs were more nearly equal and although infected specimens survived longer than noninfected specimens, they were nearly equal in size when the same age. The most reliable indication of the effect of infection by P. elegans on the

size of L. *stagnalis* is shown by those snails each exposed to 50 eggs, because there were only three infected and two noninfected specimens for 133 of the last 182 days of the experiment. The five snails were approximately equal in size and length of life. As a result of these investigations both crowding and frequent handling are considered to be contributing factors in the inhibition of growth and curtailment of life of snails maintained experimentally. It has not been conclusively established that "it is more 'expensive' in nutritional terms for a snail to produce eggs than to produce cercariae" (McClelland & Bourns, 1969).

Fig. 3.1. Faeces of L. stagnalis containing hatched and unhatched eggs of P. elegans. (E.e.) apparently embryonated egg; (H.e.) hatched egg; (N.e.) non-viable egg. Scale 40μm.

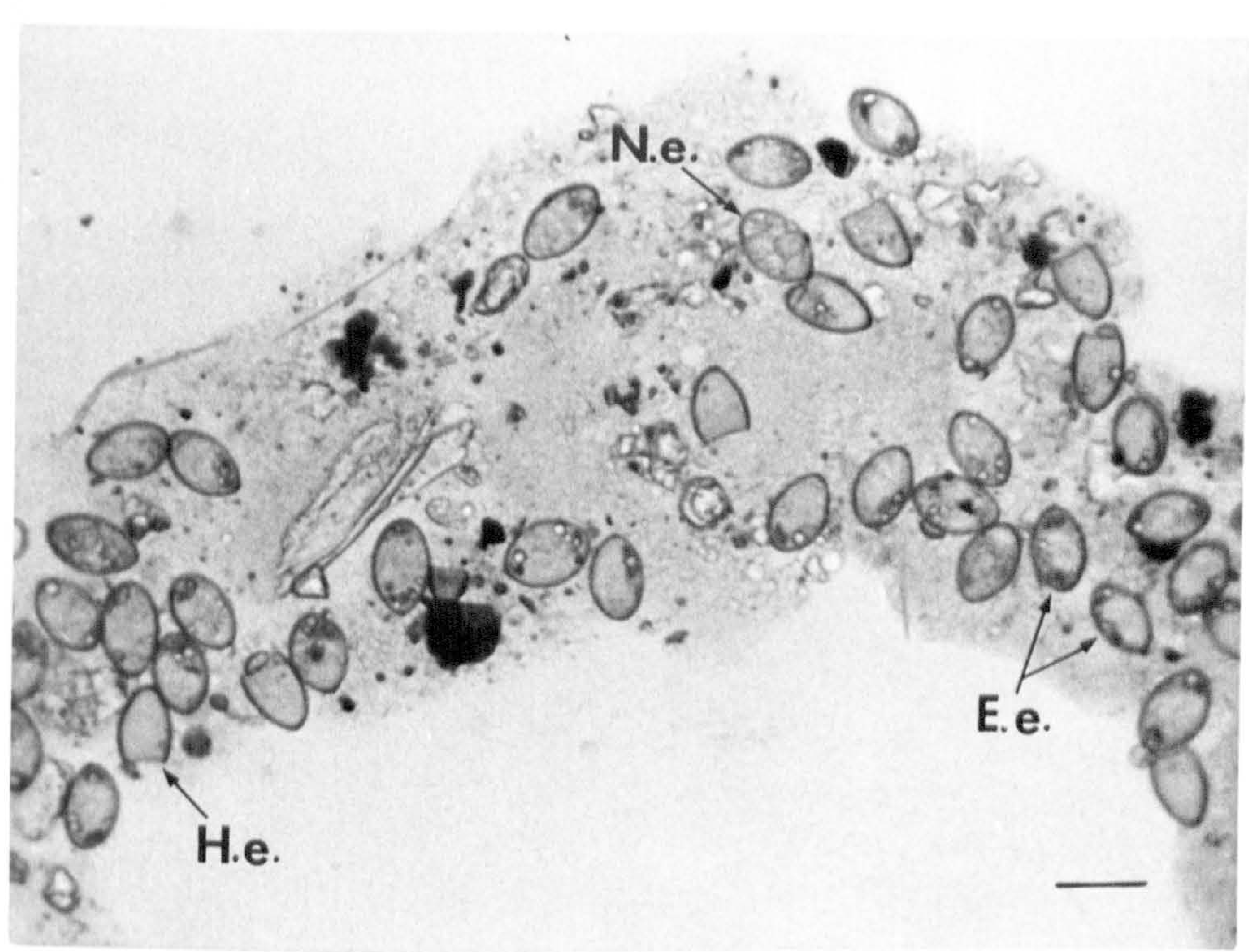
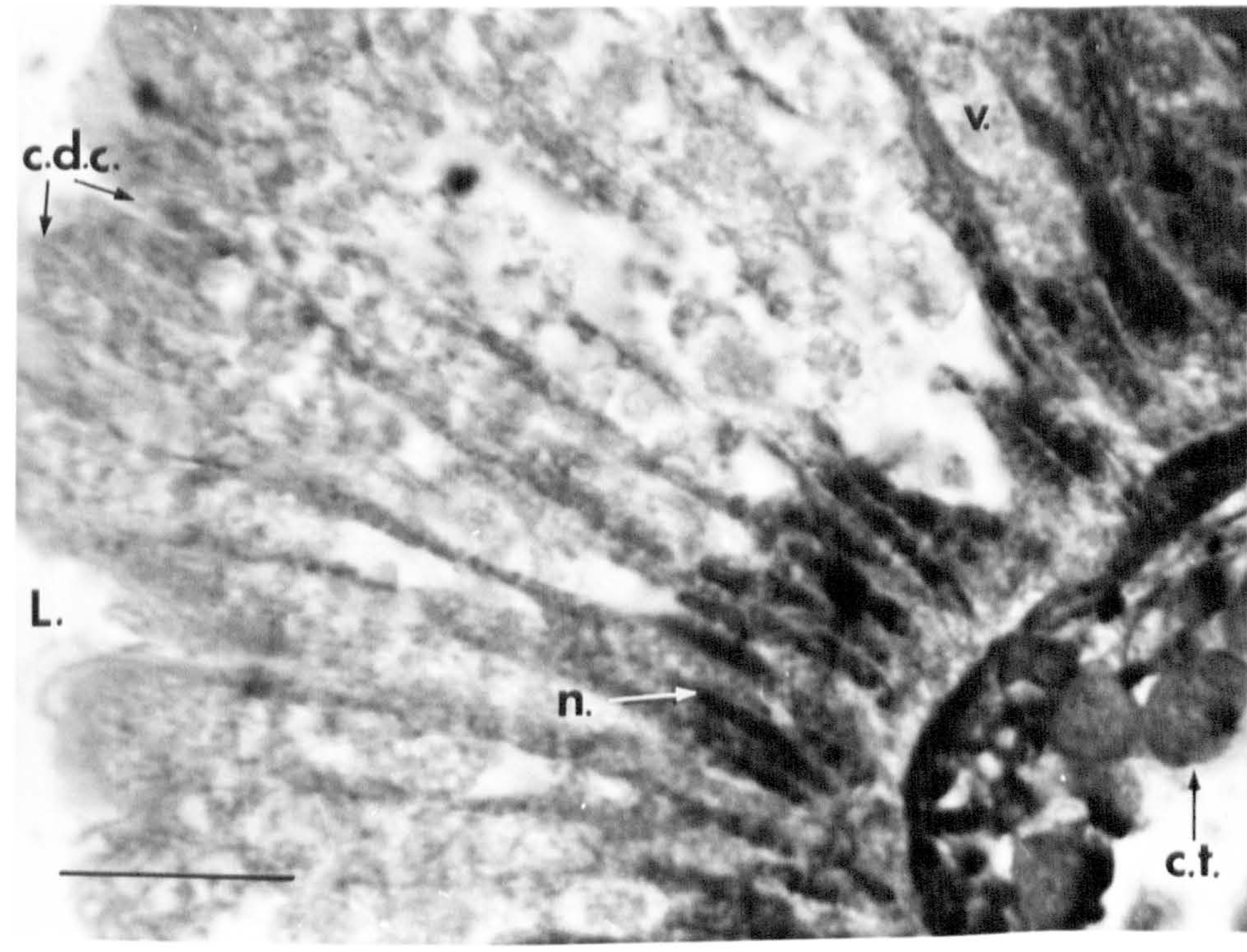

N.e.
E.e.
H.e.

Fig. 3.2. Transverse section (8μm) through normal digestive gland tubule of noninfected L. *stagnalis* stained with Mallory's Triple stain. Scale 50μm.

(c.c.) calcareous cell; (c.d.c.) columnar digestive gland cell; (c.t.) connective tissue; (L.) lumen of hepatic tubule;(n.) nucleus; (v.) vacuole.

Fig. 3.3. Transverse section (8μm) through normal digestive gland tubule of noninfected L. *stagnalis* stained with Mallory's Triple stain. Scale 50μm.


c.d.c.
v.
L.
n.
c.t.

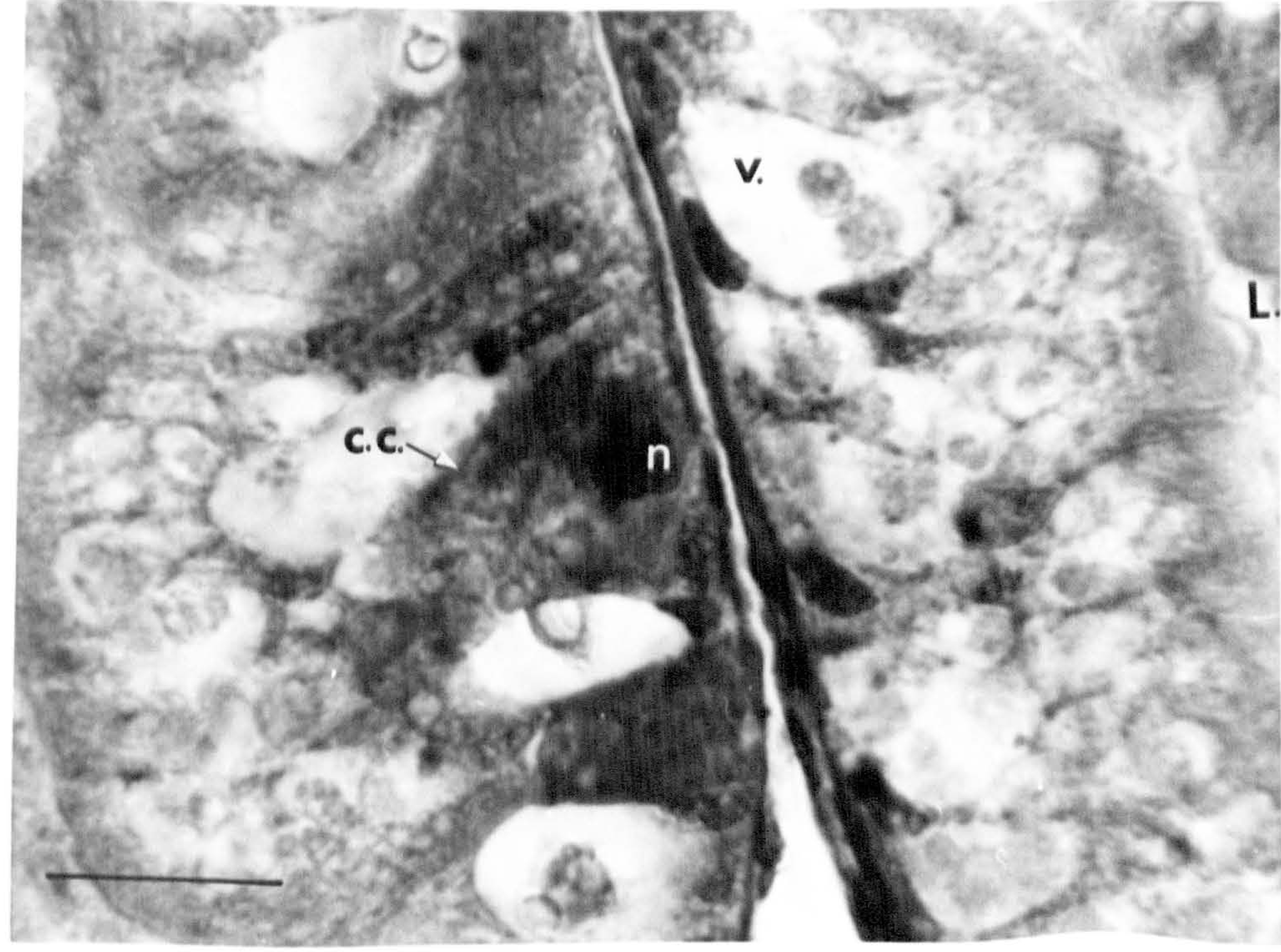
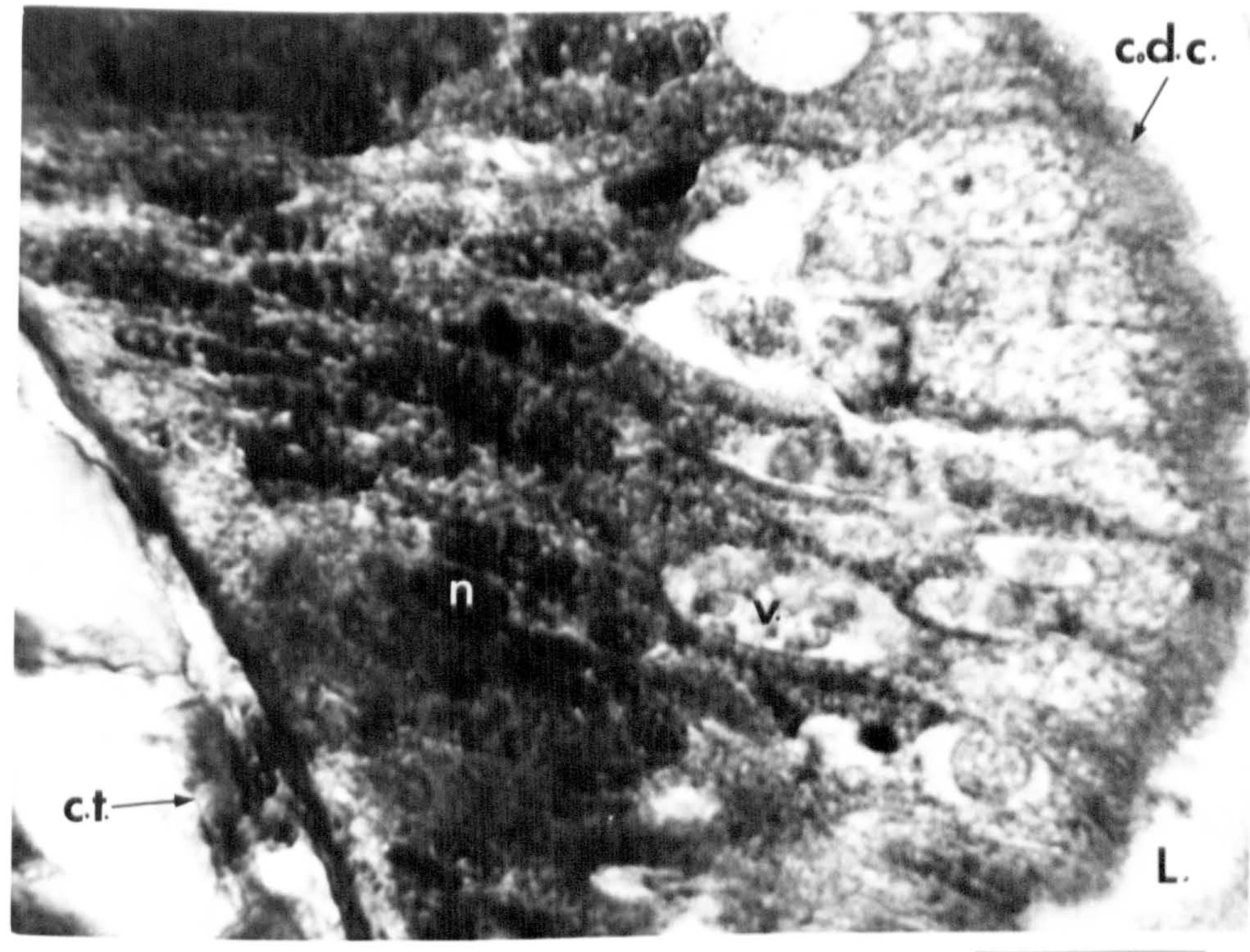

v.
L.
c.c.
n

Fig. 3.4. Transverse section (8μm) of digestive gland tubule of L. stagnalis infected for 4 months with P. elegans. Note distal migration of nuclei and destruction of connective tissue. Scale 50μm.

Sections stained with Mallory's Triple stain. (c.c.) calcareous cell; (c.d.c.) columnar digestive gland cell; (c.t.) connective tissue; (L.) lumen of digestive gland tubule; (n.) nucleus; (v.) vacuole.

Fig. 3.5. Transverse section (8μm) of digestive gland tubule of L. stagnalis infected with P. elegans for 4 months. Note cytolysis. Scale 50μm.


c.d.c.
n
v.
c.t.
L.

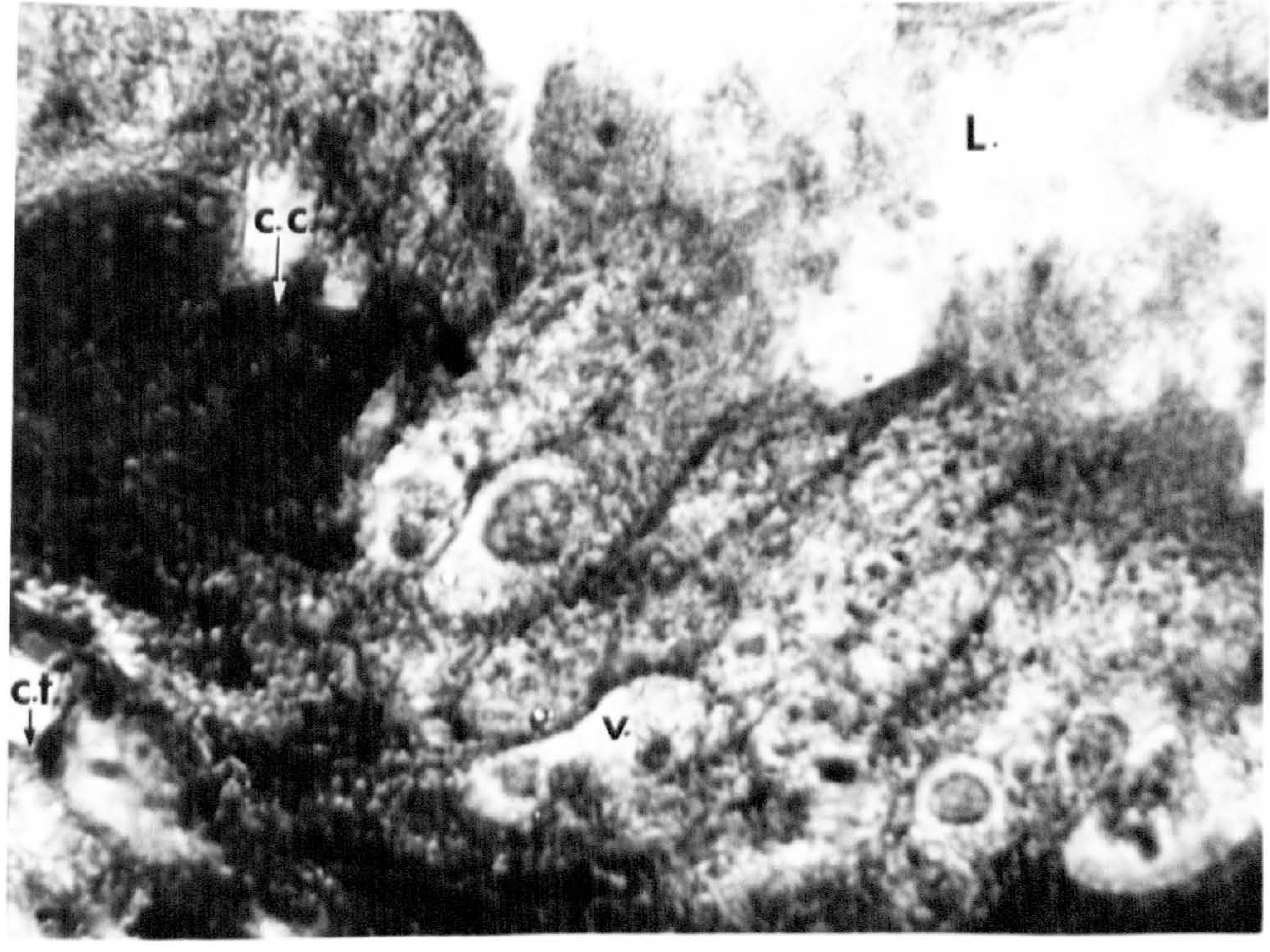
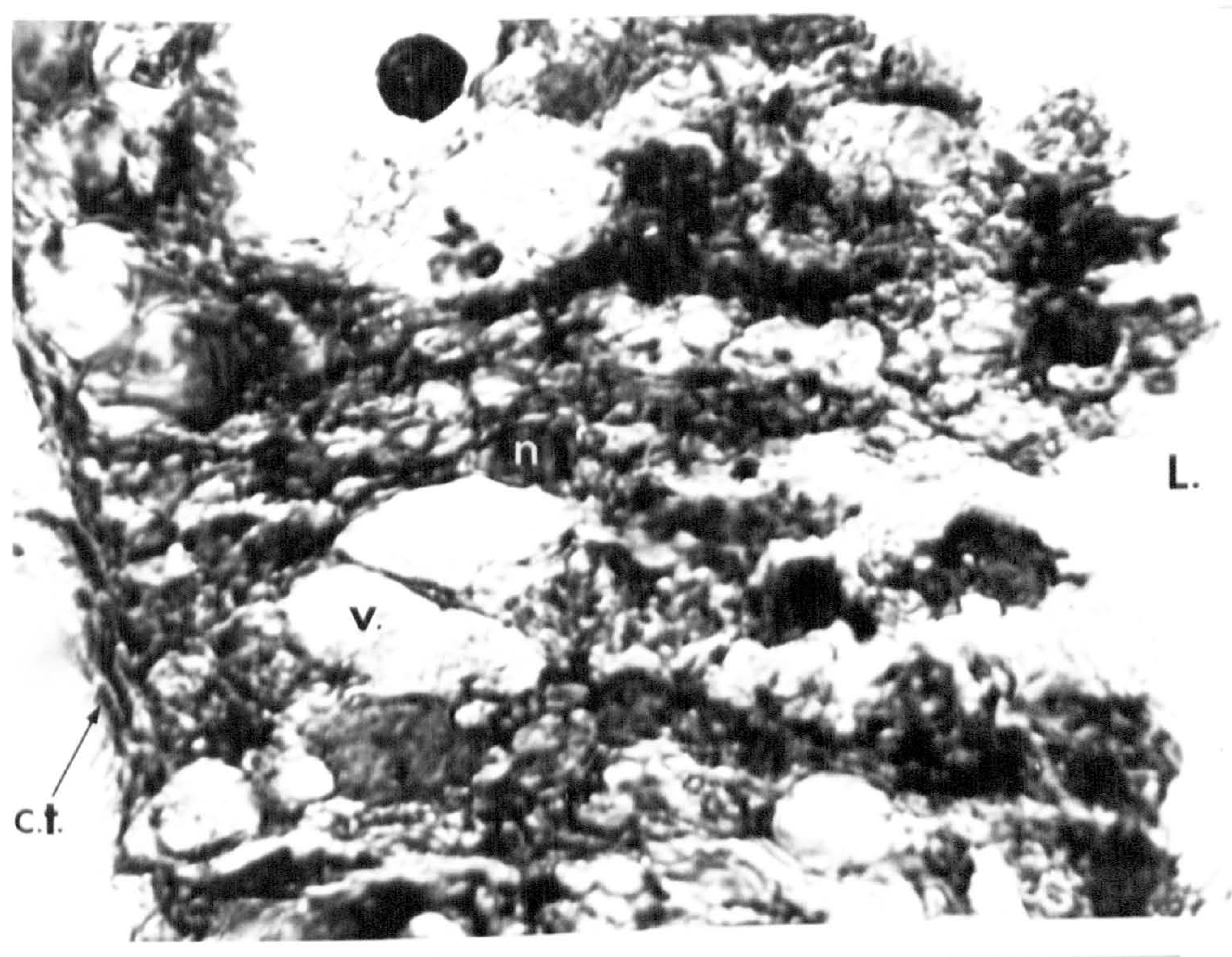

L.
c.c.
c.t.
v.

Fig. 3.6. Transverse section (8μm) through digestive gland of L. stagnalis carrying an 8-month old infection with P. elegans. Stained with Mallory's Triple stain. Lateral and distal cell membranes have degenerated; cellular contents are emptied into tubule lumen. (c.t.) connective tissue; (L.) lumen of tubule; (n.) nucleus; (v.) vacuole. Scale 50μm.

Fig. 3.7. Transverse section (8μm) through digestive gland of L. stagnalis infected for 8 months with P. elegans. Stained with Mallory's Triple stain. Damage to the tubules is apparently not related to the proximity of the daughter sporocysts. (d.t.) digestive gland tubule; (d.s.) daughter sporocyst; (L.) lumen of tubule. Scale 100μm.

59a



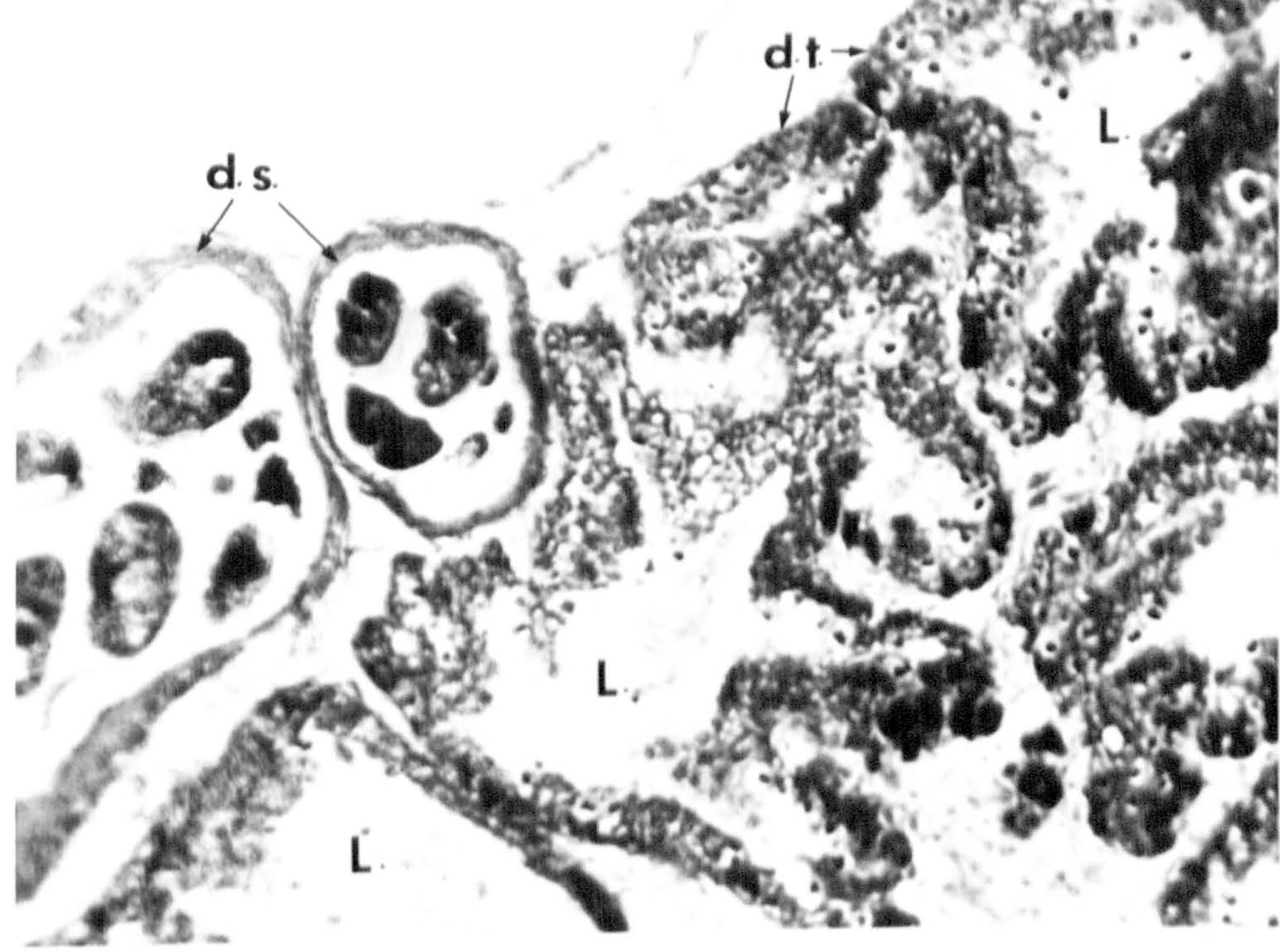


Fig. 3.8. Section through migrating cercaria of P. elegans encapsulated by amoebocytes within the digestive gland of L. stagnalis. Stained with Mallory's Triple stain. Scale 50µm. (a.) amoebocytes; (c.) cercaria.

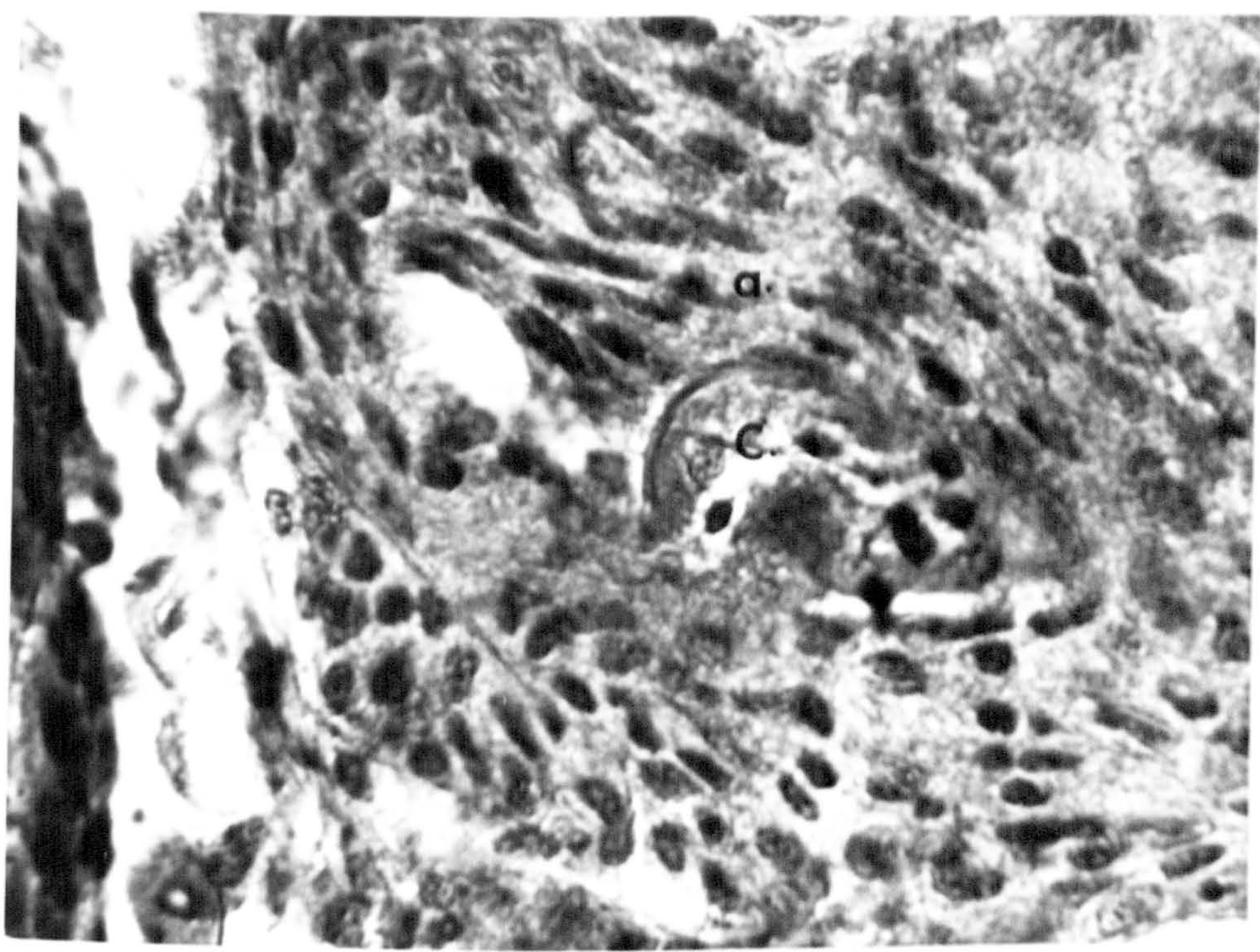
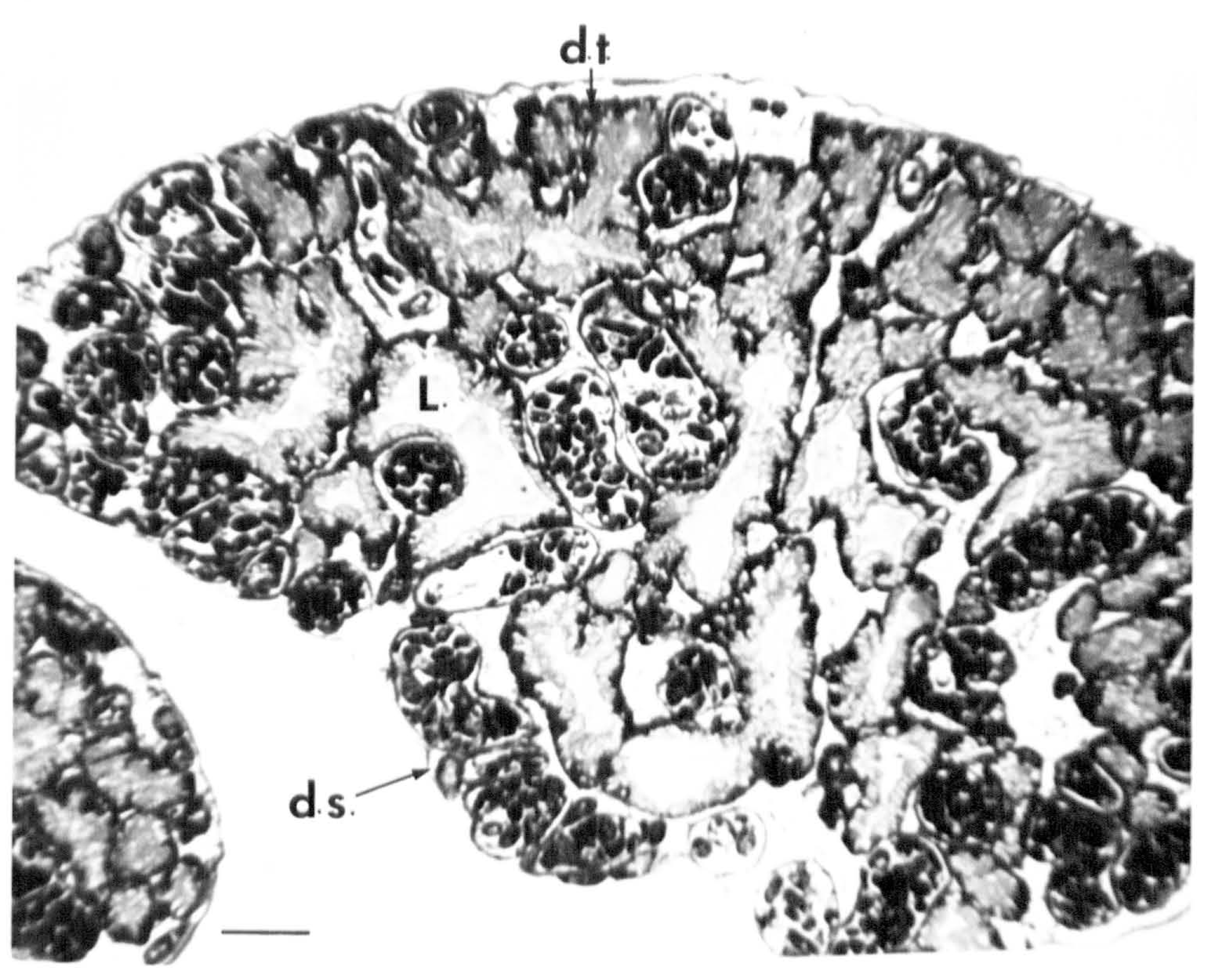

a.
c.

Fig. 3.9. Longitudinal section (8μm) through the visceral sac of L. stagnalis (approx. 7 months old) carrying a 4-month old P. elegans infection and showing the distribution of sporocysts and liver tubules. Stained with Mallory's Triple stain. (d.t.) digestive gland tubule; (d.s.) daughter sporocyst; (L.) lumen of digestive gland tubule. Scale 500μm.

Fig. 3.10. Longitudinal section (8μm) through the visceral sac of a noninfected L. stagnalis demonstrating the peripheral distribution of the tubules in a reproductively active (approx. 7-month old) specimen. Stained with Mallory's Triple stain. (a.o.) acini of ovotestis; (a.l.) acinus lumen; (o.o.) oöcyte; (S.c.) Sertoli cells with spermatagonia. Scale 500μm.

6la



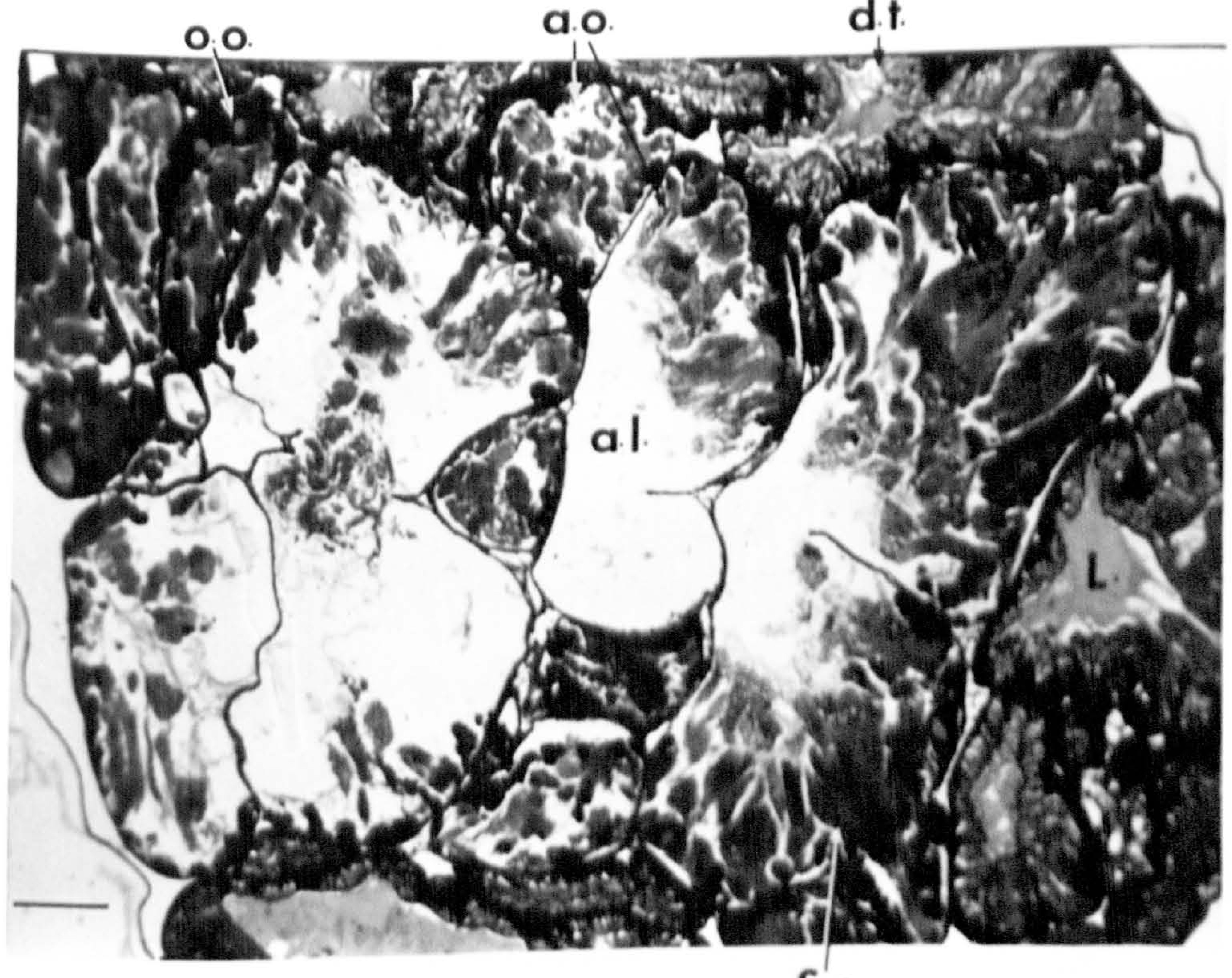
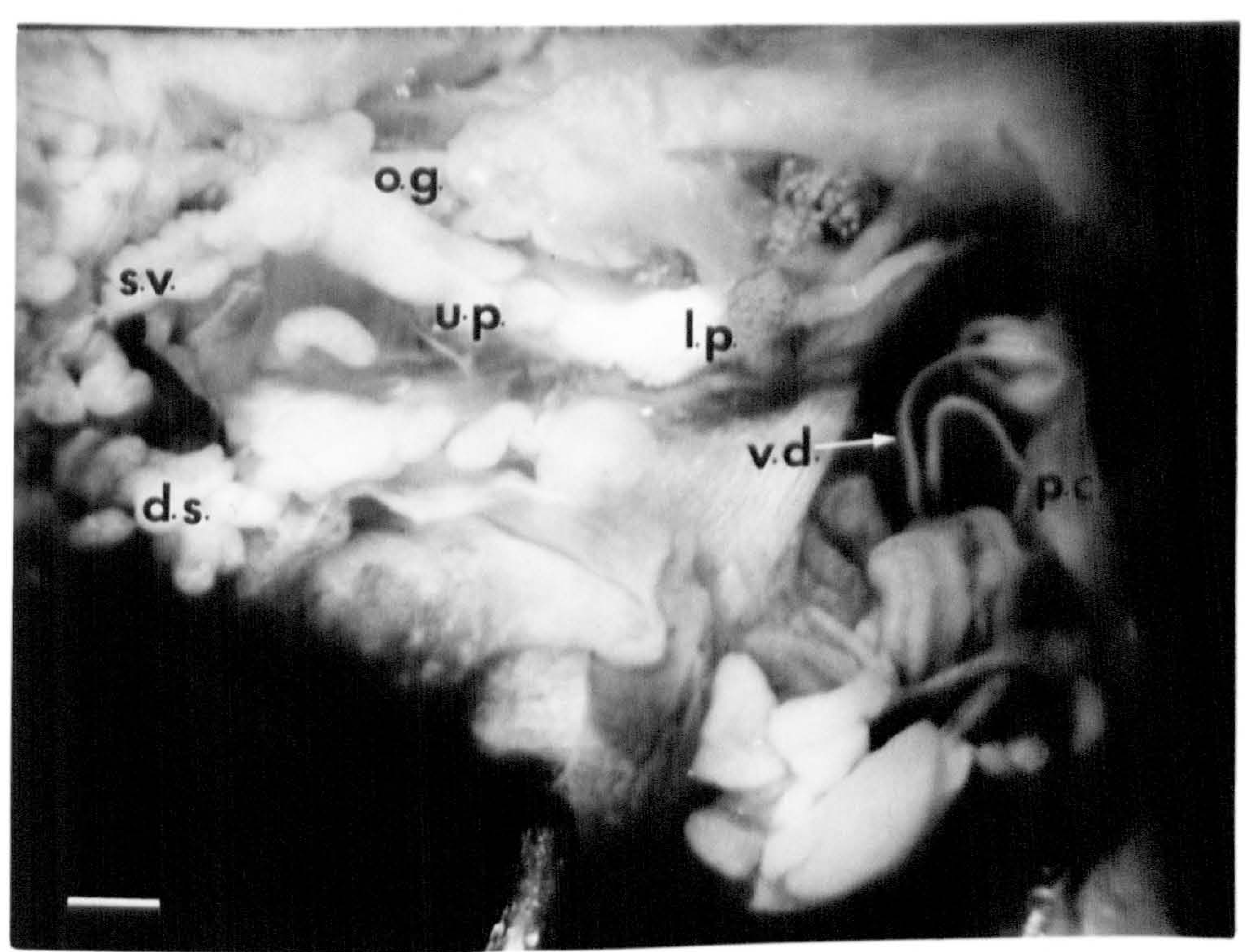


Fig. 3.11. Reproductive structures present in the head region of *L. stagnalis* (approx. 7 months old) infected with *P. elegans* for 4 months. Scale 1.0mm.

d.s.	daughter sporocyst
l.p.	lower prostate
o.g.	oöthecal gland
p.c.	penial complex
s.v.	seminal vesicle
u.p.	upper prostate
v.d.	vas deferens

Fig. 3.12. Reproductive structures present in the head region of a noninfected control *L. stagnalis* (approx. 7 months old). Scale 1.0mm.

a.g.	albumen gland
d.g.	digestive gland
h.d.	hermaphrodite duct
l.p.	lower prostate
m.g.	muciparous gland
o.g.	oöthecal gland
p.c.	penial complex
s.v.	seminal vesicle
u.p.	upper prostate
v.d.	vas deferens


o.g.
s.v.
u.p.
l.p.
v.d.
p.c.
d.s.

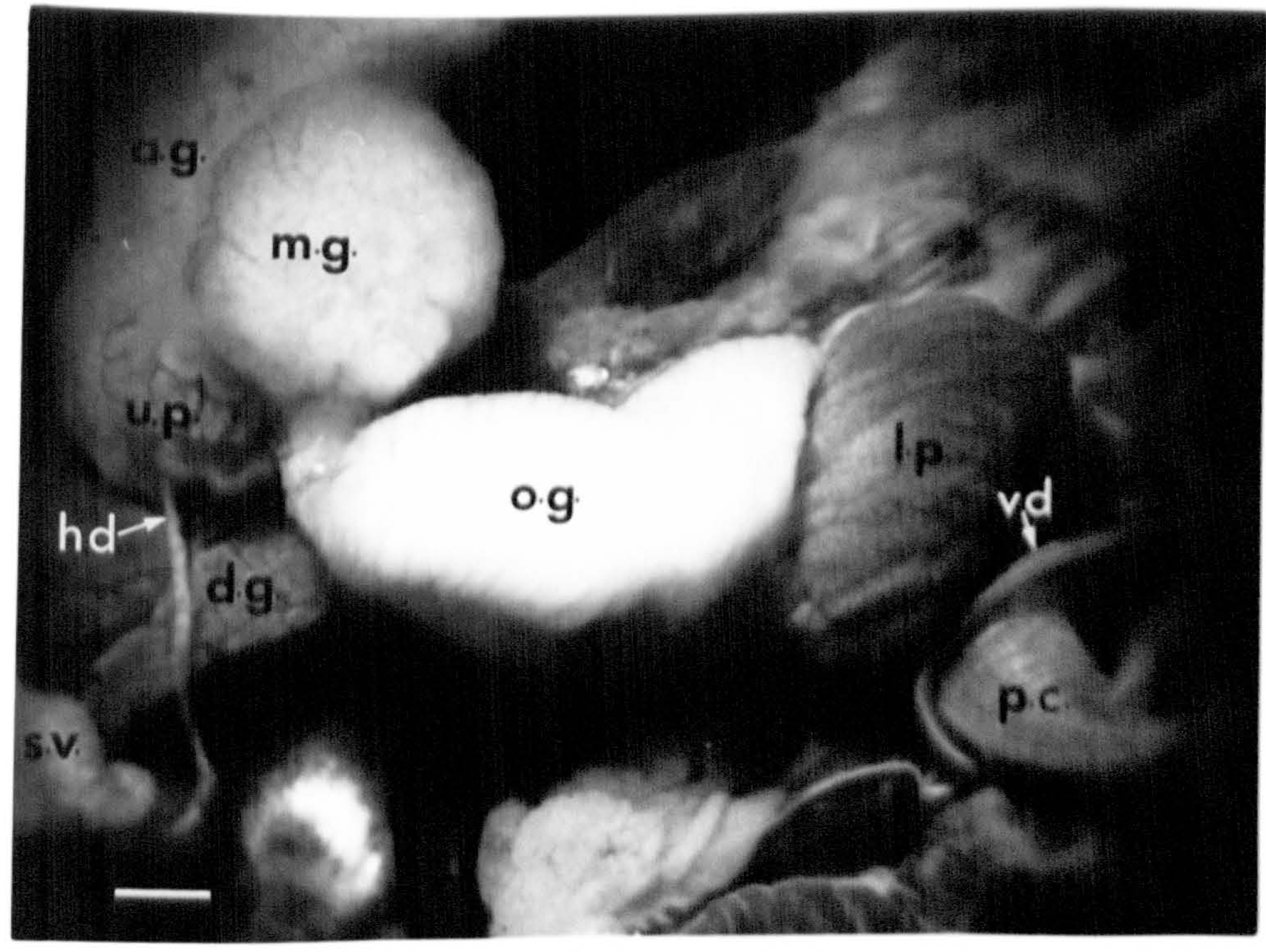
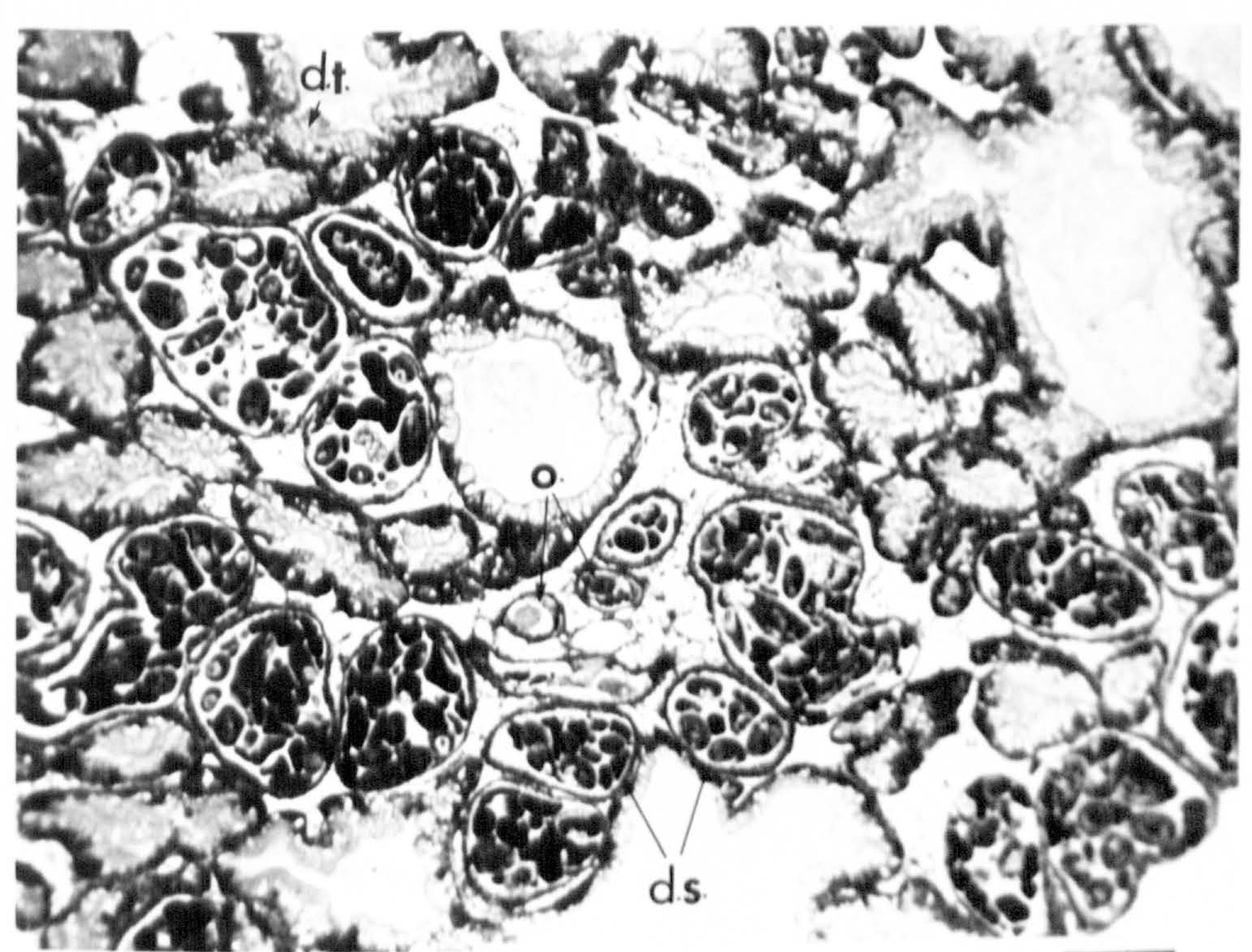

a.g.
m.g.
u.p.
hd
d.g.
o.g.
l.p.
vd
p.c.
s.v.

Fig. 3.13. Transverse section (8µm) through the visceral sac of L. stagnalis (approx. 7 months old) to show the distribution of the daughter sporocysts and digestive gland tubules in relation to the rudimentary ovotestis of a 4-month old infection with P. elegans. Stained with Mallory's Triple stain. (d.t.) digestive gland tubules; (d.s.) daughter sporocyst; (o.) ovotestis. Scale 400µm.

Fig. 3.14. Reproductive structures in the head region of L. stagnalis (approx. 13 months old) infected for 10 months with P. elegans. Scale 1.0mm.

d.t.	digestive gland tubules
d.s.	daughter sporocyst
h.d.	hermaphrodite duct
i.	intestine
l.p.	lower prostate
m.g.	muciparous gland
o.g.	oöthecal gland
p.c.	penial complex
s.g.	salivary gland
u.p.	upper prostate
v.d.	vas deferens

63a



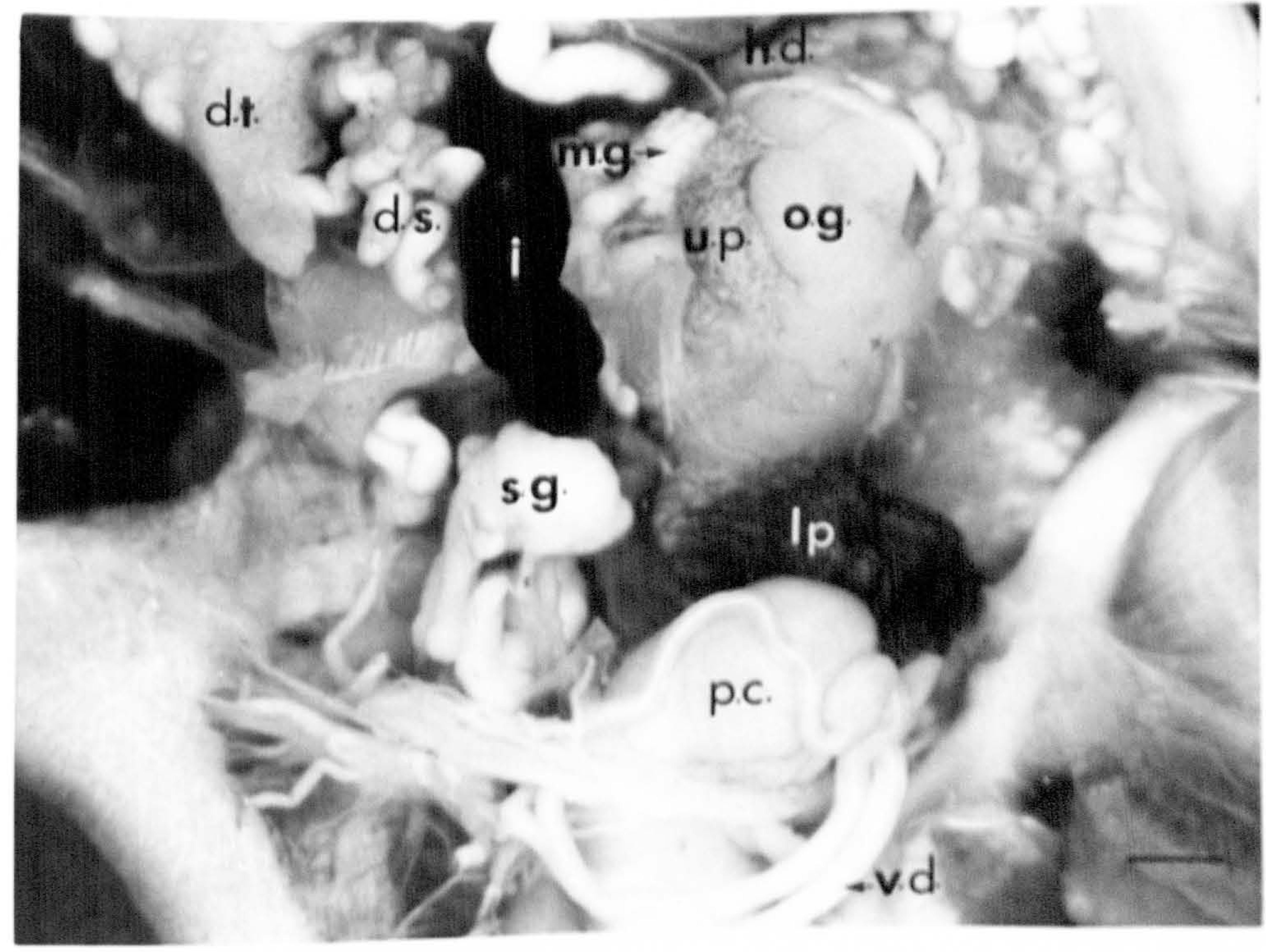
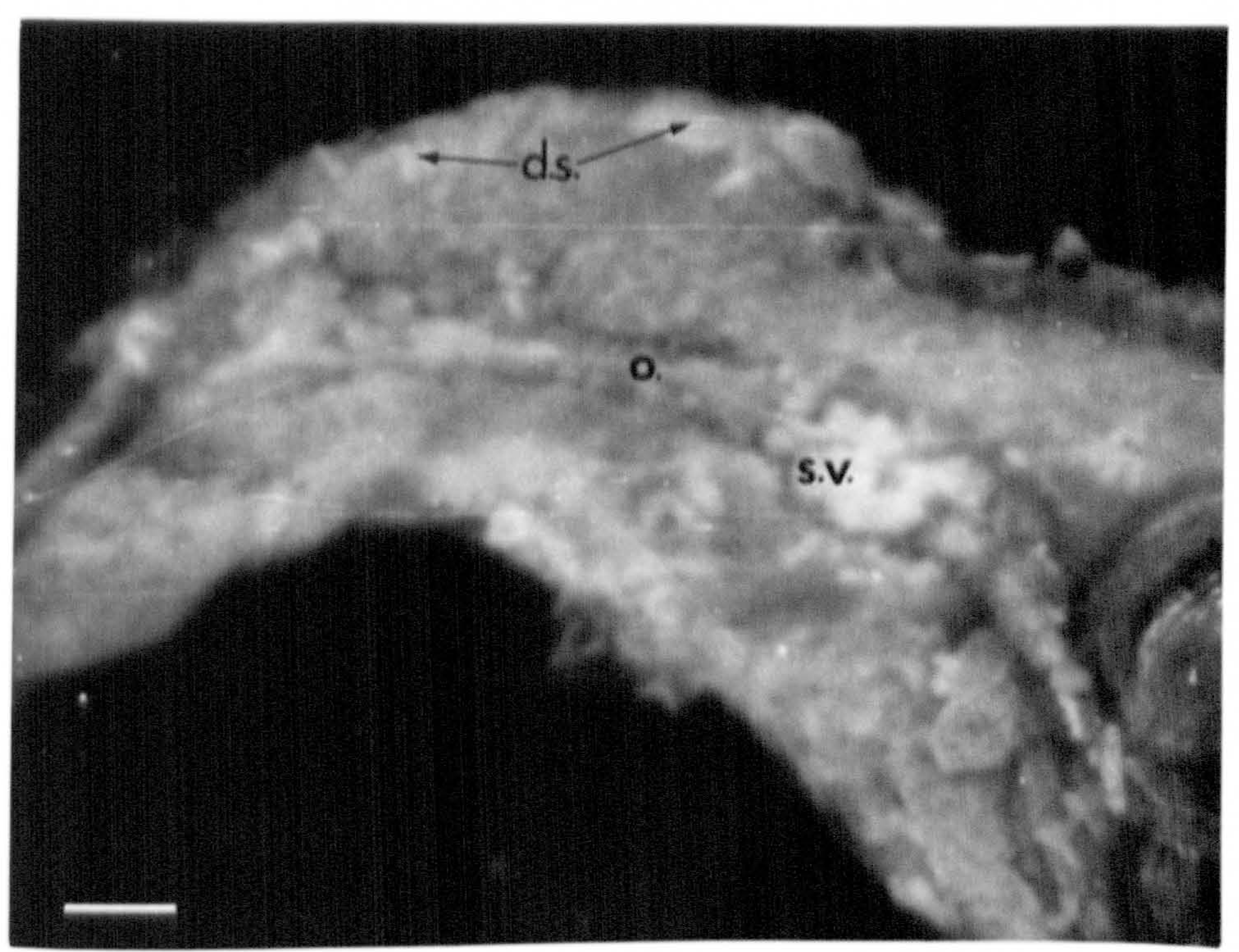
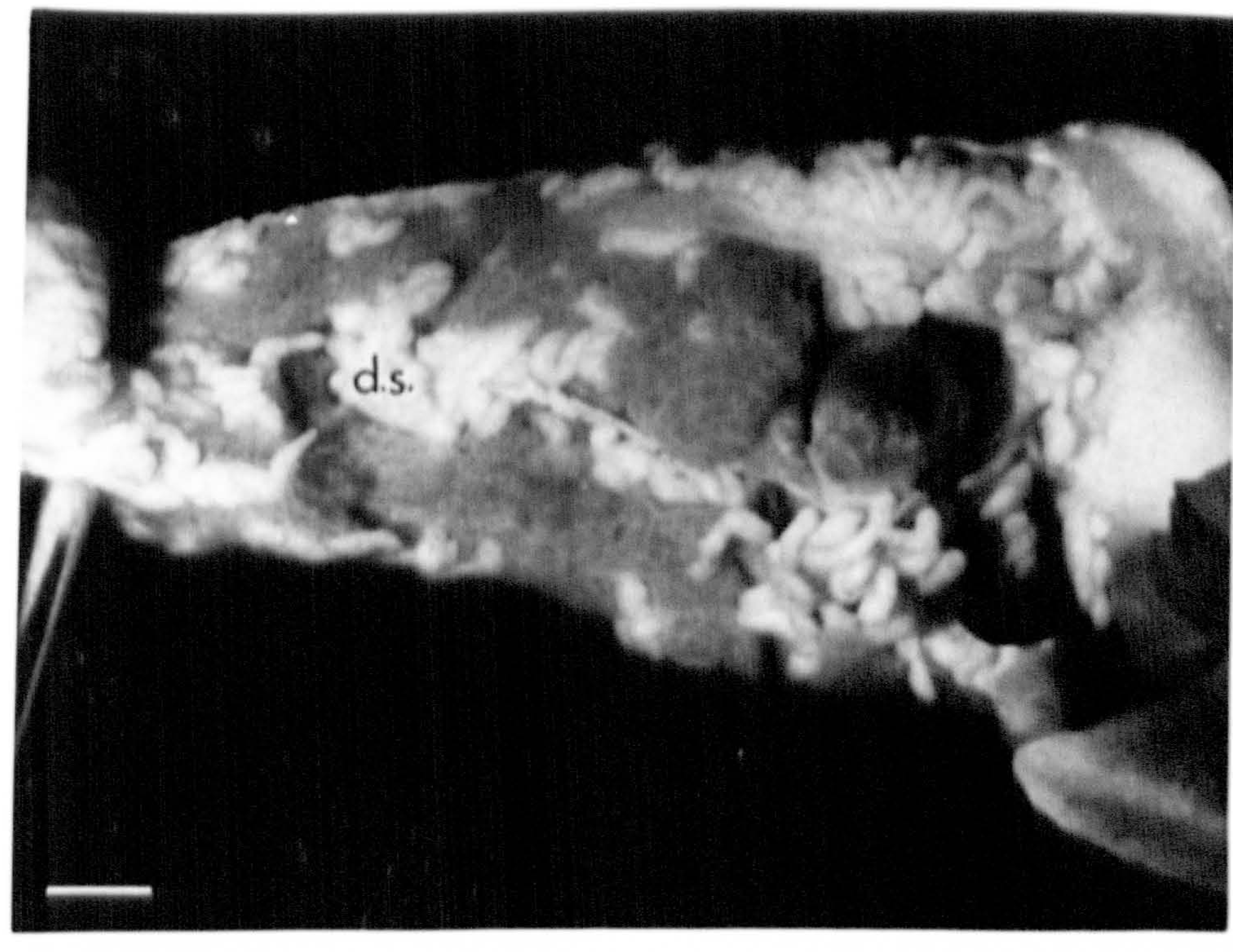


Fig. 3.15. Lymnaea stagnalis (9 months old) infected when 5 months old and sexually mature upon exposure to 50 P. elegans eggs. Note presence of ovotestis (o.) along midventral line, also the seminal vesicle (s.v.) and the distribution of the daughter sporocysts (d.s.). Scale 1.0mm.

Fig. 3.16. Lymnaea stagnalis (11 months old) infected when 3 months old by a single P. elegans egg. Note presence of daughter sporocysts (d.s.) along midventral line and the apparent lack of the ovotestis in contrast to Fig. 3.15. Scale 1.0mm.


d.s.
o.
s.v.


d.s.

Section 4

Cercaria and metacercaria

INTRODUCTION.

Previous studies have shown that several environmental factors, in particular light and temperature, affect the emergence patterns of cercariae (Cort, 1922; Rees, 1931; 1947; Giovannola, 1936; Asch, 1972). Investigations concerning species of Plagiorchis conducted by Rees (1952), Macy (1960), Wagenbach and Alldredge (1974), Theron (1976) and Blankespoor (1977) have demonstrated that within the genus there is no behavioural uniformity with regard to light. In some species cercariae are released predominantly during periods of darkness while in others they are released almost solely during periods of light; in addition cercariae once released may be positively phototropic, negatively phototropic or apparently unresponsive to light. Temperature on the other hand is considered not to be involved in the periodicity of cercarial release, rather it is thought to affect the magnitude of numbers of cercariae emerging. When the temperature exceeds a maximum or falls below a minimum the emission of cercariae ceases altogether.

Cercarial behaviour may affect the range of second intermediate hosts and thus ultimately the range of final hosts by placing the cercariae in the vicinity of potential second intermediate hosts. Species of Plagiorchis are known to utilize lymnaeid snails, aquatic insects and fresh water crustaceans as second intermediate hosts, while adults of the genus occur naturally in a wide variety of birds, mammals, amphibians and reptiles.

This study was undertaken to elucidate various aspects of the biology of the cercaria of P. elegans, in particular its release, behaviour, host specificity and encystment and thus to obtain a better understanding of the mode of transmission of the parasite.

MATERIALS and METHODS.

The present investigations were conducted using the laboratory established strain of P. elegans in Lymnaea stagnalis (p. 17). An incubator with timed cycling of illumination was employed to carry out experiments concerning cercarial release both at low temperatures and during alternating 12h periods of light and darkness at constant temperatures of either 18°C or 22°C. Cercariae were removed with a fine pipette and counted in groups of five, since dilutions were found to

be inaccurate as a result of the tendency of cercariae to congregate at one side of the bowl and to cling to the glass surfaces when the water is agitated.

Potential second intermediate hosts were obtained from a variety of sources. Chironomid larvae were purchased commercially and sampled on a regular basis (p. 16). *Xenopus laevis*, *Lymnaea stagnalis* and *L. palustris* were laboratory-reared, while *Gammarus pulex*, *Asellus aquaticus*, and *Bufo bufo* were collected locally. Since *L. stagnalis* occurred at the site where *B. bufo* was collected, 10 tadpoles were examined but harboured no metacercarial cysts.

RESULTS.

Cercarial release; monomiracidial infections.

As stated on p. 39 monomiracidial infections of *P. elegans* were established in 6 of 45 *L. stagnalis* which survived at least 42 days beyond the date of exposure. In order to determine the productivity of each mother sporocyst in terms of cercarial production by daughter sporocysts, four of the snails were individually isolated for 24h at weekly intervals in small finger bowls containing approximately 150 cm^3 of dechlorinated water and lettuce. Throughout the investigations the specimens were maintained at a constant temperature of 22°C and constant light (from fluorescent tubes). The counts were begun 12 weeks after infection and were continued until the death of the snails 23 to 25 weeks later (Fig. 4.1).

The daughter sporocyst stage of *P. elegans* is extremely prolific-a total of 111,371 cercariae were released by snail A (Table 4.1; Fig. 4.1) during the weekly isolations over a period of 24 weeks; from week 12 to week 36 as few as 425 to as many as 7070 larvae emerged during the weekly counts (Fig. 4.1).

There was considerable variation in the weekly cercarial counts not only for each snail, but also when the development of the four infections are compared. They remained fairly constant in snail A from week 16 to week 31 with 3 distinct peaks; the first occurred during week 19, while the second and third peaks occurred on the 29th and 33rd weeks after infection just prior to the death of the snail. Cercarial release increased more gradually in snail B, peaking on the 26th week post infection, but it also fell off more sharply than in snail A. The counts did not rise above 2750 in snail C until the 24th week post infection at which time 4950 cercariae were released. They then increased rapidly to 10,600 on the 28th week and fell off again, although

less rapidly than in B. It should be noted however that snail C appeared to be ill when isolated during the 16th week and this may account for the more gradual increase in cercarial release. Although there were several peaks in cercarial release during the infection of snail D the counts never rose above 4430.

Table 4.1. Production of cercariae by monomiracidial P. elegans infections of L. stagnalis during the period 12 to 37 weeks post infection.

	Snail			
	A	B	C	D
Total no. of cercariae counted (1 day/week)	111,371	100,317	100,720	58,119
Estimates of total cercarial production	779,597	702,219	705,040	406,833
No. of sporocysts present	667	-	643	-
Estimated total number of cercariae/sporocyst	1169	-	1096	-

It was considered possible that the weekly isolations of the snails in small volumes of water may have affected them physiologically, - initially by stimulating the release of greater numbers of cercariae in the freshly changed water and then later by inhibiting the release of cercariae because of the accumulation of waste products. If this were the case it should have resulted in considerable variation between the cercarial counts obtained on consecutive days. To determine whether there were very great fluctuations in the numbers of cercariae released per day, a single snail (D) was isolated for 4 consecutive 24h periods; the water was changed after each period and the cercariae released counted. As shown in Table 4.2 the variation is not substantial. Consequently, the once-weekly counts are reasonable estimates of the numbers of cercariae produced and can therefore be multiplied by the appropriate factor (7 days/week) to give the total numbers shed per snail (see Table 4.1).

On the death of snails A and C the sporocysts within each were carefully teased out and counted (Table 4.1); 667 and 643 were present in A and C respectively. There are no counts available for snails B and D because the former was sectioned and the latter died and

started to decay making a reliable count impossible.

Table 4.2. Cercariae released during 4 consecutive 24h periods by a single *L. stagnalis* (snail D, see Table 4.1) infected with *P. elegans*.

	cercariae emitted
day 1	3775
day 2	3400
day 3	3850
day 4	3750
total	14775

mean = 3693.75 S.D. = 200.39

Factors influencing cercarial release.

Temperature.

In order to investigate the influence of low temperature on cercarial release, four experimentally infected *L. stagnalis* (24-week old infections), which had been maintained at 18°C in constant illumination and were each releasing from 1500 to 2300 cercariae per day, were individually isolated, as before for total cercarial counts, and allowed to acclimate for three days at 11°C, still in continuous light. During this time one snail died despite the daily changes of water while the others produced from 45 to 500 cercariae per day. The temperature was gradually reduced over 48h from 11°C to 8°C and several cercariae emerged from one snail during this period. At 6°C a single cercaria was shed by the same individual. When returned to normal laboratory conditions, all three snails resumed cercarial emission (approximately 1500 to 2000/snail/day).

The upper temperature limit of cercarial shedding was not determined, because it entailed the possibility of killing the snails.

Photoperiodicity.

The following experiments were performed to determine the pattern of emergence of cercariae of *P. elegans* from laboratory infected *L. stagnalis* during alternating 12h periods of light and darkness at 18 or 22°C. The snails were kept individually in small glass covered vessels (150 cm^3) within the incubator and at the end of each time

interval each snail was transferred into a fresh bowl of water at the appropriate temperature; cercariae in the previous bowl were then counted.

In the preliminary investigation a single L. stagnalis (infected for 16 weeks) which had been maintained at 22°C under continual light was isolated as described above, at 22°C for 4 consecutive days without being allowed to acclimate. On the first day only, cercarial counts were made hourly during the initial 3 hours; thereafter they were performed at the end of each 12h period (Fig. 4.2). Counts of 74, 36 and 45 cercariae were obtained for hours 1, 2 and 3 respectively, while only 90 cercariae were released during the following 9 hours. Figure 4.2 clearly illustrates that the majority of cercariae were shed in darkness.

In the following experiment three L. stagnalis (infected for 15 weeks), which had been maintained at 18°C in continual light, were isolated as before but at 18°C. In contrast to the preliminary study the snails were allowed to adjust to the new conditions for five days prior to the start of cercarial counts; furthermore, the periods of light and darkness were reversed, that is, the snails were maintained in darkness during the daytime and under illumination at night. During this time the water was changed daily. The numbers of cercariae released were then determined three times at intervals of 4 hours during each dark period, but only a single count was performed at the end of each 12h light period (Fig. 4.3).

Although the numbers of cercariae emitted varied depending on the individual gastropod, cercarial emergence again occurred primarily during periods of darkness, despite the fact that the photoperiods had been reversed. The greatest emergence occurred during the first few hours of darkness and rapidly decreased thereafter.

Cercarial behaviour and longevity.

Upon emergence from the snail host the cercariae are both negatively phototropic and negatively geotropic. They tend to occur in rather dense clouds against one surface of the finger bowl rather than being evenly dispersed throughout the liquid. Cercariae swim actively for several hours after release occasionally resting dorsal surface downwards on the bottom of the container, with the body of the cercaria cupped around the ventral sucker. From this position swimming is resumed by rapid movement of the posterior extremity of the body combined with lashings of the tail. Eventually after several hours swimming ceases and the cercariae crawl along the dish bottoms. Agi-

tating the water may stimulate them to swim for short periods, although they may instead adhere very tightly to the substratum by means of their suckers.

When cercariae are placed in water containing chironomid larvae the former show no evidence of attraction by the latter. This apparent indifference exhibited by the cercariae towards the chironomids continues with the cercariae swimming actively for several hours. However, when an individual cercaria makes contact with a chironomid it immediately attaches itself utilizing its suckers and crawls over the surface of the insect apparently searching for a suitable place to penetrate; occasionally cercariae leave the chironomid and resume swimming. Normally penetration occurs through the soft intersegmental articulations of the body and is accomplished by the scraping action of the stylet against the insect cuticle combined with cytolysis of this tissue by material released from the penetration gland cells; as a result a small slit is made in the host tissue through which the cercaria enters. Frequently the tail is shed just prior to penetration, although it may be cast off while the cercariae are crawling freely on the surface of the insect or substratum; after having been shed the tail continues to move slowly for some time. Completion of the entire process of penetration takes about 10 to 15 minutes. Once within the larva some cercariae may wander away from the point of entry, but the majority appear to encyst nearby within 10 to 30 minutes of penetrating.

When maintained at room temperature, cercariae are apparently able to penetrate and encyst in chironomid larvae immediately upon release and up to 36h after emission. However the earliest formal recordings of these processes have been made not less than 90 minutes after release. Furthermore despite the fact that cercariae released in temperatures as low as 6 to 8°C swim lethargically they still encyst successfully in larvae of *Chironomus* sp.

Metacercaria.

Metacercariae rotate very actively while laying down the cyst wall, but within several days this rapid movement becomes markedly reduced and thereafter is sporadic. They have not been found to be infective within 24h of formation, although some metacercariae are within 48h. Cysts are known to remain infective in chironomid larvae for as long as one month and to retain their infectivity through metamorphosis into the adult stage. Metacercariae from *Gammarus pulex*, *Asellus aquaticus* and from sporocysts in both *Lymnaea stagnalis* and

L. palustris are also viable.

Most laboratory infections of chironomids were standardized by exposing 6 larvae to approximately 120 cercariae in a petri dish (5 cm diam.). Under these circumstances the insects each carry a burden of from 10 to 30 cysts. Larger numbers of cercariae per larva caused the death of some larvae. Yet on one occasion when chironomid larvae were exposed to an undetermined number of cercariae 75 cysts were recovered from a single individual one week later.

Host specificity.

In order to investigate the specificity of the cercariae of P. elegans for the second intermediate host, 30 specimens of each of the following species - Gammarus pulex, Asellus aquaticus and Chironomus sp. - were isolated individually in small petri dishes (5cm diam.). Each dish contained 25cm^3 of tap water and 20 cercariae which were approximately 90min old. After 60 minutes' maintenance at room temperature the arthropods were removed and kept in clean water for 3 days before examination for the presence of cysts. This delay provided time for the accumulation of concretions in the excretory bladder of the metacercariae. The presence of these highly light-refractive granules renders the metacercariae more visible within the body of the arthropod. The results are given in Table 4.3.

Table 4.3. Specificity of the cercaria of P. elegans for the second intermediate host. (Each individual exposed for 60 min to 20 cercariae which were approximately 90 min old.)

	host species		
	Chironomus sp.	Gammarus pulex	Asellus aquaticus
No. surviving	27	30	28
No. infected	27	1	3
Total no. cysts recovered	296	1	3
% recovered	54.8	0.2	0.5
Cysts/host	10.96	0.03	0.11
Range/host	1 - 18	0 - 1	0 - 1

These results show that the cercariae of P. elegans penetrate chironomid larvae more readily than either G. pulex or A. aquaticus. Even when the crustaceans are isolated with much larger numbers of cer-

cariae similar results are obtained. Data from such an experiment are given here. Ten A. aquaticus and 18 G. pulex were placed in separate vessels each containing approximately 1000 cercariae. One week later each individual crustacean was dissected. Seven of the 10 asellids were infected with from 1 to 5 metacercarial cysts each (mean 1.5) and 13 of the 18 gammarids harboured from 1 to 8 cysts each (mean 1.8).

The preferred sites of encystment within chironomid larvae are the anterior and posterior segments, particularly the false legs. When successful penetration of gammarids and asellids occurs encystment is most frequent within the appendages and antennae, although cysts are sometimes found in the gills.

Amphibians.

The reactions of cercariae to both Bufo bufo and Xenopus laevis tadpoles were examined under a low power stereomicroscope. Approximately 150 actively swimming cercariae were pipetted into each of two finger bowls containing 150cm^3 of tap water and either 5 X. laevis or 5 B. bufo tadpoles. The cercariae were evidently not attracted by the tadpoles, although some cercariae landed on the tadpoles and crawled over their surfaces. A few cercariae were even carried into the amphibians' gill chambers by the inhalent respiratory current. However in all cases the cercariae resumed swimming within a short period of time. No cysts were present when the amphibians were dissected 3 days later.

Molluscs.

Precocious encystment.

Precocious encystment occurs within the daughter sporocysts of P. elegans (Fig. 4.4) in both L. stagnalis and L. palustris. Six of 7 L. stagnalis which had been maintained at 18°C and which each harboured 6-month old experimental infections were found to contain precociously encysted metacercariae. The numbers found per snail are given in Table 4.4.

Table 4.4. Precocious encystment of metacercariae of P. elegans within experimentally infected L. stagnalis. (Each snail infected for 6 months).

snail no.	1	2	3	4	5	6	7
no. cysts	0	2	27	30	56	75	138

total = 328; mean = 46.86; S.D. = 48.42

Molluscs as second intermediate hosts.

In aquaria containing only infected snails and thus a high density of cercariae, penetration and subsequent encystment apparently occur in most of the snail surfaces accessible to the cercariae. As shown in Fig. 4.5 the cysts appear as numerous white spots on the outer surface of the snail. Examination of histological sections of infected L. stagnalis has revealed the presence of metacercarial cysts within and just below the epithelium of the head, foot, mantle and the haemocoel, including the organs within the body cavity. No cercariae were found in the process of migrating towards the exterior through the musculature of the foot; as a result it is believed that cercariae either encyst within the epithelial surfaces of the haemocoel before emerging from the snail host or encyst within the epithelium of the external body surfaces after having emerged.

Encystment without host penetration.

The conditions which trigger encystment in the absence of a host animal are unknown. On rare occasions when snails have been isolated overnight cysts have been found in the snail mucus, on the lettuce and even more exceptionally free in the water. The surrounding environment is apparently unsuitable however, because in no instance have the cysts been alive when examined within twelve to eighteen hours of formation.

Attempts to induce encystment.

The first attempt to induce encystment was performed using chironomid larvae haemolymph, since cercariae readily encyst in these larvae. Twenty-five Chironomus sp. larvae were teased apart in $5.0cm^3$ of tap water contained in a small petri dish (5.0cm diam.) and the cuticle and body tissues were carefully removed; to this solution were added 300 actively swimming cercariae in $5.0cm^3$ of tap water. No changes were noted in the behaviour of the cercariae; they continued swimming and ultimately died.

When the experiment was repeated, the procedure was altered by including the host tissue, because it is believed that the act of penetration may be the principal mechanism triggering encystment; in addition a saline solution (0.9%) was used rather than tap water. Most of the cercariae exhibited no interest in the tissue, however the cercariae were allowed to incubate overnight at $23^{o}C$ and upon examination the following morning 26 cysts were present in the tissue, although none of them contained living metacercariae.

While attempts to induce encystment and subsequently maintain the

metacercariae have been unsuccessful, cysts (6 days or older), teased from chironomid larvae, have on several occasions survived for up to 48h in a 0.9% saline solution maintained at either 18 or 22°C and have infected LACA mice.

DISCUSSION.

The large number of larval digeneans produced by daughter sporocysts of P. elegans compensates in part for the hazards encountered by cercariae during their brief free-swimming existence and ensures the transmission of infection. Other contributing factors are the low degree of second intermediate host specificity and possibly the synchronization of cercarial release to coincide with active periods of potential intermediate hosts.

It is estimated that as many as 779,597 cercariae were produced during the course of a monomiracidial infection of L. stagnalis. In fact, this figure is considered to be conservative, because the snail died within 10 months of infection. The duration of infection of a number of other specimens infected by one or more miracidia has exceeded 12 months (p. 43) although cercarial counts were not performed during this time. In one instance a specimen of L. stagnalis infected for 15½ months was known to be shedding cercariae 3 days before its death. Both frequent handling and isolation in small volumes of water probably contributed to the early death of the molluscs carrying monomiracidial infections.

Up to 10,600 cercariae have been shed during 24h by a single specimen of L. stagnalis harbouring a monomiracidial infection. Within the genus Plagiorchis such prolificity however is not unique to P. elegans. Wagenbach and Alldredge (1974) reported the release of 5,375 P. micracanthos cercariae from a naturally infected Stagnicola exilis during 12h of darkness, while Macy (1960) stated that 2000 P. vespertilionis parorchis cercariae were emitted within the peak hour of cercarial release by naturally infected L. stagnalis. One of the longest studies concerning the fecundity of larval digeneans was conducted by Meyerhof and Rothschild (1940) who observed the release of Cryptocotyle lingua cercariae from a naturally infected Littorina littorea over a period of 5 years. They estimated that on average 3600 cercariae were produced per day during the first year of their observations; this number gradually decreased until in the fifth year of the snail's captivity approximately 830 cercariae emerged daily.

From the number of daughter sporocysts produced per mother sporo-

cyst and the cercarial counts of monomiracidial infections an estimate of the number of cercariae which develop from each daughter sporocyst can be calculated. Experimental evidence presented in Section 3 (p.42) supports the existence of only a single daughter sporocyst generation. As a result each daughter sporocyst produces an average of nearly 1100 cercariae during its lifetime. Although the rate of daughter sporocyst development has not been investigated, it is known that in a 9-month old infection (p. 18) sporocysts ranged in size from 0.60 to 2.36mm in length and 0.21 to 0.68mm in width. It was also noted during the examination of snails, whether infected with one or several miracidia, that some daughter sporocysts were apparently exhausted while others contained many developing cercariae. Since neither size nor duration of cercarial production is constant, even in daughter sporocysts from a single mother sporocyst, some daughter sporocysts may produce far fewer than 1100 cercariae and others well in excess of that figure.

Before encysting the cercariae of P. elegans must under ordinary circumstances come into contact with a suitable second intermediate host. Occasionally they encyst precociously, but the vast majority of cercariae leave the snail and assume a free-swimming existence, which they are able to sustain for a limited amount of time before dying. It is therefore to their advantage that they are able to penetrate and encyst in a wide variety of hosts. Chironomus sp. larvae are not necessarily the normal or most frequent host of P. elegans; Owen (personal communication) has reported finding metacercarial cysts of P. elegans in naturally infected caddis and mayfly larvae. These data together with the experimental results presented here suggest that encystment of the cercaria of P. elegans occurs predominantly in insect larvae, although molluscs and fresh-water crustaceans may also be employed as hosts. Apparently there are multiple routes by which the life cycle may be completed and the diet of potential final hosts is not the limiting factor it would be if the cercariae were more host specific. Encystment in gastropods, with the exception of precocious encystment, probably does not occur commonly under natural conditions; in the current experiments it may be attributed to the fact that the infected snails were maintained together in water containing very dense concentrations of cercariae. Styczynska-Jurewicz (1962) has reported that under experimental conditions cercariae of P. elegans penetrate into young specimens of L. stagnalis, although with difficulty. Penetration of the crustacean integument was apparently difficult since few cysts were present when they were dissected.

Several members of the genus do readily encyst within Gammarus

pulex; these include *P. cirratus* (Buttner & Vacher, 1959), *P. laricola* (Krasnolobova, 1971) and *P. farnleyensis* (Diaz, 1976); *P. noblei* (Williams, 1963) and *P. neomidis* (Brendow, 1970) do not. It is interesting to note that Styczynska-Jurewicz (1962) reported that the cercariae of *P. elegans* penetrated *Gammarus pulex* with difficulty, yet in contrast to the present investigations it readily encysted within *Asellus aquaticus*.

Precocious encystment among species of *Plagiorchis* has not often been reported, but many species of the genus have been described solely on the basis of adult specimens without reference to the larval stages. For this reason it is believed that precocious encystment may be more widespread than the literature reveals. It has been recorded for:-

P. muris Dollfus (1925)
P. ramlianum Azim (1935) within rediae
P. muris McMullen (1937b)
P. jaenschi Johnston & Angel (1951)
P. goodmani Najarian (1961)
P. elegans Styczynska-Jurewicz (1962)
P. dilimanensis Velasquez (1964)

while metacercarial cysts have not been recovered from the sporocysts of

P. vespertilionis parorchis Macy (1960)
P. noblei Williams (1963).

McMullen(1938) suggested that precocious encystment occurs most frequently when the snails are approaching senility and death and is probably stimulated by the resulting physiological changes. He did not believe that temperature was a factor, since few cysts were present in young infected gastropods during the beginning of the summer when the water temperature was still low, while the incidence increased through the heat of summer and into the cold of autumn with the advancing age of both snail and parasite.

Attempts to induce encystment and subsequently to maintain the cysts have so far proved to be unsuccessful; although cercariae will penetrate and encyst within the tissues of dismembered chironomid larvae, the resulting metacercariae do not survive. At no time were viable cysts found within 12 to 18h of formation whether in the larval tissues, on lettuce leaves, snail mucus or when encystment occurred in the water in which the snails were isolated. Yet in the laboratory 6-day old metacercarial cysts maintained in 0.9% saline at 18 or 22°C were infective for up to 48h after having been teased from the second

intermediate host. Rees (1952) has stated that it requires approximately 12h for the cercaria of P. megalorchis to complete the formation of its cyst wall and the present author has demonstrated that metacercarial cysts of P. elegans are not infective within 24h of formation. The differences between immature potentially infective and mature infective cysts are apparently confined to the cyst wall and structures associated with its formation, such as cystogenous gland cells, since the larva is not progenetic. As a result it appears that the inability of metacercariae to survive when encystment occurs externally or at least not within a living host was due to a combination of factors:

first, the surrounding environment was unsuitable, perhaps osmotically.

second, the cyst wall was not sufficiently formed for the metacercaria to maintain its metabolism by regulating the passage of material, possibly nutrients, into or out of the cyst.

The likelihood of cercariae making contact with potential hosts may be enhanced by the behaviour of the cercariae. When first released they are negatively geotropic and swim most actively; such behaviour no doubt serves to disperse the larvae (Cable, 1965). In addition it is possible that cercarial release is synchronized with periods of host activity, as suggested by their apparently innate pattern of emergence. Throughout the present investigation most cercariae of P. elegans were shed during periods of darkness with the greatest number emerging within the first few hours after an extended period of light. While working with P. neomidis, Theron (1976) observed that the cercariae were shed in the absence of light while Sialis lutaria, the natural second intermediate host, is most active. The sudden shedding of cercariae may also have been stimulated by the change of water, because during the first few hours of both light and darkness more cercariae were released than during the hours following.

It is possible that the emission of large numbers of cercariae depends on conditions which cause the accumulation within the snail of mature cercariae that are ready to be shed, since cercariae may develop at a fixed rate throughout the day (Rees, 1947; Asch, 1972; Erasmus, 1972). On the other hand perhaps factors unknown at the present time accelerate the process of maturation under certain conditions. Asch (1972), studying infections of Schistosoma mansoni in Biomphalaria glabrata, thought that cercariae may be responding to a physiological rhythm within the snail which is photo-dependent. Lymnaea stagnalis

was reared in continual light at a constant temperature in the present investigations, yet responded immediately to alternating periods of light and darkness, even when the natural photoperiod was reversed (Fig. 4.3). Not all the cercariae of _Plagiorchis_ species are nocturnal however. The recorded relationships between cercarial release and light or darkness are listed below.

Released only in darkness:-

P. megalorchis Rees (1952)
P. vespertilionis parorchis Macy (1960)
P. noblei Daniell & Ulmer (1964)
P. peterborensis Kavelaars & Bourns (1968)
P. micracanthos Wagenbach & Alldredge (1974)
P. farnleyensis Diaz (1976)
P. neomidis Theron (1976)
P. noblei Blankespoor (1977)

Released only in light:-

P. jaenschi Johnston & Angel (1951)
P. maculosus Angel (1959)
P. noblei Williams (1963)

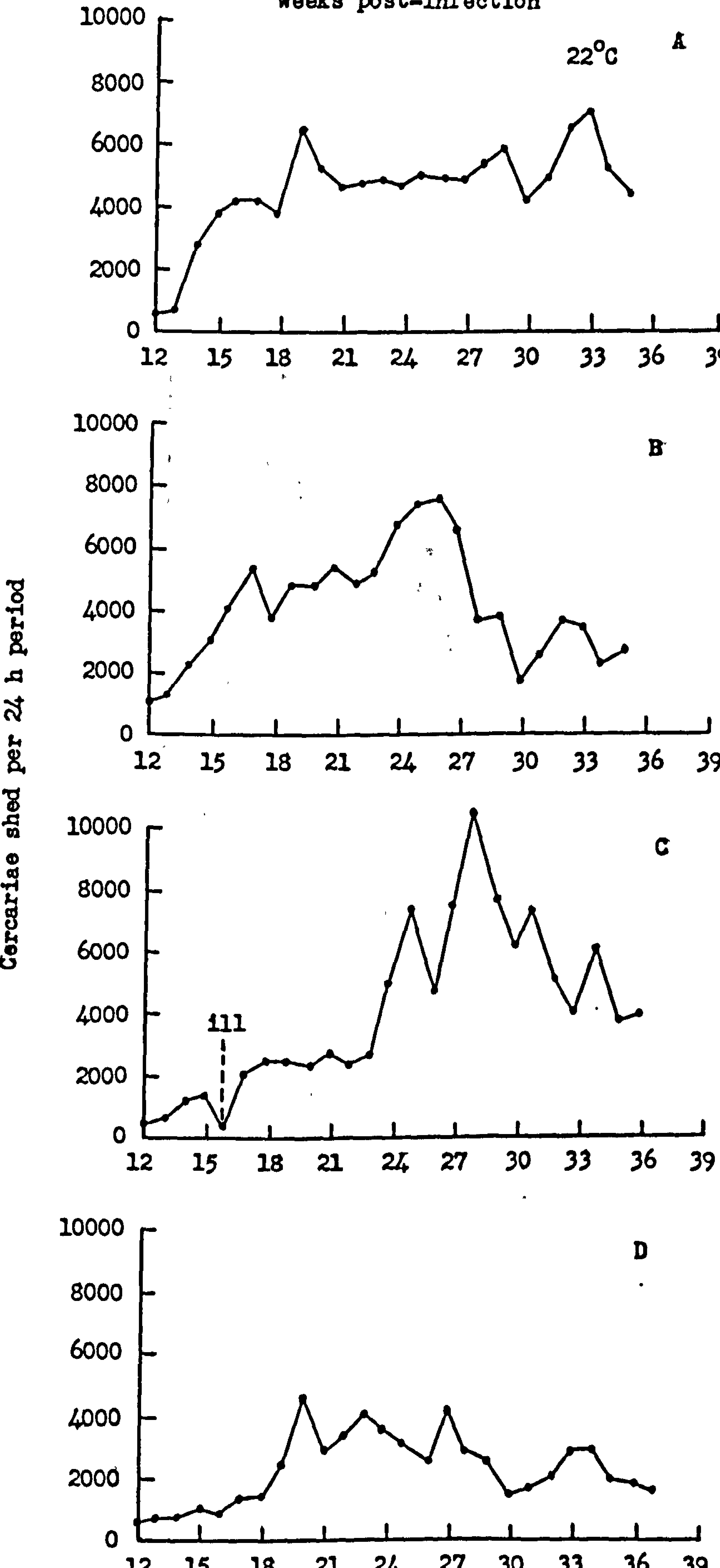
Released in both light and darkness:-

P. elegans Styczynska-Jurewicz (1962)
P. kirkstallensis Diaz (1976)

Temperature, although it may inhibit the shedding of cercariae beyond upper and lower limits, has not been implicated in the periodicity of cercarial release of _Plagiorchis_ species (Macy, 1960; Blankespoor, 1977).

Perhaps as suggested by Asch (1972) and Erasmus (1972) studies on the activity patterns of snails and on their biochemical and physiological fluctuations may reveal a rhythm which affects the maturation and emission of cercariae.

Fig. 4.1. Weekly counts of cercariae of *P. elegans* shed during 24h by monomiracidial infections of laboratory-reared *L. stagnalis*. The four graphs represent counts for individual snails - A,B,C and D.


Weeks post-infection
22°C
A
B
C
D
ill
Cercariae shed per 24 h period
10000
8000
6000
4000
2000
0
12
15
18
21
24
27
30
33
36
39

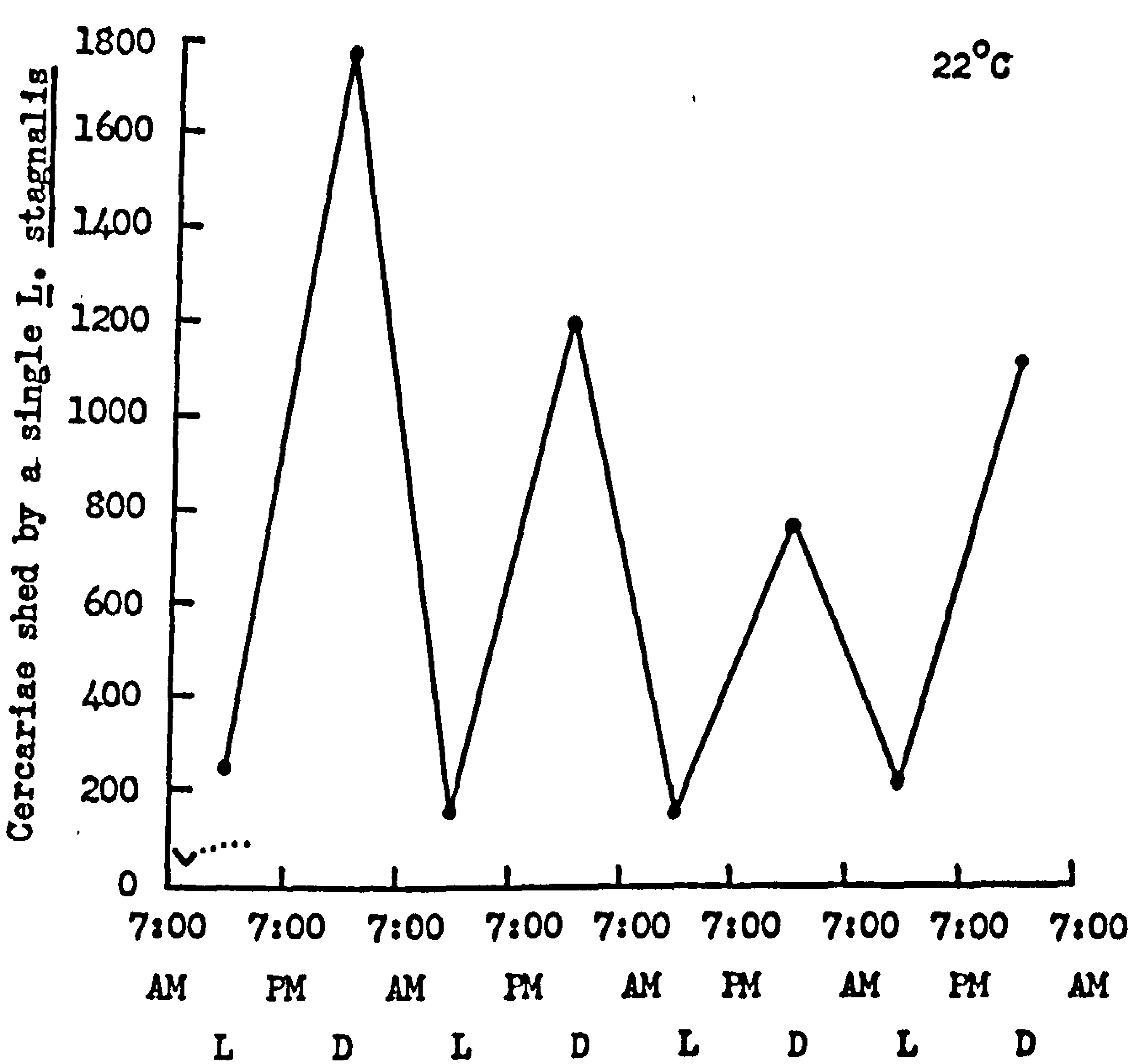
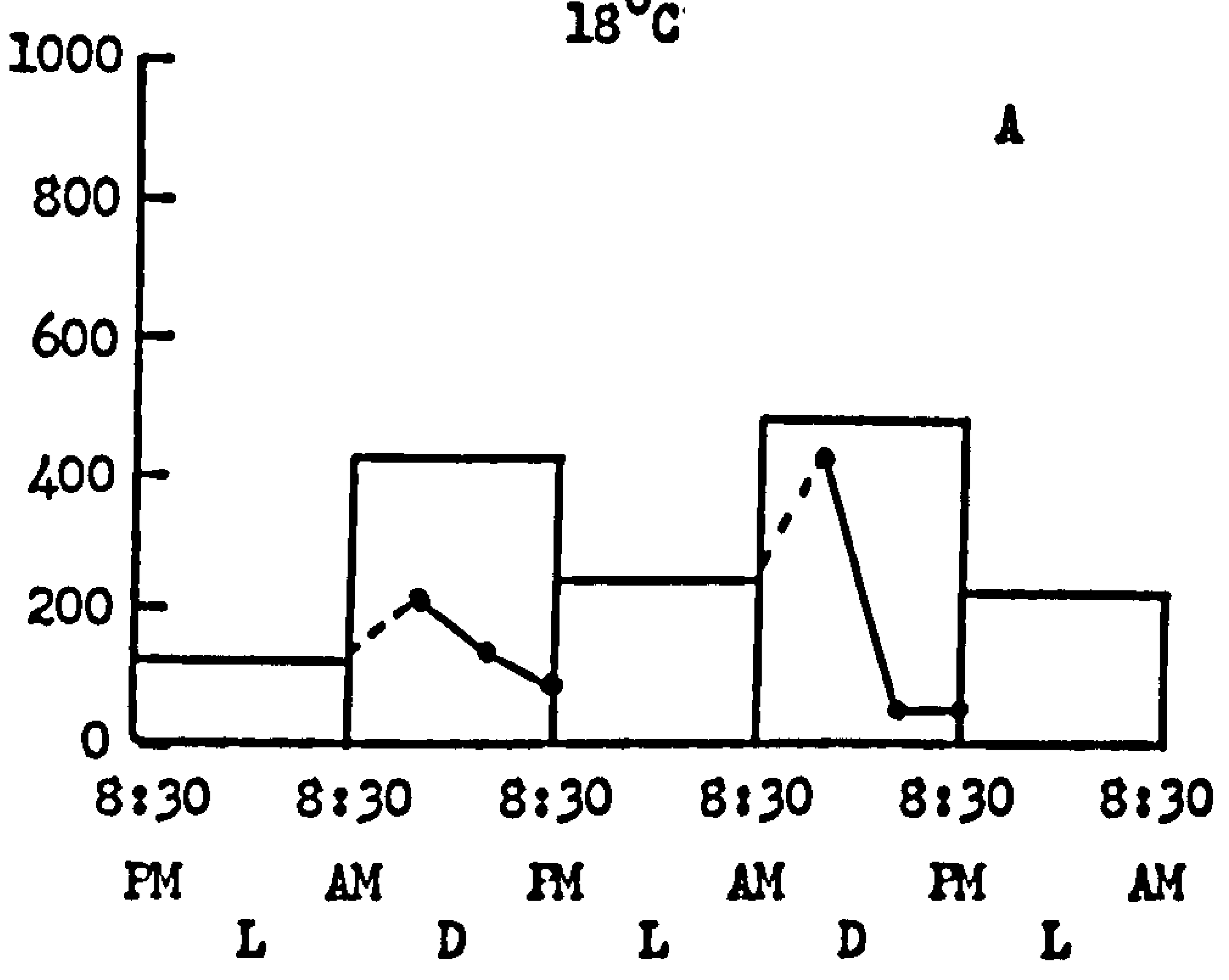
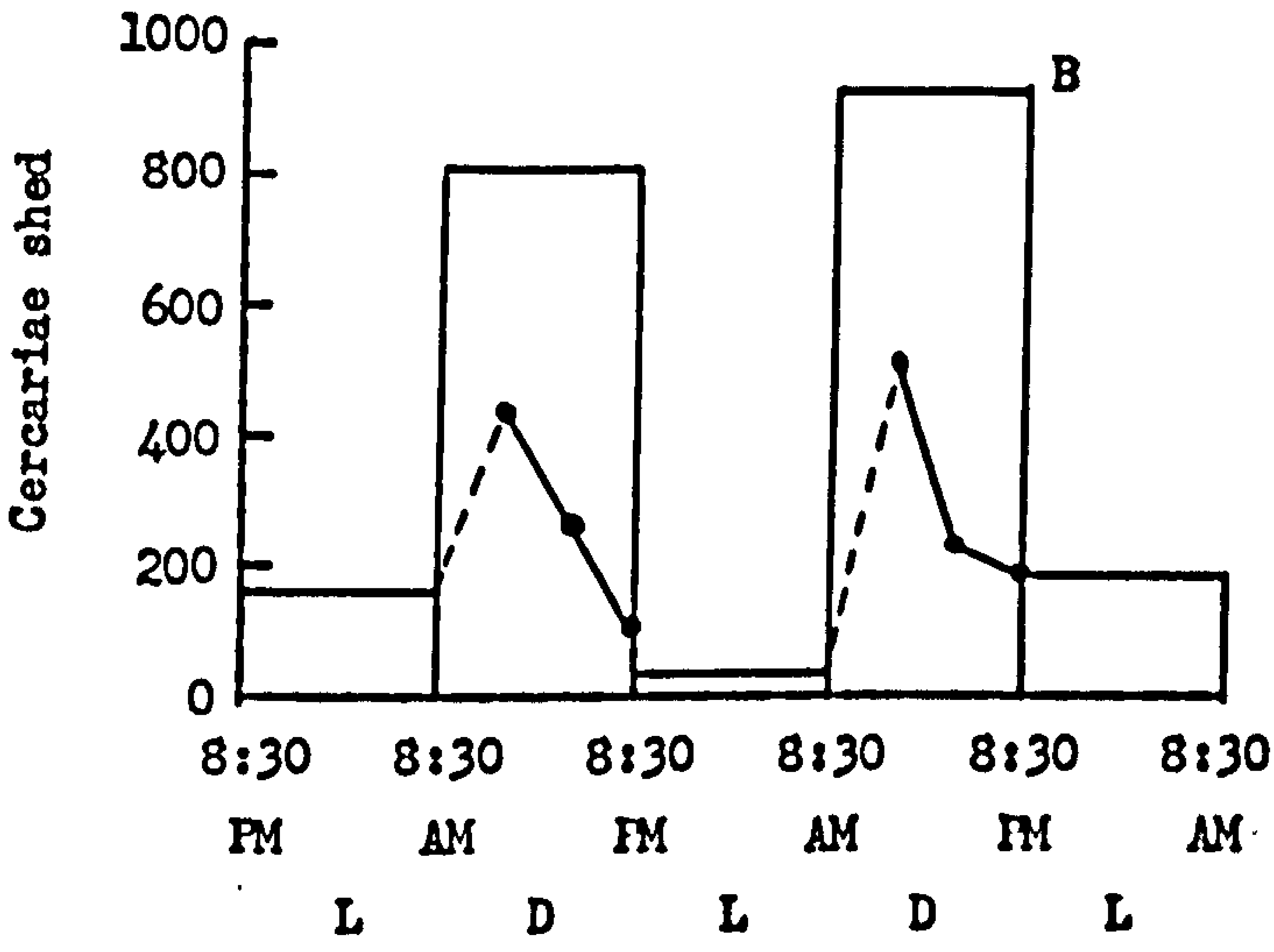


Fig. 4.2. The effect of alternating 12h periods of light (L) and darkness (D) on emergence of cercariae of P. elegans from L. stagnalis (16-week old infection). First three counts performed at hourly intervals.

Fig. 4.3. Shedding of cercariae of P. elegans from L. stagnalis (15-week old infection) during alternating 12h periods of light (L) and darkness (D). Three counts performed at intervals of 4h during each dark period. Graphs A, B, and C represent shedding by individual snails. Histograms represent total numbers of cercariae shed per 12h interval.


18°C
A
1000
800
600
400
200
0
8:30 PM
8:30 AM
8:30 PM
8:30 AM
8:30 PM
8:30 AM
L
D
L
D
L


Cercariae shed
B
1000
800
600
400
200
0
8:30 PM
8:30 AM
8:30 PM
8:30 AM
8:30 PM
8:30 AM
L
D
L
D
L

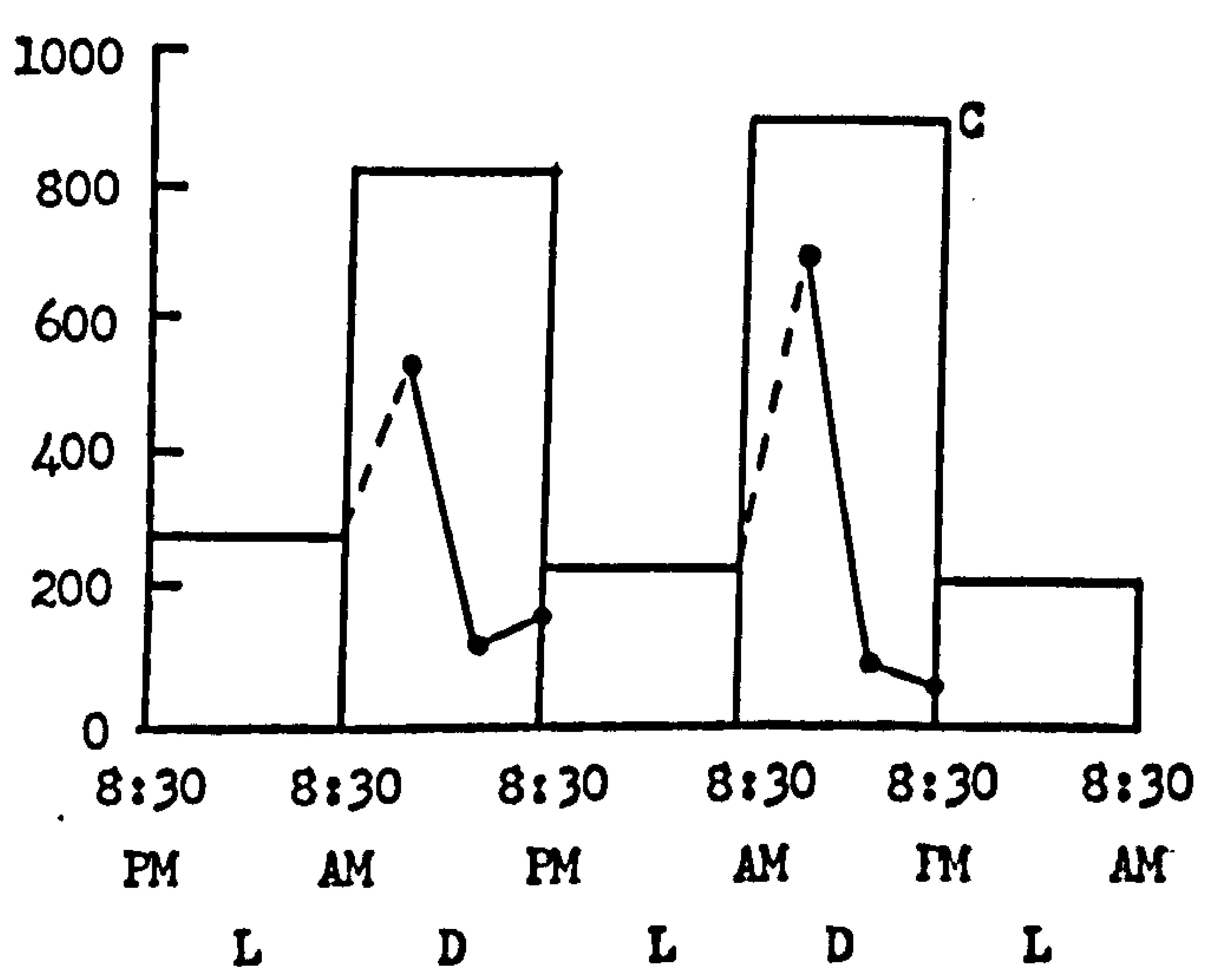
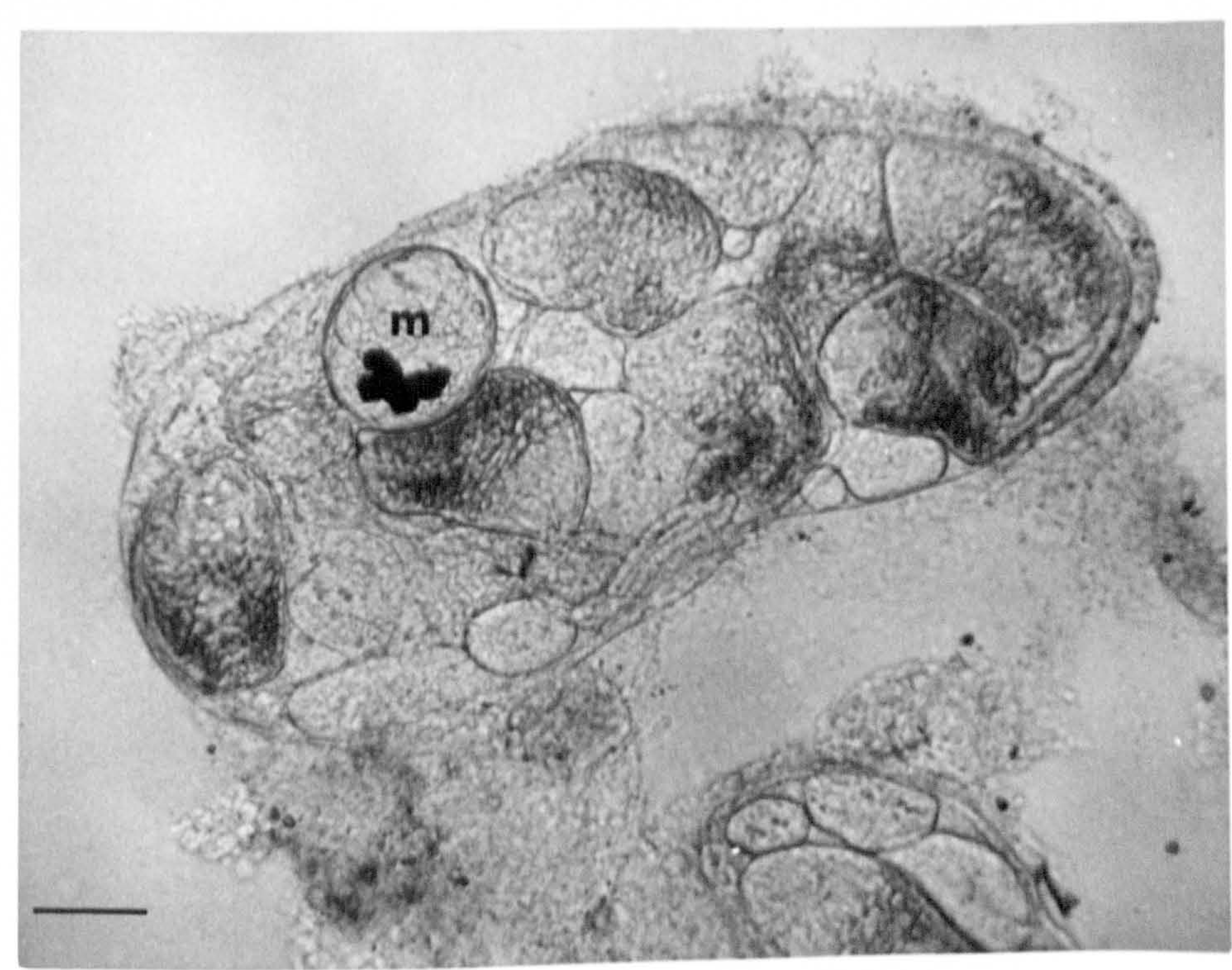
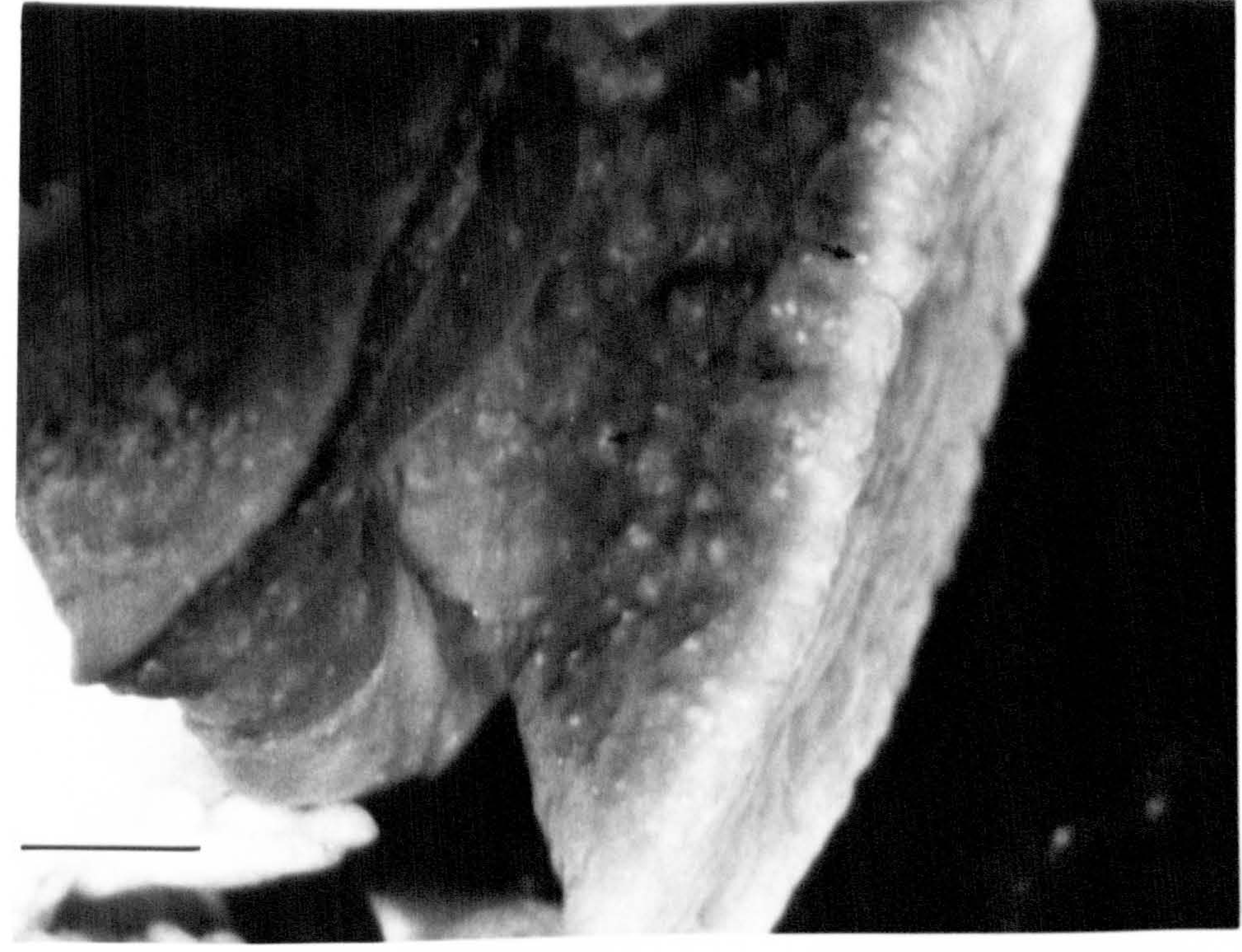

C
1000
800
600
400
200
0
8:30 PM
8:30 AM
8:30 PM
8:30 AM
8:30 PM
8:30 AM
L
D
L
D
L

Fig. 4.4. Plagiorchis elegans daughter sporocyst teased from the digestive gland of L. stagnalis harbouring a 6-month old infection. Note the presence of a precociously encysted metacercaria (m).
Scale 0.10mm.

Fig. 4.5. Head and foot region of L. stagnalis infected with P. elegans. Encysted metacercariae are visible as numerous whitish dots (arrows). The snail was maintained for 6 months in an aquarium tank containing four other infected snails.
Scale 0.25cm.


m



Section 5

The adult stage of *Plagiorchis elegans*

INTRODUCTION.

One of the most difficult tasks in taxonomy is to establish criteria by which genera and species may be defined. Guidelines set down by Looss (1902) and later applied by Stunkard (1960) conceive of the natural genus as comprising species with a common anatomical pattern. Within the genus there are essentially no morphological differences and the member species may be distinguished by variation in the size and position of the elements within the pattern.

In delineating the more than 100 species of *Plagiorchis* taxonomists have emphasized variation in body and egg size, extent of the vitellaria, position of the internal structures and the ratios, particularly of the suckers and gonads to one another (Dollfus, 1960; Massino, 1927; Park, 1936; Mehra, 1937; Schulz and Skworzow, 1931; Olsen, 1937). Yet the possible effect of the host on the morphology of the adult has often been overlooked (Styczynska-Jurewicz, 1962; Sharpilo and Sharpilo, 1972; Blankespoor, 1974), although it is well established that the rate of development of digenetic flukes may vary depending upon the definitive host and that such variation in rate of development has a direct effect upon the relative sizes of the body and internal structures (Willey, 1941; Stunkard, 1957; Dawes, 1962; Kinsella, 1971). Furthermore, the method of fixation utilized may affect the position and shape of the organs (Styczynska-Jurewicz, 1961, 1962; Groschaft and Tenora, 1974).

In many instances species have been established on insufficient data; for example, because the presence or absence of a rudimentary seminal receptacle is considered a distinguishing feature between species (Yamaguti, 1971), the examination of histological preparations is necessary. However when species have been established on the characteristics of only single specimens (Macy, 1931; Mehra, 1937; Sandground, 1940; Strom, 1940; Furmaga, 1956) it has not been possible to section the type specimen. In numerous other instances *in toto* preparations provide the only material for specific descriptions.

Besides morphological variation, host specificity has often been cited as a means of distinguishing between species of *Plagiorchis* (Skrjabin and Massino, 1925; Schulz and Skworzow, 1931; Ogata, 1938; Tang, 1941; Chalupsky, 1954; Richard, 1965/1966; Brendow, 1970; Theron, 1976). However several species of the genus are recorded to be polyxenous (Braun, 1902; McMullen, 1937; Angel, 1959; Blankespoor, 1974).

In Section 1 a historical review of the taxonomy of the genus Plagiorchis was presented, mentioning particularly the criteria on which taxonomists have based the erection of new species of Plagiorchis. In this section an attempt is made to reevaluate these parameters by investigating host specificity and the effects of various first, second and definitive hosts on the morphology and rate of development of a pure laboratory strain of P. elegans and to compare it with established species.

MATERIALS and METHODS.

Laboratory-reared Lymnaea stagnalis and L. palustris were used as alternate first intermediate hosts; Chironomus sp. larvae, Gammarus pulex, Asellus aquaticus and L. stagnalis served as second intermediate hosts. Potential final hosts were laboratory-reared Xenopus laevis, LACA mice, rats and gerbils, commercially supplied chicks, ducklings and hamsters and adult pigeons and shrews (Sorex araneus) caught in the vicinity of Leeds.

Before attempting to infect them, animals not bred in the laboratory were isolated for a period of at least ten days and their faeces checked daily to ascertain whether they harboured natural trematode infections. Although all the hamsters carried cestode infections and the shrews and several rats were found to be infected with both cestodes and nematodes, no digenean eggs were recovered.

All the animals were provided with food (non-antibiotic) and water ad libitum. Mice, rats, ducklings, hamsters and gerbils were fed Oxoid Pasteurized Breeding Diet. The diet of the last two hosts was supplemented with carrots. Xenopus laevis were fed liver pellets, chicks chick starter crumbs, and pigeons hen corn. The shrews were fed blowfly larvae.

Each of two X. laevis adults was fed 150 cysts by stomach tube; two other toads and specimens of S. araneus were allowed to ingest approximately 300 and 400 cysts respectively, which were encysted within living chironomid larvae. Mice, rats, gerbils, and hamsters were fed 1 to 20 cysts, while chicks, ducklings and pigeons were fed 25, 100, 300 or 400 cysts all by stomach tube. The cysts were 6 to 15 days old except when L. stagnalis served as second intermediate host, in which case their age was unknown.

Mice and rats were sacrificed after varying periods of time, that is 4, 7, 14 and 21 days and 7, 14, 21 and 28 days respectively, so that the rate of development of the flukes in the two hosts could be

compared. All other hosts were examined 7 days after infection.

Immediately upon killing the animals the small intestine was removed, placed flat in an extended but not taut position and, with the exception of toads and shrews, measured. To facilitate the search it was then cut into 6 or 12 equal sections depending upon its length; each section was placed in a petri dish containing 0.9% saline, slit longitudinally and examined under a dissecting microscope for P. elegans.

Specimens of P. elegans were examined live under high power magnification (oil immersion; x 900) and for whole mounts were individually flattened under the slight pressure of a cover slip, heat killed and fixed in 10% formol saline (Lillie, 1965). They were stained with alum carmine and mounted in XAM (Gurr), with half of the specimens mounted dorsally and the other half ventrally. Measurements were noted and drawings were made with the aid of a camera lucida. Sucker ratios were calculated by dividing the approximate mean diameter of the oral sucker $(\frac{\text{length + width}}{2})$ by that of the ventral sucker $(\frac{\text{length + width}}{2})$. Because the oral sucker may be either terminal or subterminal, probably as a result of fixation, the preacetabular distance was measured from the middle of the oral sucker to the middle of the ventral sucker, rather than from the anterior extremity of the worm. Measurements of the cirrus sac were not made because this structure is frequently displaced and distorted during fixation, so accurate comparative measurements are difficult if not impossible to obtain. Adults for sectioning were fixed in hot Bouin's solution; serial longitudinal and transverse paraffin wax sections (m.p. 58°C) were cut at either 5 or 7µm in thickness and stained with Mallory's Triple stain (Humason, 1967). Adult specimens were compared with co-types of P. megalorchis Rees, 1952, which were lent by the British Museum.

Egg counts were performed on both infected rats and mice using the McMaster method (Ministry of Agriculture, Fisheries and Food, 1971). The animals were kept individually in cages provided with grated floors through which their faeces could easily fall and faecal samples were collected at 24h intervals. Total egg output per 24h period per host was calculated rather than eggs per gramme of faeces, because the daily weight of faeces per mouse was not often in excess of 3.0g. Daily faecal samples from rats were weighed and egg counts performed using 3.0g; total daily egg release was then calculated.

RESULTS.

Anatomy of adult P. elegans (Figs. 5.1 to 5.6).

The following description is based on 7-day old specimens recovered from LACA mice; the first and second intermediate hosts in the life cycle were Lymnaea stagnalis and Chironomus sp. larvae respectively.

Body elongate, oval to elliptical in outline; 2.16 to 3.12mm long by 0.59 to 0.81mm wide. Tegument covered by transverse rows of spines, spines densest anteriorly, hooked, curving posteriad, 7 to 10μm long by 3 to 4μm wide at base. Oral sucker ventral, terminal or subterminal, larger than ventral sucker. Preacetabular distance 1/4 to 1/5 body length.

Mouth opening on mid-ventral surface of oral sucker, leading into short prepharynx; oesophagus approximately length of prepharynx following trilobed muscular pharynx, bifurcating into intestinal caeca; caeca continuing laterally toward sides of body, turning caudad and running along lateral body margins, terminating near posterior extremity.

Three layers of muscle existing: circular, longitudinal and innermost oblique. Dorsoventral muscles also well developed.

Brain dorsal to oral sucker and anterior to pharynx. Three pairs of nerves, situated on either side of oral sucker: dorsal, lateral and ventral.

Excretory vessel characteristic Y-shape of genus Plagiorchis; main stem extending anteriorly from ventral, nearly terminal excretory pore, bifurcating at mid level of anterior testis; cornua of vessel not reaching level of ovary in section, in whole mounts extending as far anteriorly as posterior 1/3 of ovary. Flame cells often obscured by internal structures; established formula for genus as in cercaria (Diaz, 1976) $2\ (3 + 3 + 3) + (3 + 3 + 3) = 36$.

Ovary postero-dorsal and to the right of the ventral sucker; oviduct arising from postero-lateral border of ovary, enlarging into rudimentary seminal receptacle; Laurer's canal taking slightly sinuous course from seminal receptacle to dorsal surface and exterior; union of transverse yolk ducts forming vitelline reservoir dorsally in mid-line; median yolk duct arising from reservoir and entering oviduct just distal to seminal receptacle, continuing as ovovitelline duct, expanding to form oötype surrounded by Mehlis' gland. Descending limb of uterus functioning as seminal receptacle, following S-shaped route posteriorly, dorsal to anterior testis, ventral to posterior testis, bending back upon itself near posterior extremity of body; ascending limb retracing route of descending limb ventrally; uterus proceeding from approximately mid level of ovary as thick-walled metraterm; unicellular gland cells sur-

rounding metraterm proximally; uterus terminating at female pore in common genital atrium.

Anterior extent of vitellaria varying from slightly posterior to origin of caeca to anterior border of oral sucker; yolk glands continuing along lateral sides of body, overlying caeca to near posterior extremity of body; vitellaria not confluent antero-ventrally; antero-dorsal commissure present or absent; majority of specimens possessing posterior vitellarian commissure, often encircling body.

Testes situated obliquely in posterior half of body; anterior testis ventral and to left of midline, posterior testis dorsal and to right of midline; both vasa efferentia extending anteriorly from anterior border of testes; entering cirrus sac separately though close together; expanding to form seminal vesicle, consisting of long proximal cylindrical part and smaller spherical distal body; prostatic gland cells surrounding pars prostatica; ejaculatory duct and unarmed, coiled, invaginated cirrus in distal portion of cirrus sac; evaginated cirrus hollow cylinder approximately 0.4mm in length; in whole mounts cirrus sac generally extending anteriorly from mid-level of ovary around right side of ventral sucker, terminating at male gonopore in common genital atrium; cirrus sac dorsal to both acetabulum and metraterm in section.

Morphological and anatomical variation due to age (Figs. 5.8 and 5.9).

LACA mice and laboratory rats were infected with P. elegans to compare the effects on morphology of the two hosts in addition to the rate of growth of the parasites. The animals were fed known numbers of cysts and were sacrificed at set intervals from the fourth to the twenty-eighth day post-infection. Comparative measurements of the fixed specimens are given in Tables 5.1 and 5.2.

LACA mice.

Plagiorchis elegans is protandric; 3 of the 19 4-day old specimens examined had sperm in the seminal vesicle, but none of them possessed eggs in utero. However, it is of note that during preliminary studies eggs were found in the faeces of mice 4 days after infection. The largest immature fluke recovered, that is with neither eggs nor a developed seminal vesicle, was 1.60mm long and 0.43mm wide; the smallest mature fluke was 1.04 by 0.34mm.

By day 7 the parasites had grown to their maximum mean length (2.68 mm), the gonads were well developed and the uterus filled with eggs. When 2 and 3 weeks of age the mean over-all dimensions of the flukes were 2.61 by 0.74mm and 2.66 by 0.73mm respectively. With advancing age the gonads decreased in size and the uterus was no longer replete with

Table 5.1. Anatomical measurements, expressed as means, used in the taxonomy of *Plagiorchis*. Specimens were harvested from LACA mice 4,7,14 or 21 days after infection. Each mouse was fed 10 or 20 cysts, 6 to 12 days old. (1 standard error given in parentheses.) Measurements in mm.

age of worms	4 days	7 days	14 days	21 days
no. measured	19	28	40	8
no. mice	2	4	6	3
body length	1.34 (0.030)	2.68 (0.042)	2.61 (0.063)	2.66 (0.131)
body width	0.42 (0.009)	0.69 (0.011)	0.74 (0.009)	0.73 (0.032)
oral sucker length	0.15 (0.002)	0.21 (0.002)	0.24 (0.002)	0.24 (0.003)
oral sucker width	0.15 (0.002)	0.20 (0.002)	0.23 (0.002)	0.23 (0.003)
pharynx length	0.08 (0.001)	0.10 (0.002)	0.12 (0.002)	0.11 (0.002)
pharynx width	0.09 (0.001)	0.12 (0.002)	0.14 (0.001)	0.13 (0.003)
ventral sucker length	0.10 (0.002)	0.15 (0.002)	0.17 (0.002)	0.18 (0.002)
ventral sucker width	0.10 (0.001)	0.15 (0.001)	0.17 (0.001)	0.18 (0.002)
ovary length	0.09 (0.004)	0.22 (0.004)	0.21 (0.006)	0.19 (0.012)
ovary width	0.08 (0.004)	0.22 (0.004)	0.21 (0.005)	0.19 (0.014)
anterior testis length	0.19 (0.005)	0.35 (0.008)	0.30 (0.008)	0.26 (0.019)
anterior testis width	0.15 (0.006)	0.27 (0.007)	0.23 (0.005)	0.18 (0.019)
posterior testis length	0.20 (0.006)	0.37 (0.011)	0.30 (0.008)	0.28 (0.014)
posterior testis width	0.16 (0.006)	0.28 (0.007)	0.22 (0.005)	0.17 (0.018)
preacetabular distance	0.40 (0.008)	0.59 (0.019)	0.60 (0.018)	0.63 (0.047)

Table 5.2. Summary of routes by which the experimental life cycle of P. elegans was completed with data on the parasite's dimensions and the distribution of the vitellaria anteriorly. Measurements in mm.

final host (no.)	cysts dose/host	age worms days	first intermediate host	second intermediate host	no. meas-ured	body length mean (1SD)	body width mean (1 SD)	ant. vit.* bridge +	-
LACA mice (2)	20	4	L. stagnalis	Chironomus sp.	19	1.34 (0.307)	0.42 (0.096)	9	5
LACA mice (4)	10	7	"	"	28	2.68 (0.506)	0.69 (0.130)	6	22
LACA mice (6)	10 or 15	14	"	"	40	2.61 (0.413)	0.74 (0.117)	18	22
LACA mice (3)	10 or 20	21	"	"	8	2.66 (0.941)	0.73 (0.258)	2	3
rats (4)	15	7	"	"	27	1.54 (0.296)	0.45 (0.087)	12	13
rats (2)	15	14	"	"	14	1.83 (0.489)	0.52 (0.139)	2	12
rats (3)	15	21	"	"	19	2.40 (0.551)	0.70 (0.161)	5	14
rats (2)	15	28	"	"	6	1.90 (0.776)	0.56 (0.229)	3	1
LACA mice (1)	10	7	"	G. pulex	9	2.42 (0.807)	0.71 (0.237)	2	7
LACA mice (3)	10	7	"	A. aquaticus	27	2.22 (0.427)	0.65 (0.125)	8	19
LACA mice (1)	20	7	"	L. stagnalis	9	2.53 (0.843)	0.69 (0.230)	1	8
LACA mice (3)	10 or 20	7	L. palustris	Chironomus sp.	29	2.65 (0.492)	0.64 (0.119)	17	12
hamsters (4)	10 or 20	7	L. stagnalis	"	10	1.99 (0.629)	0.53 (0.168)	5	5
gerbils (3)	10	7	"	"	7	2.93 (1.107)	0.79 (0.299)	2	5
chicks (3)	400	7	"	"	10	2.07 (0.655)	0.57 (0.180)	0	10
ducklings (1)	400	7	"	"	7	1.38 (0.522)	0.37 (0.140)	1	6
pigeons (3)	300 or 400	7	"	"	7	1.86 (0.703)	0.59 (0.223)	1	6

* It was not possible in all cases to determine whether an anterior vitelline bridge was present or absent.

eggs, in some instances it was nearly empty. However, it is evident from Table 5.1 that there was on the whole an increase in size of the non-reproductive structures (oral sucker, pharynx and acetabulum) with age. The relationships of the internal organs to one another are given in Appendix II. At different ages the mean sucker ratios (Table 5.3) of specimens recovered from LACA mice are quite constant, being 1.33:1 to 1.46:1, but they occur over a considerable range - 1.16 to 1.60:1.

Laboratory rats.

Maturation occurs more slowly in the laboratory rat as demonstrated by the fact that the maximum length was not attained until the third week of infection (Table 5.2; Fig. 5.9) and even then the worms were not as large as 7-day old P. elegans from mice. In addition, few eggs were present in the uterus of week old flukes; of the 27 specimens recovered and fixed 10 had no eggs in utero and in 3 of those 10 the seminal vesicle was not yet apparent. The largest immature worm was 1.62mm long by 0.40mm wide, while the smallest adult specimen was 1.22 by 0.38mm respectively.

As exhibited previously in mice (Appendix II) the size relationships of the various organs to one another change during the course of the infection. Although the mean sucker ratios (Table 5.3) are, at 1.49:1 to 1.57:1, somewhat larger than in mice, they are consistent within the rat final host, and again occur over a wide range, 1.30:1 to 1.63:1.

Effects of various hosts on morphology.

Definitive hosts.

Three gerbils were each fed 10 cysts and 8 hamsters were each fed either 10 or 20 cysts (Fig. 5.10 B and C). Specimens of P. elegans recovered from gerbils were the largest flukes observed during this study (Table 5.2) and all the worms remaining after 7 days in both hamsters and gerbils were mature.

The sucker ratios (Table 5.3) of worms from these hosts were like those of week-old adults from mice. While the relative dimensions of the internal organs are quite similar in flukes from mice, gerbils and hamsters (Appendix II), the size of the oral sucker of specimens recovered from hamsters differs from that of 7-day old worms harvested from mice, because there is no consistent size relationship noted between it and the testes; in adults from gerbils the oral sucker was occasionally found to be larger than the ovary, but this was never the case in 7-day old specimens from mice. Also, the ventral sucker of flukes from hamsters was noted to be larger, smaller or occasionally

Table 5.3. Sucker ratios of P. elegans obtained from a variety of experimentally infected hosts. (Life cycle normally completed using L. stagnalis and Chironomus sp. + alternative first intermediate and * second intermediate hosts.)

final host	age worms days	no. meas.	OS:VS mean (1 SE)	OS:VS range
LACA mice	4	19	1.46:1 (0.08)	1.25:1 to 1.60:1
LACA mice	7	28	1.33:1 (0.06)	1.16:1 to 1.44:1
LACA mice	14	40	1.36:1 (0.07)	1.24:1 to 1.55:1
LACA mice	21	8	1.33:1 (0.04)	1.28:1 to 1.40:1
rats	7	27	1.51:1 (0.04)	1.38:1 to 1.59:1
rats	14	14	1.53:1 (0.07)	1.43:1 to 1.63:1
rats	21	19	1.49:1 (0.08)	1.30:1 to 1.61:1
rats	28	6	1.57:1 (0.08)	1.42:1 to 1.62:1
LACA mice (G. pulex)*	7	9	1.35:1 (0.10)	1.25:1 to 1.56:1
LACA mice (A. aquaticus)*	7	27	1.32:1 (0.08)	1.17:1 to 1.43:1
LACA mice (L. stagnalis)*	7	9	1.28:1 (0.07)	1.16:1 to 1,37:1
LACA mice (L. palustris)+	7	29	1.35:1 (0.05)	1.23:1 to 1.42:1
hamsters	7	10	1.38:1 (0.08)	1.29:1 to 1.54:1
gerbils	7	7	1.32:1 (0.04)	1.28:1 to 1.38:1
chicks	7	10	1.48:1 (0.09)	1.30:1 to 1.58:1
ducklings	7	7	1.52:1 (0.04)	1.45:1 to 1.58:1
pigeons	7	7	1.39:1 (0.07)	1.29:1 to 1.50:1

the same size as the ovary, while it was always smaller than the ovary in mice. The posterior testis was uniformly larger than the anterior testis in gerbils, but in mice no such relationship was regularly observed.

Nine chicks (1-day old), 4 ducklings (7 days old) and 6 pigeons (adult) were each fed 300 or 400 cysts and killed for examination after 7 days (Fig. 511).

Despite the mean body dimensions of the specimens recovered from ducklings being only 1.38mm long by 0.37mm wide, all the flukes were mature (Table 5.2). The sucker ratios of specimens from the avian hosts

agreed more closely on the whole with those of rats than mice (Table 5.3) and the relative sizes of the internal organs in flukes harvested from chicks and pigeons were quite similar to those from mice (Appendix II). However, it is apparent from these data (Table 5.2; Figs. 5.10 and 5.11) that P. elegans matured more quickly in both mice and gerbils than in avian hosts. The oral sucker of specimens from chicks and pigeons exhibited no consistent size relationship with the testes and ovary respectively; moreover in flukes from the latter host the ovary was never larger than the testes.

Specimens from ducklings were very small and poorly developed, consequently when comparing the relative sizes of the internal organs with those of specimens from mice several differences were noted. When ducklings were employed as hosts the oral sucker was larger than the gonads and the ventral sucker was of greater dimensions than the ovary; furthermore the ovary was the same size or smaller than the pharynx, yet in parasites from mice the ovary was without exception larger than both the pharynx and ventral sucker.

Second intermediate hosts:

Gammarus pulex, Asellus aquaticus and Lymnaea stagnalis were used as alternative intermediate hosts to chironomid larvae, while L. stagnalis and LACA mice were maintained as first intermediate and definitive hosts respectively.

All the parasites recovered were sexually mature. The flukes were the largest when L. stagnalis was the second intermediate host and smallest when cysts teased from Asellus were used (Table 5.2).

Although the sucker ratios (Table 5.3) and general relationships of the various internal organs to one another are similar to those when when Chironomus sp. is utilized, particularly in the case of L. stagnalis, there are differences (Appendix II). When both G. pulex and A. aquaticus were second intermediate hosts the ovary displayed no consistent size relationship with the oral sucker, or the ventral sucker in the case of the latter host; whereas in parasites passaged using Chironomus sp., the ovary was never smaller than either the oral or ventral sucker.

First intermediate hosts:

It has been possible to infect L. palustris experimentally (p.40) and since little is known of the role of the first intermediate host on adult morphology, it was used as an alternative host to L. stagnalis. When the life cycle was completed using L. palustris, Chironomus sp. and LACA mice the specimens recovered closely resembled those obtained under similar conditions using L. stagnalis as the first inter-

mediate host. The flukes were mature and had attained an average length and width of 2.65 by 0.64mm respectively (Table 5.2).

The sucker ratios are in agreement with those of specimens for which L. stagnalis was the first intermediate host, being 1.35:1 (Table 5.3). However when comparing the relationships of the internal organs it is apparent that the oral and ventral suckers were either larger or smaller than the ovary in worms passaged through L. palustris, but not larger than the ovary when L. stagnalis was employed (Appendix II).

The size relationships of the internal organs of P. elegans obtained when using various intermediate and final hosts can be summarized as follows:

1. The oral sucker is always larger than the ventral sucker and pharynx.
2. The ventral sucker is always larger than the pharynx.
3. The oral sucker, pharynx and ventral sucker exhibit no regular size relationship with the gonads.
4. The ovary, anterior and posterior testes exhibit no regular size relationships among themselves.

Statements 3 and 4 depend upon the degree of development of P. elegans.

Anatomical variation of P. elegans. (Observations made on in toto preparations).

Oral and ventral suckers (Fig. 5.13).

The oral sucker is most often subterminal, although it may be situated terminally, probably due to the pressure exerted on the specimen during fixation. The mouth assumes various shapes, normally appearing in the form of an inverted bulb, although sometimes ranging from triangular to circular in outline. The ventral sucker is located within the anterior third of the body and its aperture may also be triangular to circular in outline.

Caeca (Figs. 5.13, 5.14).

In general the caeca extend from the level of the posterior border of the pharynx along the lateral margins of the body to near the posterior extremity where they end blindly; occasionally however they may almost touch in the mid-line just behind the posterior testis. Variation also occurs in the posterior limit attained by the caeca. While they normally exceed the uterus in posterior extent, in some instances the uterus alone may occupy the posterior extremity of the body. In a single specimen the left caecum was considerably longer than the

right caecum.

Gonads (Fig. 5.15).

Although the borders of the gonads are generally smooth, they, and most frequently the borders of the testes, may be irregular in outline. This is probably a result of fixation and is more commonly observed in specimens fixed in formol saline without prior heat killing than in flukes first heat killed. However, it may also be a result of senility, when the contents of the gonads are nearly exhausted.

The ovary is usually posterior and to the right of the acetabulum, being separated from the ventral sucker by the cirrus sac, but if the cirrus sac is displaced during fixation, the ovary may be forced anteriorly and as a result overlap the posterior limit of the ventral sucker.

In all but one of the hundreds of specimens of _P. elegans_ examined by the author, the testes were obliquely situated in the mid to posterior part of the body. The sole exception (Fig. 5.7) was an adult recovered from a pigeon in which the testes were tandem and the uterus rather than running between the testes was to the right side, most probably having been displaced there during fixation.

Uterus (Fig. 5.14).

The uterus generally takes the sinuous course previously described (p. 88), turning anteriorly near the posterior end of the body. It is a simple uncoiled tube in immature and senile specimens, containing few or no eggs and highly coiled in mature gravid specimens (Fig. 5.1). Only exceptionally is the uterus confined to the region anterior to the anterior testis; more frequently it does not extend beyond the posterior testis. In a single instance, as stated above, the uterus was to the right of both testes making them appear tandem (Fig. 5.7). Further variation was observed in the course followed by the uterus of one fixed specimen in which it passed on both sides of the anterior testis and in another specimen in which it encircled the posterior testis. However, it did not deviate from its normal course as a distinct tube; instead when these specimens were observed while alive from the ventral aspect the uterus appeared to be abnormally well-developed and obscured either the ovary or posterior testis. During fixation it was displaced, as the specimens were being dorso-ventrally compressed, and was then seen to pass on both sides of either the ovary or testis. Free yolk and ova were present in several _P. elegans_ of different ages obtained from a variety of definitive hosts (Fig. 5.16).

Cirrus sac (Fig. 5.15 D-I).

In the majority of in toto preparations the cirrus sac extends from approximately the anterior third of the ovary around the right side of the acetabulum, terminating in front of the ventral sucker at the male gonopore, slightly to the left of the midline. However, it may originate beyond the posterior limit of the ovary. The cirrus sac is sometimes displaced to the left of the ventral sucker and in some instances it overlaps and does not extend posteriorly beyond the anterior border of the acetabulum. Rarely the seminal vesicle appears to comprise only a single proximal cylinder; the distal spherical body is apparently absent.

Vitellaria (Fig. 5.18).

The vitellaria are poorly developed in juvenile P. elegans, while in mature gravid flukes they may partially obscure some of the internal structures and in senescent specimens the follicles are nearly spent.

The most frequently observed range of the anterior limit of the vitellaria is from shortly behind the bifurcation of the oesophagus to the posterior border of the oral sucker. However in specimens from LACA mice the yolk glands may reach the anterior margin of the oral sucker. The vitellaria extend caudad along the lateral margins to near the posterior extremity of the body. In a single specimen it was noted that the vitellaria were discontinuous at the level of the ventral sucker on the right side of the body (Fig. 5.18A) and in another specimen they did not reach as far as the posterior extremity on the left side (Fig. 5.18B).

Although within the species P. elegans the vitellaria may or may not be confluent between the oral and ventral suckers, it is interesting to note that none of the specimens from chicks possessed an anterior commissure and in only 2 of the 14 worms harvested from ducklings and pigeons was it present (Table 5.2). Yet from all the mammalian hosts specimens were observed both with and without the anterior vitellarian bridge. In addition the author developed a pure strain of P. elegans by infecting one laboratory-reared Lymnaea stagnalis with a single egg teased from a self-fertilized adult in which the anterior vitelline commissure was present. The passage was then completed using chironomid larvae and LACA mice and the results are of particular interest. In 5 of the nine 7-day old adults which were recovered the vitellaria joined in the midline between the suckers while in the other 4 the bridge was clearly absent.

Table 5.4 is a summary of the anatomical abnormalities observed

during the present study.

Table 5.4. Incidence of anatomical abnormalities observed during the study of a pure laboratory strain of P. elegans. (In all cases the first intermediate host was Lymnaea stagnalis.)

	hosts		no. specimens;
	2nd intermediate	definitive	(% sample): age-days
uterus not posterior to anterior testis	Asellus aquaticus	LACA mice	1 (3.7): 7
uterus not posterior to posterior testis	Lymnaea stagnalis	"	4 (44.4): 7
	A. aquaticus	"	3 (11.1): 7
	Chironomus sp.	"	1 (2.5): 14
	"	pigeon	2 (28.6): 7
	"	chick	1 (10.0): 7
free yolk and ova in utero	A. aquaticus	LACA mice	3 (11.1): 7
	Gammarus pulex	"	1 (11.1): 7
	Chironomus sp.	pigeon	1 (14.3): 7
	"	rat	1 (5.3): 21
	"	"	3 (50.0): 28
uterus on both sides of anterior testis	A. aquaticus	LACA mice	1 (3.7): 7
uterus on both sides of posterior testis	A. aquaticus	LACA mice	1 (3.7): 7
seminal vesicle apparently not bipartite	A. aquaticus	LACA mice	1 (3.7): 7
caeca of unequal length	A. aquaticus	LACA mice	1 (3.7): 7
testes tandem uterus on right side only	Chironomus sp.	pigeon	1 (14.3): 7

Final host specificity (Table 5.5).

Amphibians.

Four laboratory-reared Xenopus laevis adults were exposed to infection with P. elegans. Two toads were isolated in small volumes of water containing 15 Chironomus sp. larvae; each larva harboured from 15 to 20 week-old cysts (total of 225 to 300 cysts per container). The two re-

maining X. laevis were each fed 150 cysts by stomach tube. When they were sacrificed and dissected a week later no P. elegans were recovered.

Birds.

Successful infections of pigeons, chicks and ducklings were obtained with doses of 300 or 400 metacercarial cysts, although few specimens were present 7 days after infection. To ascertain whether the low infection rate was due to doses exceeding an assumed optimum, eight 1-day old chicks and eight 7-day old ducklings were each fed 25 or 100 cysts; upon dissection a week later there was no trace of infection in any of the birds.

Mammals.

Two adult specimens of Sorex araneus were captured in the Leeds area and when brought into the laboratory were maintained in separate cages. Eighty chironomid larvae (40 per shrew), each infected with approximately 10 cysts, were mixed with blow fly larvae normally provided and placed on a piece of dampened filter paper. This procedure was adopted, rather than feeding the shrews by stomach tube, because they are extremely sensitive and apt to die from excessive handling. The shrews ate the larvae as evidenced by the presence of chironomid exoskeletons in their faeces; even so, a week later neither shrew was infected.

Three laboratory-reared gerbils in addition to eight hamsters, obtained from a local pet shop, were each fed 10 or 20 cysts by stomach tube and dissected a week later. Although both gerbils and hamsters were subject to infection by P. elegans, after 7 days only 5 to 27% of the infecting dose had survived in either host. Furthermore, while all the gerbils were infected, in only 50% of the hamsters were flukes present. The age of these mammalian hosts was unknown, however they all appeared to be in good health.

Mice and rats are evidently more susceptible to infection by P. elegans than either hamsters or gerbils. When 4 rats were each fed 15 cysts and examined a week later, all 4 were infected and harboured from 1 to 8 parasites; the total recovery rate approached 50%. However, of all the hosts employed during the present investigations LACA mice were the most susceptible. When only a single metacercarial cyst was fed to each of 20 LACA mice 13 (65%) of the mice were infected a week later. Moreover, the high rate of infection appears to be independent of either the first or second intermediate host. Table 5.5 shows that when the first intermediate host is either Lymnaea stagnalis or L. palustris and the second intermediate host Asellus aquaticus, Gammarus

Table 5.5. Host specificity of P. elegans. In all cases the cysts were 6 to 12 days old and the animals sacrificed after 7 days. Lymnaea stagnalis and Chironomus sp. served as first and second intermediate hosts respectively unless otherwise indicated (+ alternate first intermediate host; * alternate second intermediate host).

definitive host (no.)	infecting dose/host	no. worms recovered (%)	no. hosts infected
Xenopus laevis (4)	150 to 300	0 (0)	0
Sorex araneus (2)	400 (approx.)	0 (0)	0
LACA mice (4)	10	31 (77.5)	4
LACA mice (20)	1	13 (65.0)	13
LACA mice (3) *Asellus aquaticus	10 or 15	31 (88.6)	3
LACA mice (1) *Gammarus pulex	10	9 (90.0)	1
LACA mice (2) *L. stagnalis	unknown	14 (?)	2
LACA mice (1) *L. stagnalis	20	9 (45.0)	1
LACA mice (3) +L. palustris	10	29 (96.6)	3
LACA mice (1)	20	17 (85.0)	1
gerbils (3)	10	8 (26.7)	3
hamsters (4)	10	8 (20.0)	2
hamsters (4)	20	4 (5.0)	2
rats (4)	15	38 (47.5)	4
pigeons (6)	300 or 400	7 (0.4)	4
ducklings (4)	25	0 (0)	0
ducklings (4)	100	0 (0)	0
ducklings (4)	400	8 (0.5)	1
chicks (4)	25	0 (0)	0
chicks (4)	100	0 (0)	0
chicks (15)	400	11 (0.2)	5

pulex or Chironomus sp. more than 77% of the infecting dose survive the first week of infection. However when L. stagnalis served as the second intermediate host in one instance the mice ingested a portion of an infected digestive gland containing an unknown number of cysts, so it is not possible to say what percentage of the infecting dose survived. On another occasion a single mouse was fed 20 cysts teased from daughter

sporocysts within *L. stagnalis* and only 9 (45.0%) of the worms were present 7 days post-infection. But it is perhaps of note that in neither experiment using *L. stagnalis* could the age of the cysts be determined.

Intestinal length (Table 5.6).

The length of the small intestine in the above avian and mammalian hosts varied considerably. It was shortest in gerbils and longest in ducklings. Because in shrews there is no superficial demarcation between the large and small intestine the latter structure was not measured.

Table 5.6. Length of the small intestine of birds and mammals employed as experimental definitive hosts of *P. elegans*. (Measurements in cm).

host	no. measured	mean (1 SE)
chicks	26	76.2 (1.71)
ducklings	12	127.0 (3.25)
pigeons	6	83.0 (3.72)
rats	11	98.9 (2.98)
mice	15	42.2 (2.82)
gerbils	3	27.2 (3.02)
hamsters	8	42.1 (2.29)

Egg counts.

Experimental results obtained during a previous study (Table 5.5) clearly demonstrate that fewer *P. elegans* survive the first week of infection in the laboratory rat than in LACA mice; also those flukes that do survive appear to mature more slowly in the former than the latter host (p. 91). Egg counts were performed to compare the development of *P. elegans* in rats and mice in terms of reproductive activity.

Seven mice (6 to 10 weeks old) were each fed 10 cysts (15 days old) and seven rats (6 to 10 weeks old) were each fed 15 cysts (15 days old). A higher dose was given to each rat in an attempt to infect them with approximately the same number of flukes as the mice; preliminary experiments had indicated that infection rates of 45 to 50% and 70 to 80% could be expected for rats and mice respectively. The data presented here concerning egg counts performed using mice are repeated in Section

6 (p.135) when a comparison of dose versus egg counts is made.

A single mouse was sacrificed on each of the following days post-infection: 5, 7, 9, 11, 14, 17 and 25 - thus the course of egg production was followed for the entire 25 days in only one mouse. The schedule was similar for rats, but the last rat was killed after 21 and not 25 days because it was thought that the animal was about to lose its infection. When each host was killed the worms present were counted, fixed and subsequently measured. The experimental results are given in Tables 5.7 to 5.9 and the egg counts are represented graphically in Figures 5.19 and 5.20.

It is apparent from these results that P. elegans matures more quickly in the mouse than the rat, since eggs were present in the faeces of the former host by day 5 but not until days 6, 7 or 8 in the latter host. Similarly, although the 5-day old specimens were somewhat longer in rats than mice (Table 5.7), by day 9 the P. elegans from mice were nearly 1.0mm longer than those from rats. As shown in Table 5.7, P. elegans in the rat host appears to sustain its growth at a slow rate until day 17 while the maximum size of specimens from mice was attained by day 9. It was thus considered possible that egg counts would increase over a longer period of time in rats than in mice. This was not substantiated by the data. Egg release peaked in the rat host from day 7 to day 16 (Table 5.9) and in the mouse from day 8 to day 14 (Table 5.8); it was noted that only the maximum count on day 16 (rat no. 6) fell later than the range of maximum egg release for the individual mice.

Based on the number of worms present when the hosts were sacrificed (Table 5.9), it is estimated that during the infection most worms within the rat deposited between 50 and 300 eggs per day; the maximum estimate, assuming that 4 to 6 worms were present, is 500 (day 14, rat no. 7). Most worms within the mouse host (Table 5.8) released from 200 to 700 eggs per day, with the maximum estimate, assuming that 8 to 10 worms survived, being 863 (day 12, mouse no. 6; days 13 and 14, mouse no. 7).

Table 5.7. Comparison of body length and width of _P. elegans_ recovered from LACA mice and laboratory rats during egg counts. Student's _t_ test was used as a test of significance where possible. (Measurements in mm).

	mice			rats		
		mean			mean	
days post-infection	no. meas.	length (1 SE)	width (1 SE)	no. meas.	length (1 SE)	width (1 SE)
5	7	1.93 (0.05)$^{+}$	0.57 (0.02)$^{+}$	2	1.95 (0.06)	0.52 (0.01)
7	8	2.76 (0.04)^{o}	0.73 (0.02)*	9	2.01 (0.08)	0.51 (0.02)
9	9	2.90 (0.06)^{o}	0.78 (0.02)^{o}	9	1.94 (0.05)	0.57 (0.02)
11	9	2.58 (0.06)^{o}	0.66 (0.02)	1	2.25	0.58
14	0			5	2.63 (0.19)	0.58 (0.09)
17	2	2.78 (0.07)	0.73 (0.03)	1	2.96	0.85
21				4	2.57 (0.02)	0.66 (0.02)
25	1	cut during recovery				

$^{+}$ not significantly different

^{o} significantly different $p < 0.001$

* significantly different $p < 0.020$

Table 5.8. Total daily egg counts; each mouse fed 10 P. *elegans* cysts (15 days old). Single mouse sacrificed on days 5,7,9,11, 14,17 and 25 post-infection. (No. worms present when mice were killed.)

day post-infection	mouse no. 1	2	3	4	5	6	7
4	0	0	0	0	0	0	0
5	150 (8)	100	150	50	50	600	700
6		3300	3000	400	3100	1650	2850
7		4900 (9)	7100	1050	3450	3100	3750
8			5400	1350	4800	5700	5400
9			8400* (10)	3300	4050	5850	4050
10				2100	2300	6150	5250
11				1100 (10)	1500	6450	4800
12					150	6900*	6600
13					0	5700	6900*
14					0 (0)	3900	6900*
15						900	4350
16						1350	4650
17						900 (2)	2250
18							4700
19							2500
20							5925
21							6450
22							5025
23							2100
24							1875
25							300 (1)

*peak of egg release.

Table 5.9. Total daily egg counts; each rat fed 15 P. *elegans* cysts (15 days old). Single rat sacrificed on days 5,7,9,11,14,17 and 21 post-infection. (No. worms present when rats were sacrificed.

day post-infection	rat no. 1	2	3	4	5	6	7
4	0	0	0	0	0	0	0
5	0 (2)	0	0	0	0	0	0
6		0	0	0	0	0	400
7		0 (9)	200	400	0	400	1200
8			0	0	200	0	700
9			600 (10)	0	150	300	900
10				0	900	450	900
11				0 (1)	1950*	300	800
12					300	1050	1050
13					450	300	800
14					1000 (6)	750	2000*
15						600	1350
16						1200*	900
17						100 (1)	500
18							200
19							1000
20							200
21							225 (4)

* peak of egg release.

DISCUSSION.

The majority of taxonomists must rely on adult morphology as the basis for systematic studies due to the scarcity of life history data. Stunkard (1957) and Yamaguti (1971) supported this approach, because the resultant "taxonomic" species, although subjective, is as Simpson (1943) stated " an inference as to the most probable characters and limits of the morphological species from which a given series of specimens has been drawn." Stunkard (1960) noted that a general anatomical pattern is common to all members of a particular genus and that species within the genus may be distinguished by differences in the size or location of the various elements within the pattern. However, he (1957) stressed that before a taxonomic work is complete all the stages in the life cycle of a species must be known. Problems in taxonomic studies have arisen from the establishment of species on the characteristics of only one or very few adult specimens; in these cases the limits of the taxonomic species have not been properly defined since they have not been properly observed.

As discussed in Section 1 (pp. 3 to 14) emphasis has been placed upon adult morphology in the taxonomy of the genus Plagiorchis. Odening (1959) suggested that species of Plagiorchis in which the uterus did not extend beyond the posterior testis or into the fourth quarter of the body be placed in the subfamily Opisthioglyphinae, while Timofeeva (1962) erected the genus Metaplagiorchis to accommodate those species in which the caeca and/or vitellaria did not extend to the posterior extremity of the body. Although Park (1936) noted that a seminal receptacle may be apparent in young specimens of P. noblei but difficult to demonstrate in older worms, Mehra (1937) and Olsen (1937) independently erected the genera Neoplagiorchis and Plagiorchoides respectively for those species of Plagiorchis in which a seminal receptacle is present. In addition four subgenera have been erected. Schulz and Skworzow (1931) considered the presence or absence of an anterior vitellarian commissure a sufficiently important and regular characteristic to divide the genus into the subgenera Multiglandularis and Plagiorchis respectively. Yamaguti (1971) believed that the criteria of Timofeeva (1962) stated above did not warrant the establishment of a genus and thus relegated Metaplagiorchis to subgeneric rank; the subgenus Pseudoplagiorchis in which the uterus passes lateral to the testes and the genital atrium is median instead of submedian was also erected by Yamaguti (1971). However the results of the present experiments have clearly shown that the criteria of Odening (1959),

Timofeeva (1962). Schulz and Skworzow (1931) and Yamaguti (1971) are not valid, since they emphasize morphological variations which are found within a pure laboratory strain of Plagiorchis. The works of Mehra (1937) and Olsen (1937) have not been widely accepted and the presence or absence of a rudimentary seminal receptacle is considered to be a specific rather than a generic characteristic (Yamaguti, 1958; Najarian, 1961; Velasquez, 1964; Diaz, 1976). Moreover, because this organ can only really be demonstrated by serial sections, is difficult to see in old living specimens (Park, 1936) and is not evident in whole mounts, it is probably present in more species than indicated by the literature.

It has been clearly demonstrated that morphological and anatomical features of parasitic flatworms vary according to both the method of fixation (Stunkard, 1957) and the degree of maturation of the specimen (Angel, 1959; Styczynska-Jurewicz, 1961,1962; Dawes, 1962; Blankespoor, 1974; Groschaft & Tenora, 1974). Stunkard (1957) noted that the measurements of a specimen when flattened may be as much as 100% greater than the same specimen in a contracted state. Unfortunately the method of fixation is frequently omitted from descriptions and it is often not possible to ascertain the degree of maturation because specimens for comparison are not available.

The continued growth of flukes after they have attained sexual maturity is cited by Stunkard (1957) as the principal factor contributing to the difficulties in delineating helminth species. His point is well illustrated during the present study; the smallest mature fluke was 1.04mm long and 0.34mm wide, while the largest specimen was 3.89 by 0.96mm. With increase in body size there is initially an increase in the dimensions of both the reproductive and non-reproductive structures, but in senescent worms the gonads decrease in size. Although the measurements of the suckers generally increase with age the sucker ratios vary considerably in specimens from different hosts, but within the same host-species they are remarkably consistent. This phenomenon was also noted by Blankespoor (1974) to occur in P. noblei.

Most species of Plagiorchis have been described from a few in toto preparations of adult specimens. Such a practice only serves to obfuscate further an already difficult subject, because structures of diagnostic value in distinguishing species, for example, the vas deferens, seminal receptacle, Laurer's canal, prepharynx and oesophagus, although discernable in both living specimens and sectioned material, are not often apparent in whole mounts.

In agreement with previous studies (Braun, 1902; Najarian, 1961;

Styczynska-Jurewicz, 1962; Gupta, 1963; Velasquez, 1964; Yamaguti, 1971; Blankespoor, 1974) this investigation has demonstrated that the presence or absence of an anterior vitellarian commissure is not a valid criterion on which to establish subgenera and is not even consistent within a pure strain of P. elegans. It has also been shown that in the hosts utilized the anterior extent of the vitellaria falls within a narrow range, that is from just beyond the origin of the caeca to the anterior border of the oral sucker. However, Blankespoor (1974) provided conclusive evidence that the anterior vitellarian extent is of little value in separating species. Using a pure laboratory strain of P. noblei he infected 17 different final host species and found that in flukes recovered from two robins the anterior limit of vitellaria varied from the posterior border of the acetabulum to the anterior border of the oral sucker; in worms harvested from all other species of experimental host the anterior limit fell within this range.

Blankespoor (1974) also stated that "it is apparent that the major factor contributing to the problem in identifying species of the genus Plagiorchis has been a lack of sound experimental data on host-induced morphological modifications." Taxonomists have tended to adhere to a concept of strict host specificity and as a result have not recognized individuals within a species because flukes have been recovered from different hosts and perhaps differed somewhat morphologically from described species.

During the present study the highest incidence of anatomical variation occurred when Asellus aquaticus served as the second intermediate host. Nine of the worms recovered from the three infected mice exhibited some sort of abnormality, but it should be noted that six of those specimens were recovered from a single mouse. Thus the observed, apparently host-induced, abnormalities cannot be ascribed with certainty to either the second intermediate or definitive host.

Members of the genus parasitize amphibians, reptiles, birds and mammals (Yamaguti, 1971) and some species are able to infect either amphibians and reptiles or birds and mammals. However there is no firm evidence, based on experimental life-history studies, that species of Plagiorchis are able to infect both homeothermic and poikilothermic vertebrates, but there is evidence to the contrary. In the process of determining the definitive hosts of different species of Plagiorchis several authors have attempted to infect both poikilothermic and homeothermic animals. They, including this writer, have not been able to do so (Buttner & Vacher, 1960; Najarian, 1961; Blankespoor, 1974). Yet one should be cautioned that not all species of trematode are so re-

stricted (Rausch, 1947) and perhaps the experimental conditions employed by the above authors were not suitable.

Noting morphological similarities Sharpilo and Sharpilo (1972) considered _P. elegans_, a known parasite of birds and mammals, and _P. mentulatus_, a parasite of amphibians and reptiles (Yamaguti, 1958), to be synonymous. It appears however, as a result of their host-specificity, that these flukes differ substantially in their physiological requirements and are thus reproductively isolated. Whether or not they are capable of cross-fertilization, if harboured by the same host host, is at present unknown. They are best retained as independent species until it can be shown experimentally that they are able to infect the same definitive host species. Cross-fertilization is obviously more difficult to demonstrate, although not impossible (Nollen, 1968).

The natural host of _P. elegans_ is believed to be a bird (Braun, 1902; Styczynska-Jurewicz, 1962), but during the present investigation _P. elegans_ exhibited various degrees of specificity towards the definitive host and mammals were far more susceptible to infection than the avian hosts utilized. Of the mammalian hosts tested the parasite was most successful in terms of infection rate in LACA mice and least successful in shrews, which were completely refractory. Despite their apparent resistance to infection, development occurred most rapidly in gerbils. It is believed that the structure of the gut may have played a considerable part, although it was not the only factor, in the variation in establishment of infections. The small intestine is approximately 42.0cm long in LACA mice; it was on average the same length in hamsters, substantially longer in rats and smaller in gerbils. Studies conducted on the distribution of _P. elegans_ in mice (p. 147) revealed that 45 to 60 minutes after infection nearly 50% of the excysted juveniles recovered were more than half way along the small intestine. Possibly in the gerbil the cysts or worms were carried posteriorly into the large intestine by peristalsis before they were able either to excyst or become established. A valve separates the small intestine from the large intestine of most mammals, but it is absent in shrews; as a result, the gut takes the form of a rather simple tube. Not being used to captivity the shrews were very nervous, although they were handled as little as possible in the laboratory. It is suggested that in their case the infected chironomid larvae passed through the small intestine too rapidly for the parasites to establish themselves.

From the chicks, pigeons and ducklings given 300 or 400 cysts

only 0.2, 0.4 and 0.5% respectively of the infecting dose were recovered 7 days after feeding. Since neither 25 nor 100 cysts per bird produced infections, it is considered that high doses are required to infect the birds and that the low recovery rates are probably not the result of a host response. Diaz (1976) also unsuccessfully attempted to infect chicks, ducklings and pigeons with low doses of P. kirkstallensis (25 to 30 cysts/ bird), but did not try higher doses. Rees (1952) on the other hand, working with P. megalorchis used high doses and obtained results similar to those of the present author. Although Blankespoor (1974) infected approximately 90% of the chicks exposed with an average of more than 100 P. noblei each, only very young birds were susceptible. Unfortunately he gives neither the method of infection nor the doses administered. It is interesting to note that Blankespoor (1974) was unable to infect ducks and pigeons. Perhaps in the present instance many of the cysts were damaged while in the host gizzard and thus rendered susceptible to digestion.

Other factors such as the health of the host (Stunkard, 1957), composition of the host's bile (Smyth and Hazelwood, 1963), temperature, pH and salt concentrations (Cheng, 1964) within the vertebrate gut are undoubtedly involved in host specificity.

Until experimental evidence can prove otherwise, species of Plagiorchis which parasitize amphibians and reptiles are best maintained separately from those species of Plagiorchis which parasitize birds and mammals. On this basis P. elegans is distinguished from those flukes listed below which have been recorded from cold-blooded vertebrates (Yamaguti, 1971):

P. horridum (Leidy, 1850), Stossich, 1904
P. ramlianum (Looss, 1899), Stossich, 1904
P. serpenticola Massino, 1927
P. luehei Travassos, 1927
P. hepatica Lutz, 1928
P. ramlianum Azim, 1935
P. isaodena Deblock, Capron & Brygoo, 1935
P. himalayii (Jordan, 1930), Mehra, 1937
P. molini Lent and Freitas, 1940
P. lenti Freitas, 1941
P. africanus Dollfus, 1950
P. berghei Vercammen-Grandjean, 1960
P. laurenti Vercammen-Grandjean, 1960
P. rangeli Artigas and Zerpa, 1961

P. limnogale Richard, 1965/1966
P. bilorchis Fischthal and Thomas, 1968
P. momplei (Dollfus, 1932), Richard _et al._, 1968
P. taiwanensis Fischthal and Kuntz, 1975

Plagiorchis elegans shares a number of features in common with the other species of _Plagiorchis_ recorded in Britain from birds and mammals, but can be differentiated from most of them by certain significant morphological characteristics. It should be noted however that many of the descriptions are incomplete.

Although no reference is made to the presence or absence of a seminal receptacle, vas deferens or common genital aperture, _P. laricola_ Foggie (1937) can be differentiated from _P. elegans_ by the nearly equal size of the suckers in the former species. _Plagiorchis lutrae_ Fahmy (1954) and _P. muris_ Fahmy and Rayski (1963) have not been adequately enough described to make an accurate comparison possible. On the other hand an excellent description of _P. megalorchis_ was written by Rees (1952) and the two species are clearly separate. _Plagiorchis megalorchis_ lacks both a seminal receptacle and common genital atrium and possesses a vas deferens. Furthermore, although Rees described _P. megalorchis_ as a member of the subgenus _Multiglandularis_, an examination of co-types showed that even in some of these specimens an anterior vitelline bridge was absent. _Plagiorchis vitellatus_ Fraser (1974) however is closely similar to _P. elegans_ and it should be noted that it was examined after having been deep frozen. The described features do not set these species apart. In _P. notabilis_ (Nicoll, 1909) the apparent absence of a seminal receptacle and presence of a vas deferens are sufficient to distinguish it from _P. elegans_. Of the two species described by Diaz (1976) in Leeds, the suckers are nearly equal in _P. farnleyensis_ and it possesses a vas deferens, while _P. kirkstallensis_ is morphologically indistinguishable from _P. elegans_.

Of those species reported from birds and mammals in other parts of the world, sucker ratios of 1:1 or less and 2:1 or greater separate the following species from _P. elegans_:

P. cirratus Braun, 1902
P. permixtus Braun, 1902
P. arcuatus Strom, 1924
P. arvicolae Schulz and Skworzow, 1931
P. maculosus Yamaguti, 1935
P. ferruginum Mehra, 1937
P. orientalis Park, 1939 (a)

P. magnacotylus Park, 1939 (b)
P. multiglandularis Chang-Tung Ku, 1940
P. javensis Sandground, 1940
P. strictus Strom, 1940
P. upupae Strom, 1940
P. linkuolangi Tang, 1941
P. raabei Furmaga, 1956
P. vespertilionis Sogandares-Bernal, 1956

Plagiorchoides potamonades Tubangui, 1946

Moreover P. arcuatus, P. linkuolangi, and Plagiorchoides potamonades were found in aberrant sites within the definitive host - the oviduct of a hen, urinary bladder of a musk shrew and gall bladder or bile ducts of the rat experimental host respectively.

A number of species differ from P. elegans because a seminal receptacle is apparently absent:

P. proximus Barker, 1915
P. micracanthos Macy, 1931
P. bulbuli Mehra, 1937
P. casarcii Mehra, 1937
P. ferruginum Mehra, 1937
P. gonzalchavenzi Zercercero, 1949
P. cirratus Dollfus, 1960
P. vespertilionis parorchis Macy, 1960
P. dilimanensis Velasquez, 1964

In addition, the oesophagus is long in P. dilimanensis and the uterus alone occupies much of the posterior of the body. The oesophagus of P. koreanus Ogata (1938) is also characteristically long, but the presence or absence of a seminal receptacle, vas deferens and common genital atrium were not determined.

In P. elegans the cirrus is a simple hollow tube; however this is not the case in P. noblei Park (1936), P. maculosus Yamaguti (1935) or or Plagiorchoides potamonades Tubangui (1946) in which it is depicted as being covered by minute spines, button-like thickenings and wart-like elevations respectively.

Plagiorchis goodmani Najarian (1961) can be distinguished from P. elegans by the presence of separate genital pores opening to the outer surface of the worm. Although P. neomidis bears a close morphological similarity to P. elegans, it has only been recovered from shrews (Neomys fodiens), originally by Brendow (1970) and later by

Theron (1976). While shrews are apparently refractory to P. elegans, a different species (Sorex araneus) was tested in the current study, so sufficient experimental data are not at this time available to separate these species either on the basis of adult morphology or host specificity.

Plagiorchis noblei Williams (1963) is morphologically indistinguishable from P. elegans; a rudimentary seminal receptacle is present and there is a common genital atrium. Although the vasa efferentia were not noted in the text they were illustrated by Williams as entering the cirrus sac separately. Blankespoor (1974) himself suggested that P. noblei was probably synonymous with a previously described species.

Although in the descriptions of most of the following species no reference is made to the seminal receptable, vas deferens or common genital atrium, based on the available information, they cannot be separated from P. elegans. It is the belief of the author that when adult morphology and host specificity are taken into account their validity as independent species has yet to be demonstrated:

P. elegans Braun, 1902
P. nanus Braun, 1902
P. triangularis Braun, 1902
P. vitellatus Braun, 1902
P. muris (Tanabe, 1922) translation Dollfus, 1925
P. micromaculosus Skrjabin and Massino, 1925
P. muris McMullen, 1937 (b)
P. obtusa Strom, 1940
P. jaenschi Johnston and Angel, 1951
P. blatnensis Chalupsky, 1954
P. proximus Grabda, 1954
P. stefanski Furmaga, 1956
P. elegans Styczynska-Jurewicz, 1962
P. laricola Zdarska, 1966
P. peterborensis Kavelaars and Bourns, 1968
P. praevitellaris Matskasi, 1973
P. noblei Blankespoor, 1974
P. cirratus Matskasi, 1974

Plagiorchoides rhinolophi Park, 1939 (a)

The anatomical abnormalities summarized in Table 5.4 were ob-

served during this investigation in specimens of P. elegans which had been passaged using a variety of intermediate and final hosts. They are therefore known to be manifestations of intraspecific variation within a pure strain. The list is by no means considered to be definitive, but the features listed have in many instances been used by taxonomists to distinguish between species of Plagiorchis and in some instances as criteria for the erection of subgenera or even new genera.

The experimental evidence presented here supports the following adult features as apparently the only anatomical criteria by which species of Plagiorchis may be distinguished:

1. The relative sizes of the suckers and pharynx.
2. The presence or absence of a seminal receptacle, vas deferens and common genital atrium.
3. To a limited extent egg size (p. 23).

Host specificity has also been implicated and further investigations into its limits and validity as a taxonomic criterion may help to resolve the confused state of the genus.

Fig. 5.1. Dorsal view of adult P. elegans, typical 7-day old specimen recovered from LACA mice. Scale = 0.30mm.

a.t.	anterior testis
c.s.	cirrus sac
met.	metraterm
o.s.	oral sucker
ov.	ovary
ph.	pharynx
p.t.	posterior testis
s.v.	seminal vesicle
ut.	uterus
vit.	vitellaria
v.s.	ventral sucker

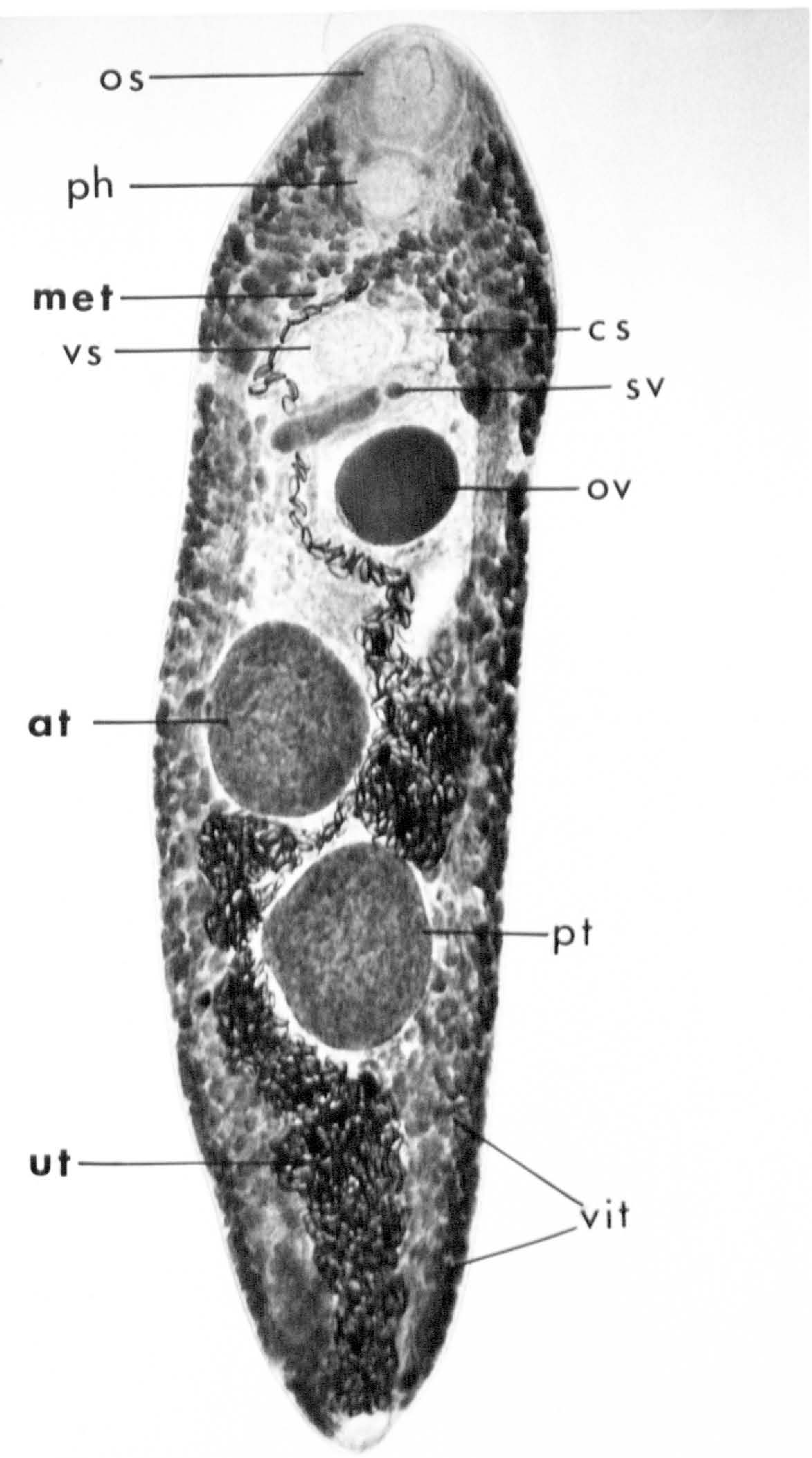

os
ph
met
vs
cs
sv
ov
at
pt
ut
vit

Fig. 5.2. Plagiorchis elegans lateral view. Caeca and cornua of excretory bladder have been omitted for clarity.

a.t. anterior testis
a.ut. ascending limb of uterus
c.s. cirrus sac
d.ut. descending limb of uterus
ex.bl. excretory bladder
ex.p. excretory pore
l.c. Laurer's canal
met. metraterm
met.c. metraterm gland cells
oes. oesophagus
o.s. oral sucker
ov. ovary
ovd. oviduct
ph. pharynx
pph. prepharynx
pr. prostate gland cells
p.t. posterior testis
s.v. seminal vesicle
v.ef. vas efferens
vit.d. vitelline duct
v.s. ventral sucker

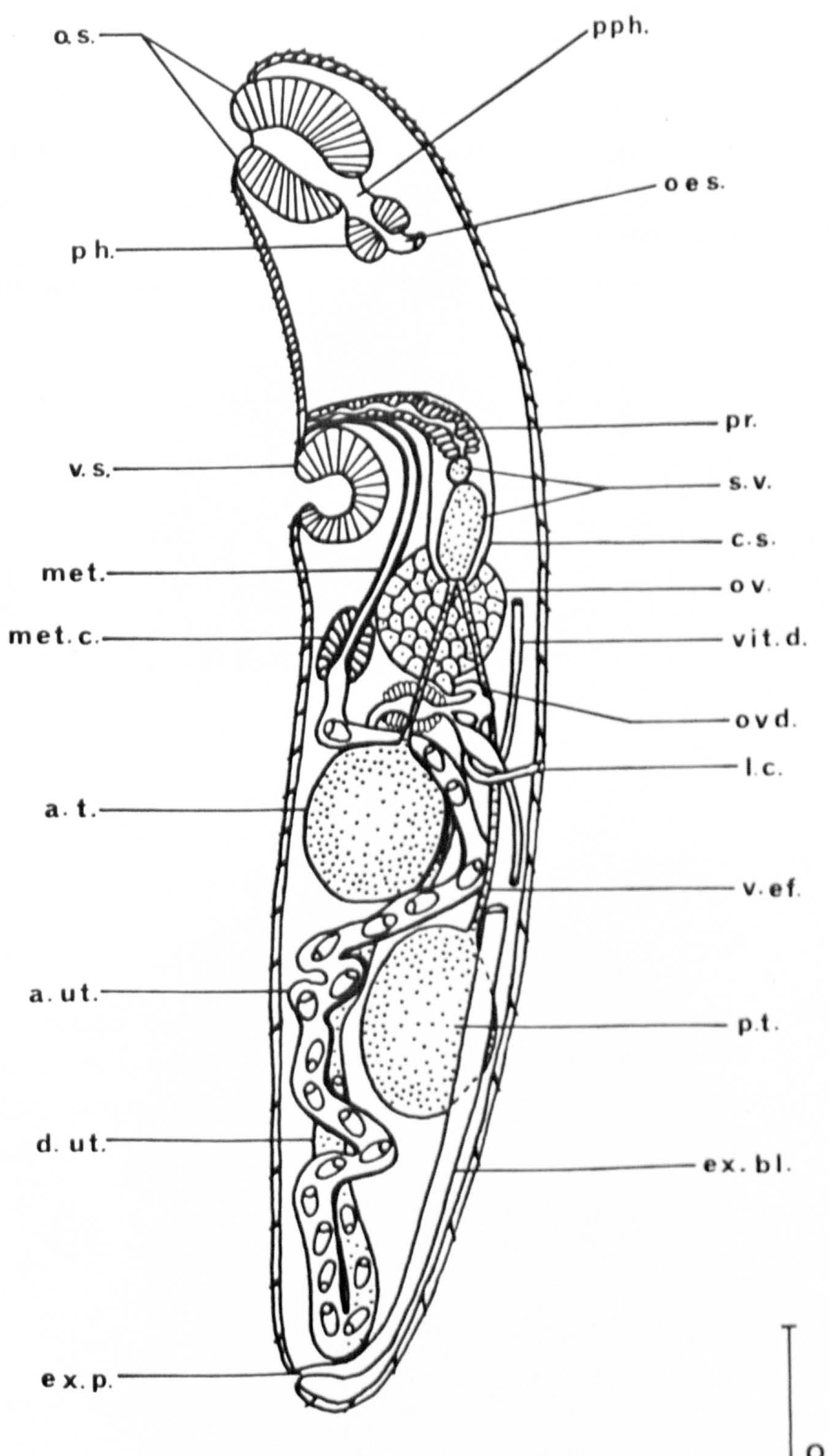
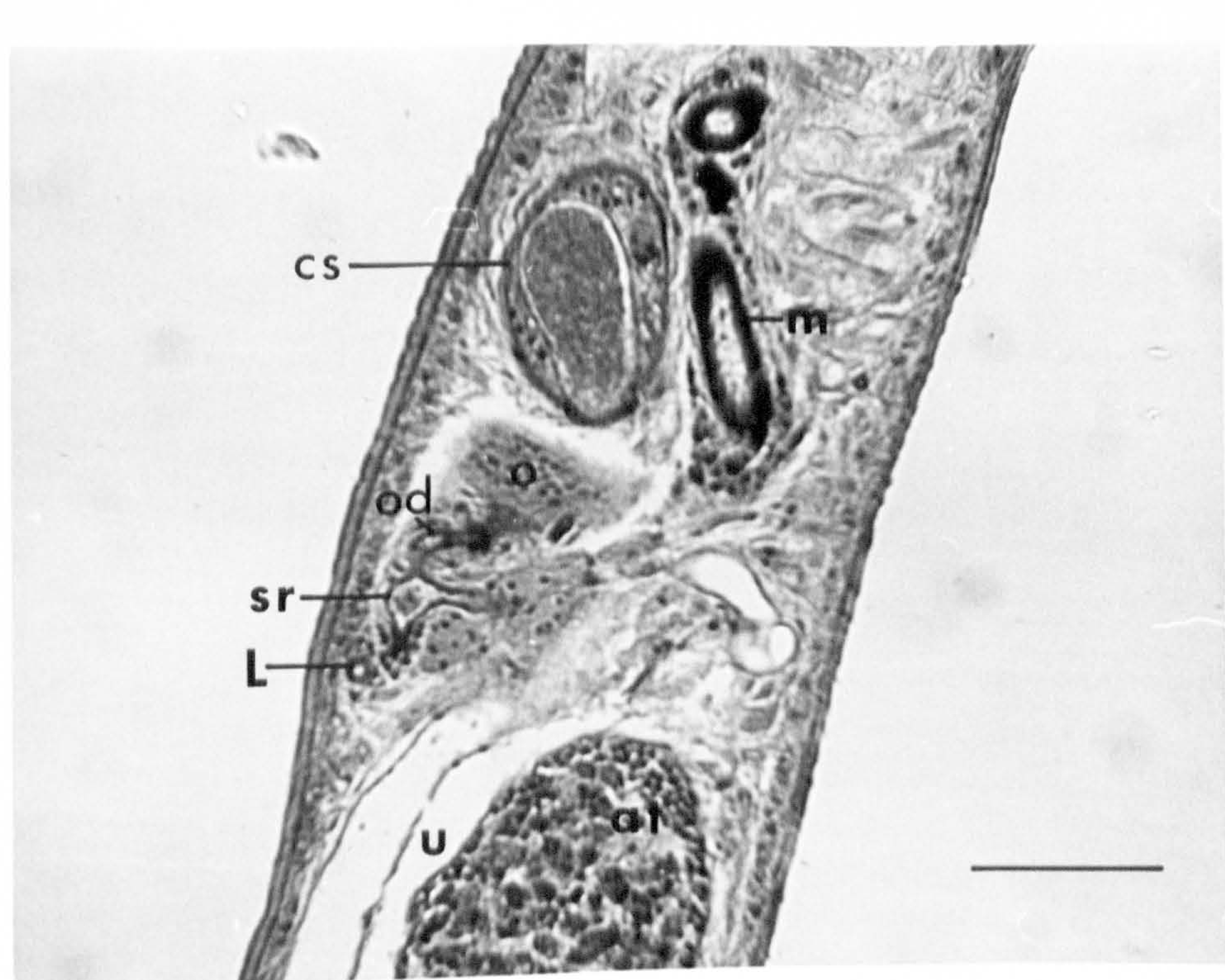

o.s.
pph.
oes.
ph.
pr.
v.s.
s.v.
c.s.
met.
ov.
met.c.
vit.d.
ovd.
l.c.
a.t.
v.ef.
a.ut.
p.t.
d.ut.
ex.bl.
ex.p.
0.40mm

Fig. 5.3. Longitudinal section through proximal part of female reproductive system. a.t., anterior testis; c.s., cirrus sac; Laurer's canal; m., metraterm; o., ovary; od., oviduct; s.r., seminal receptacle; u., uterus. Scale 0.2mm.

Fig. 5.4. Transverse section through the ventral sucker (v.s.). The caeca (c.), cirrus sac (c.s.), dorsoventral muscles (d.m.), metraterm (m.), ovary (o.) and vitelline follicles (v.) are also present in this section. Scale 0.10mm.


cs
m
o
od
sr
L
u
ai

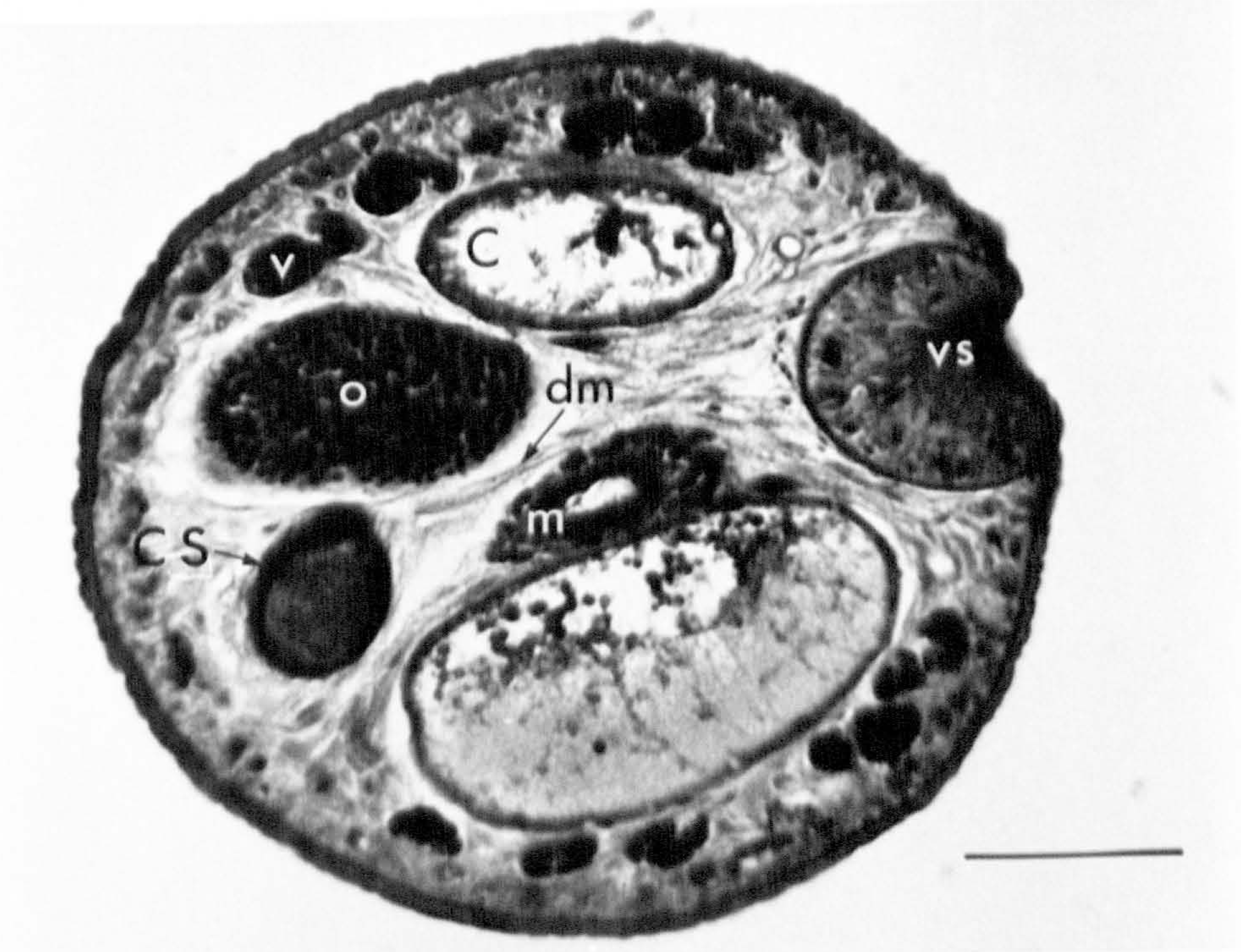
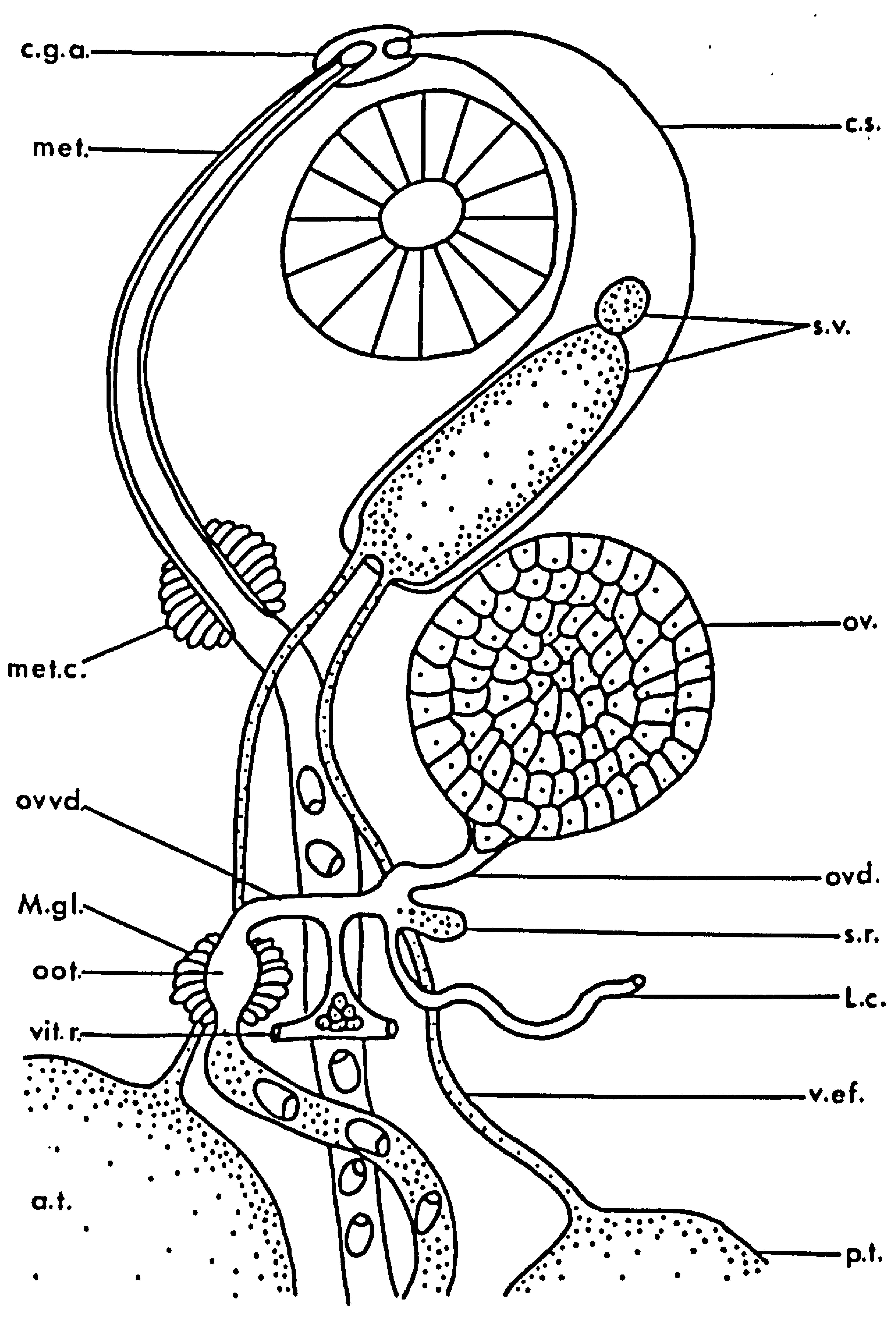

C
V
vs
O
dm
m
CS

Fig. 5.5. Schematic drawing of the male and female reproductive systems of *P. elegans*.

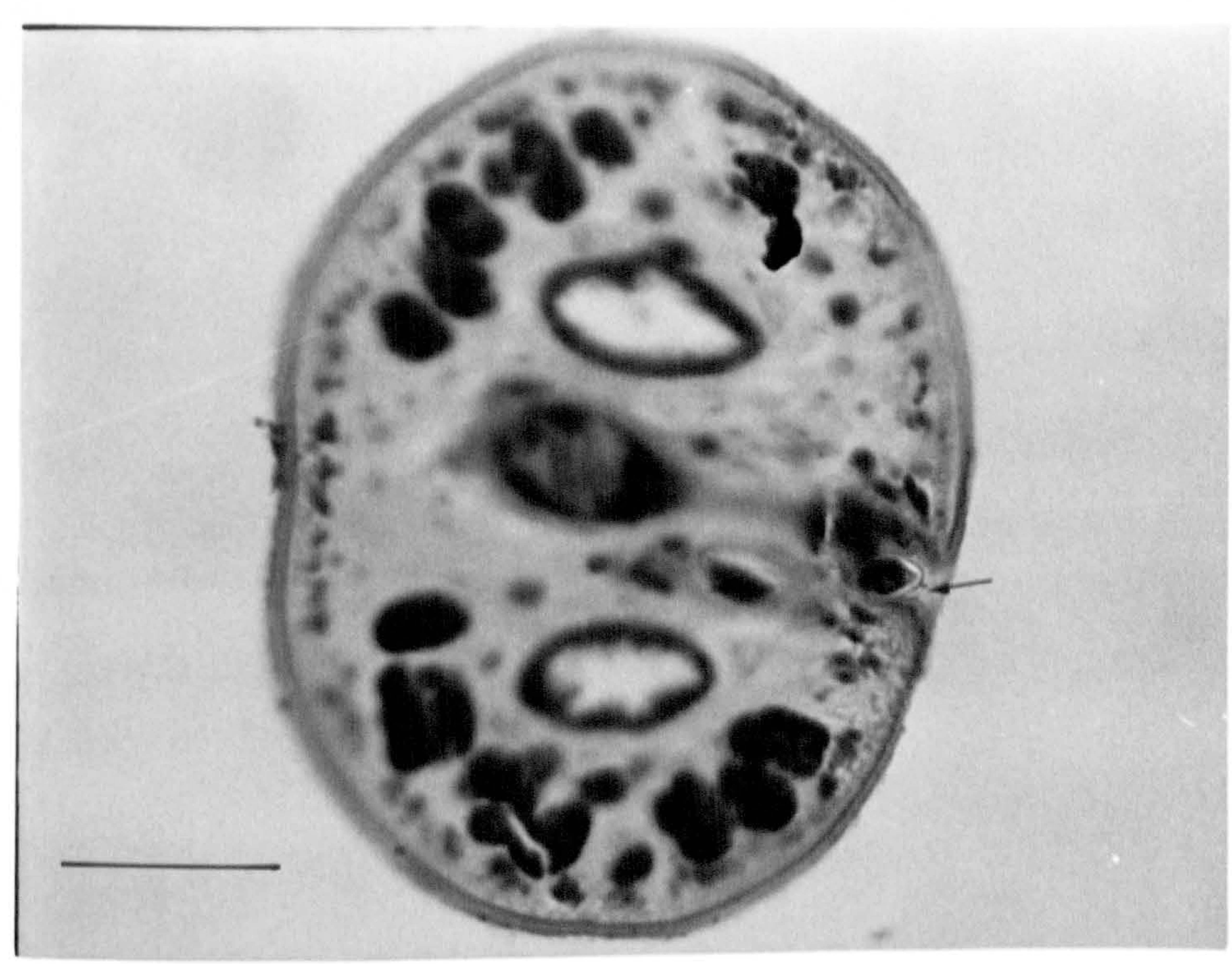
a.t. anterior testis
c.g.a. common genital atrium
c.s. cirrus sac
L.c. Laurer's canal
met. metraterm
met.c. metraterm gland cells
M.gl. Mehlis' gland
oöt. oötype
ov. ovary
ovd. oviduct
ovvd. ovovitelline duct
p.t. posterior testis
s.r. seminal receptacle
s.v. seminal vesicle
v.ef. vas efferens
vit.r. vitelline reservoir



0.10mm

Fig 5.6. Transverse section of P. elegans just anterior to ventral sucker showing common genital atrium. Scale 0.10mm.

Fig. 5.7. Plagiorchis elegans 7 days old harvested from a pigeon. Note tandem testes and uterus to the right side. Scale 0.30mm.



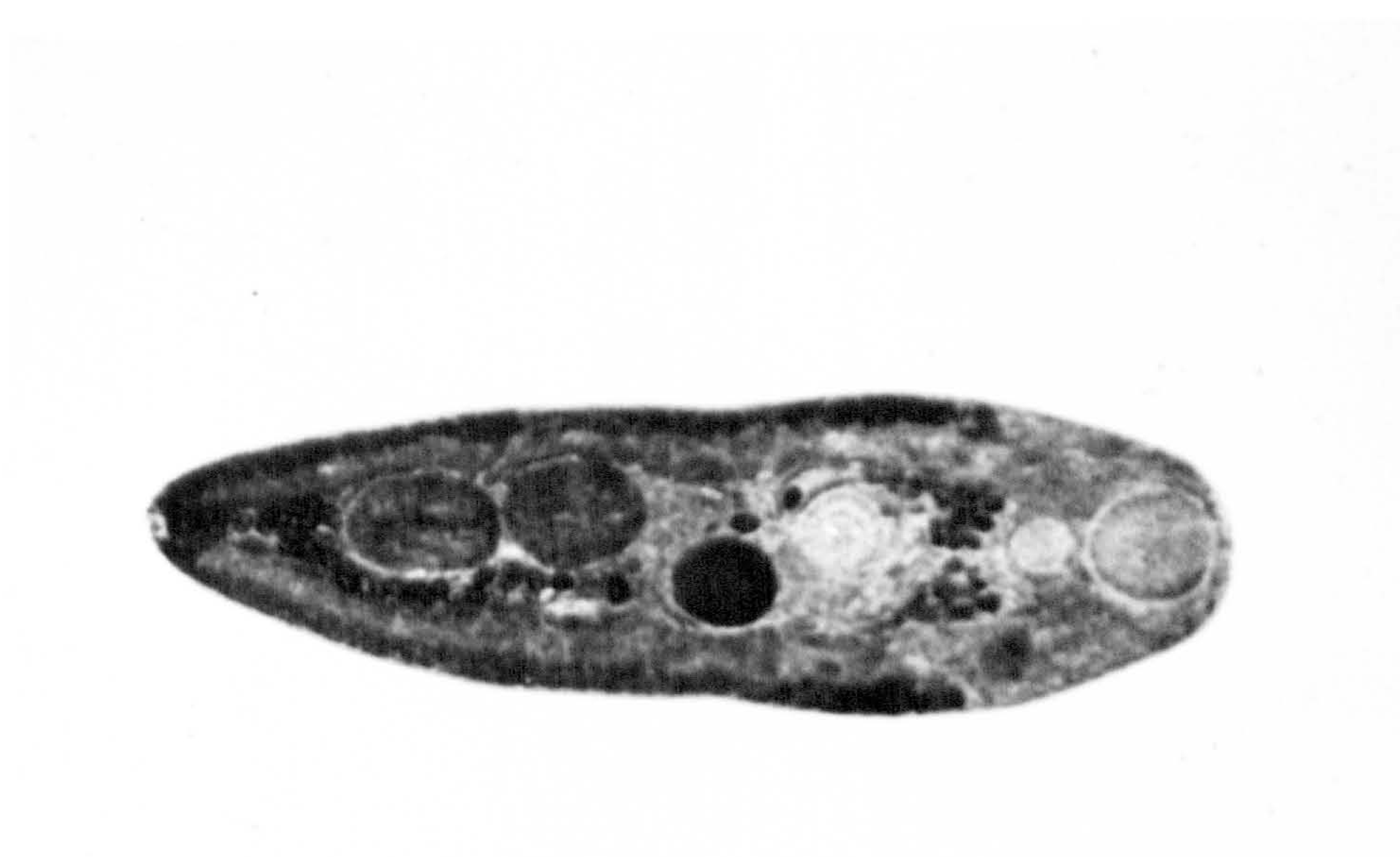
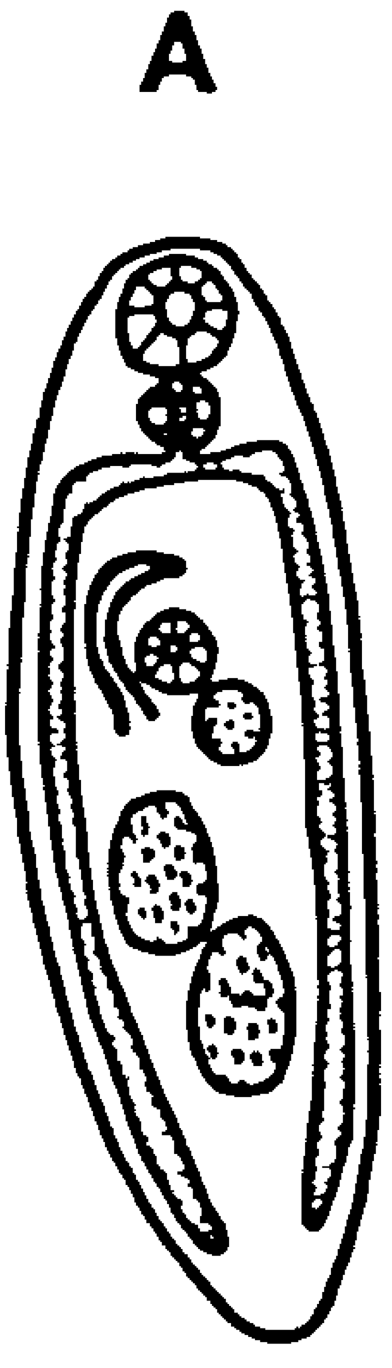
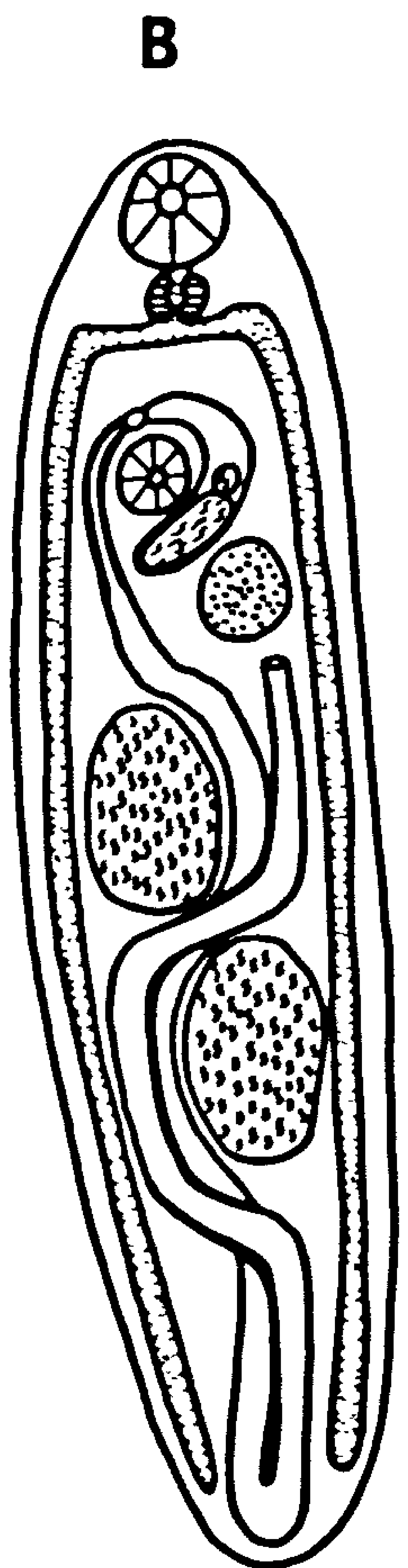
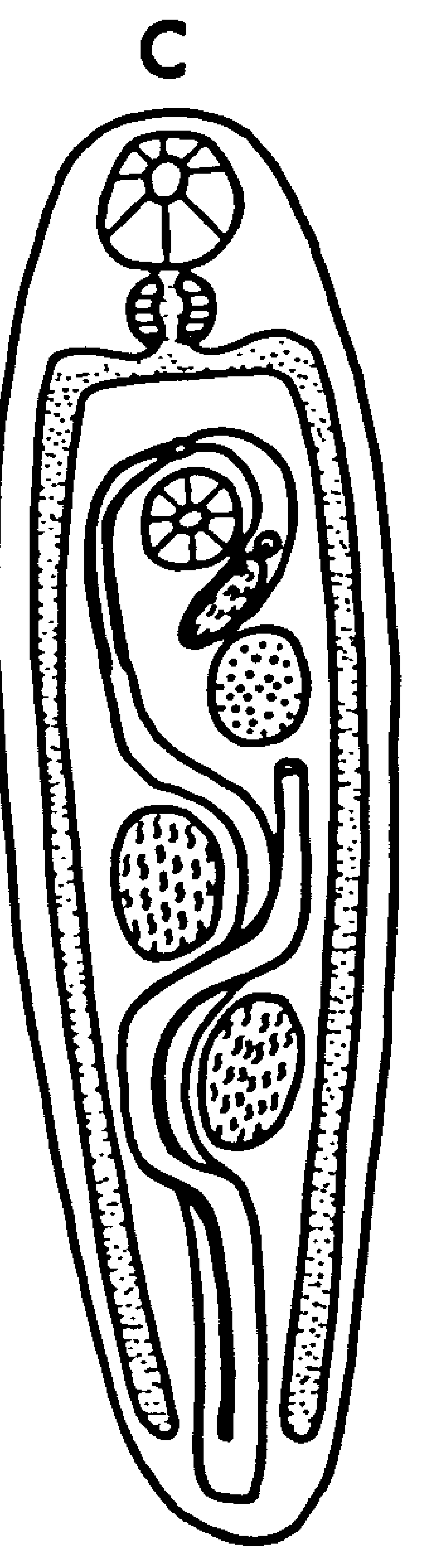


Fig. 5.8. Plagiorchis elegans harvested from LACA mice 4(A), 7(B), 14(C), 21(D) days after infection. Specimens approaching the mean for each group. Scale 0.40mm.


A


B


C

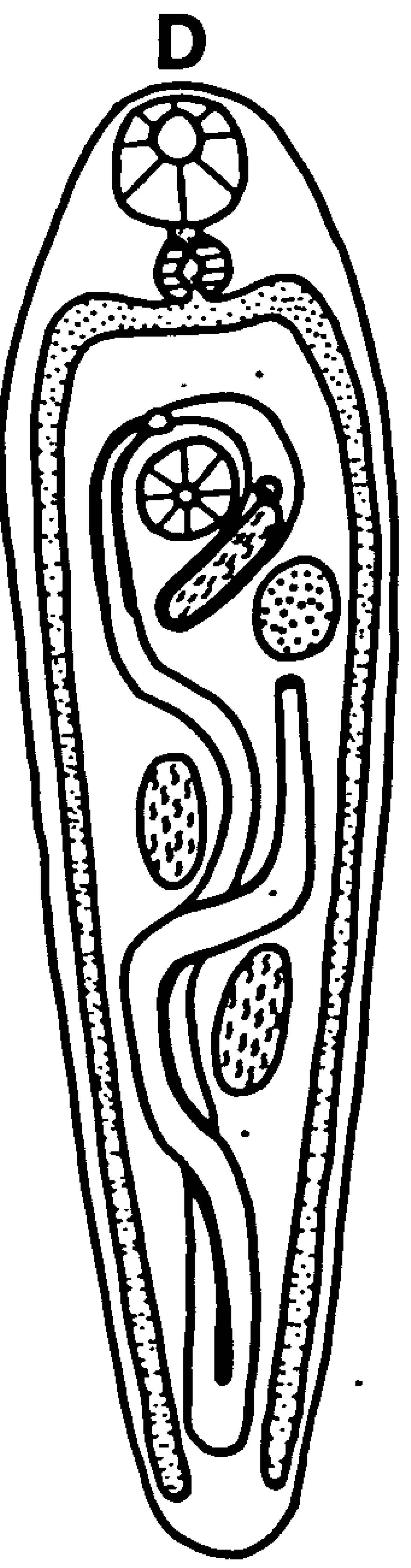
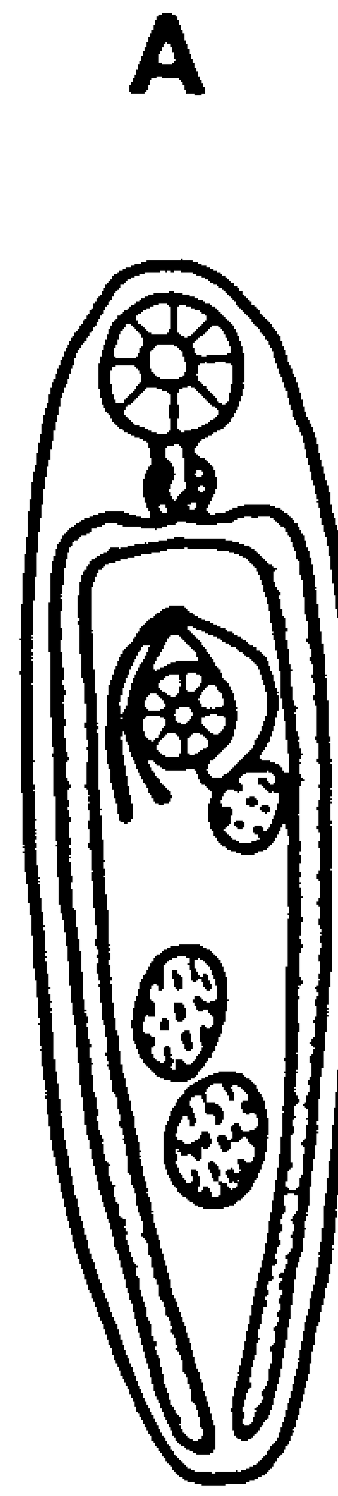
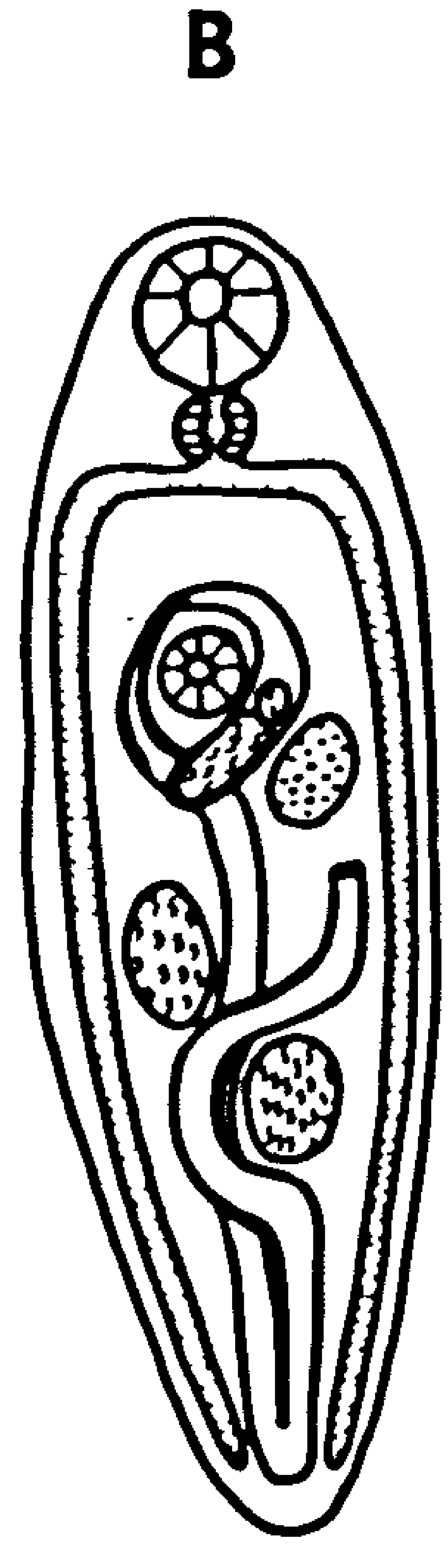
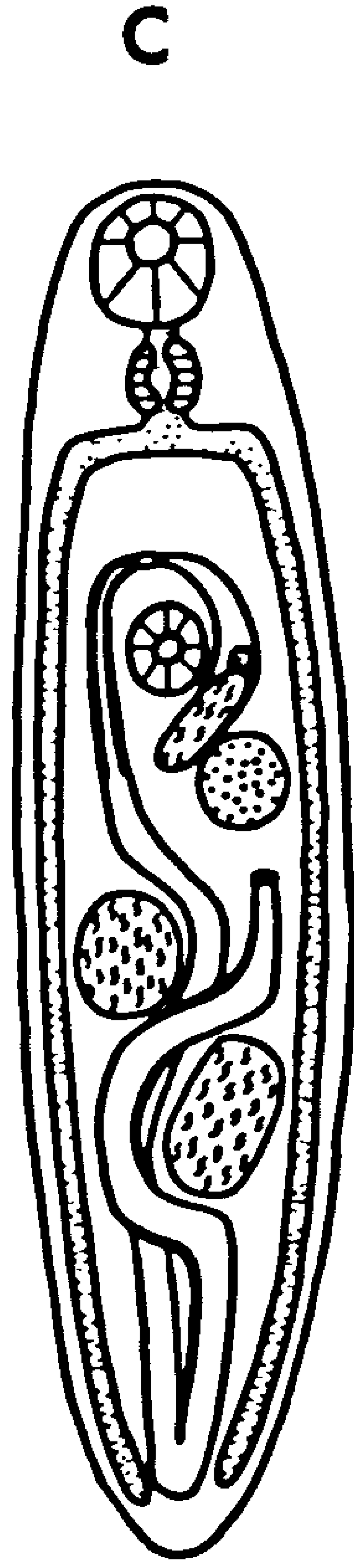

D

Fig. 5.9. Plagiorchis elegans harvested from laboratory rats 7(A), 14(B), 21(C), 28(D) days after infection. Specimens approaching the mean for each group. Scale 0.40mm.


A


B


C

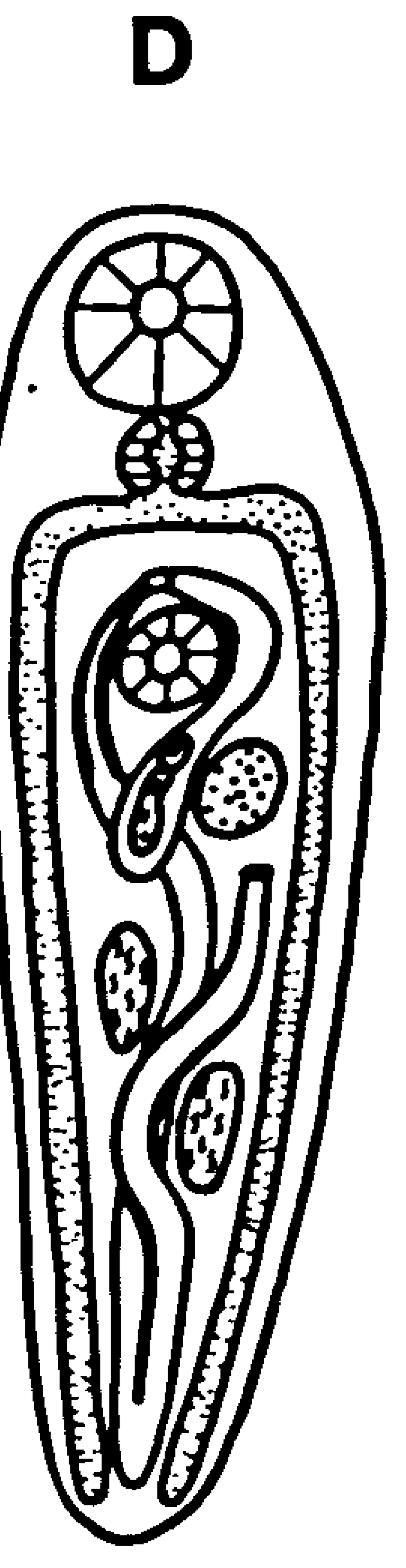
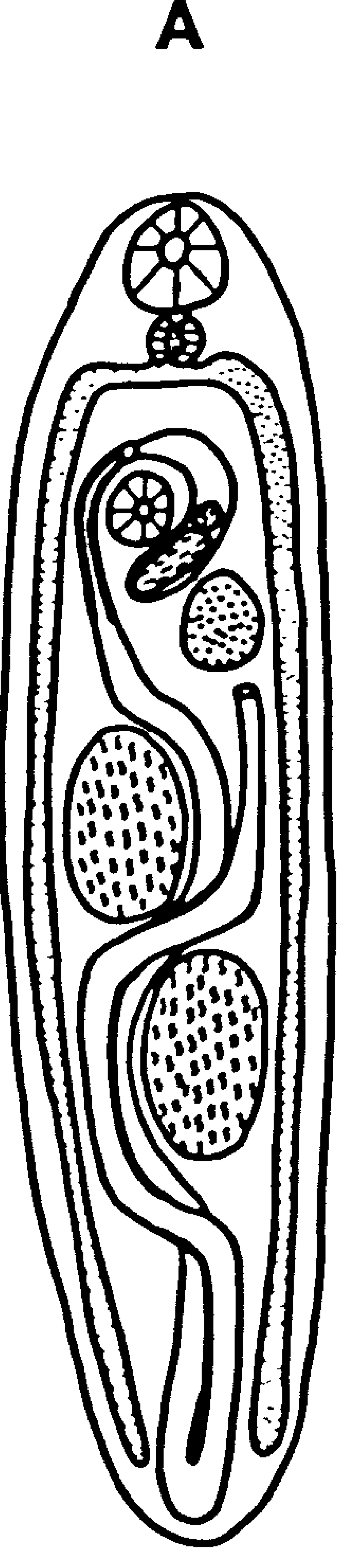
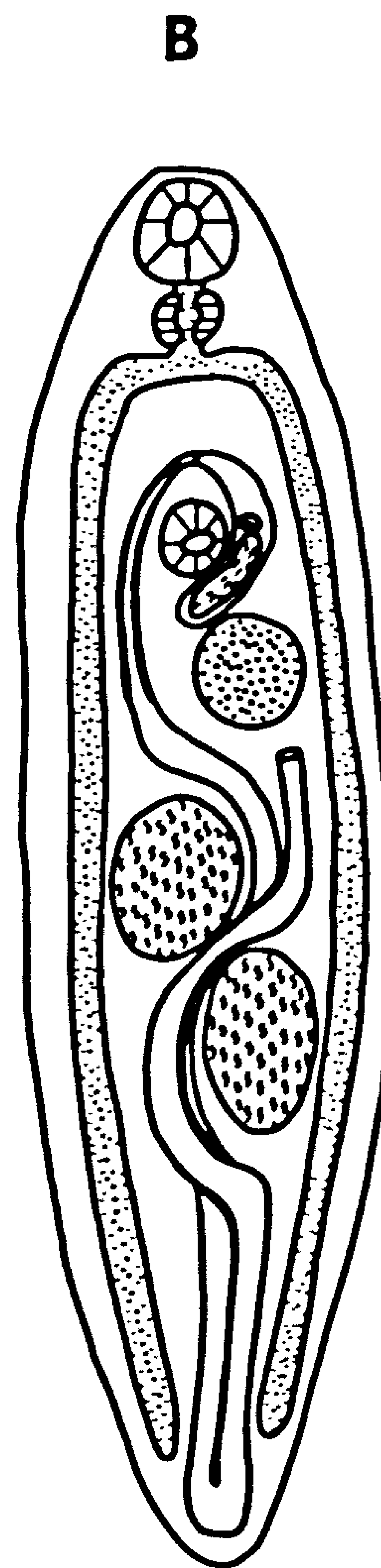
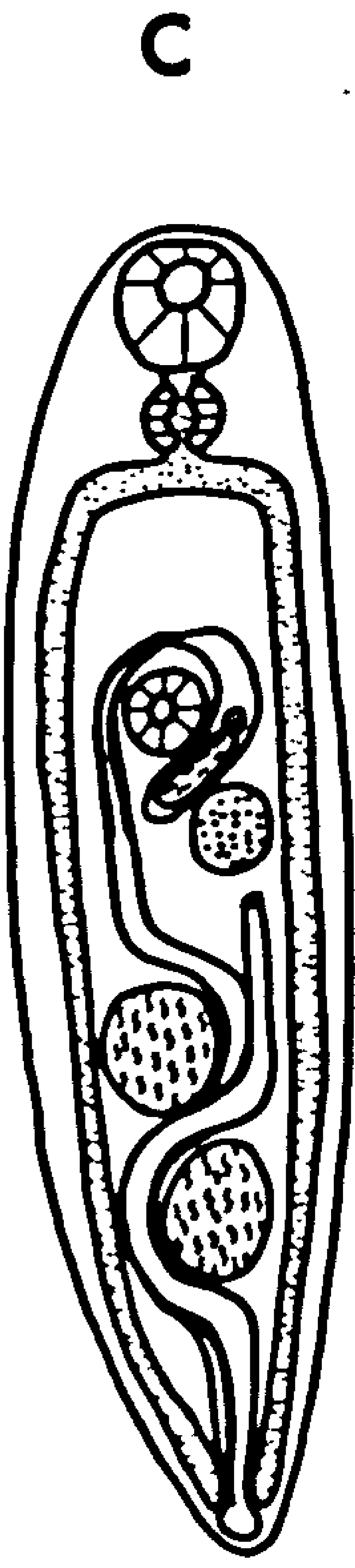

D

Fig. 5.10. Plagiorchis elegans 7 days old recovered from mammalian hosts. Mouse (A), gerbil (B), hamster (C), rat (D). Specimens approaching the mean for each group, Scale 0.40mm.


A


B


C

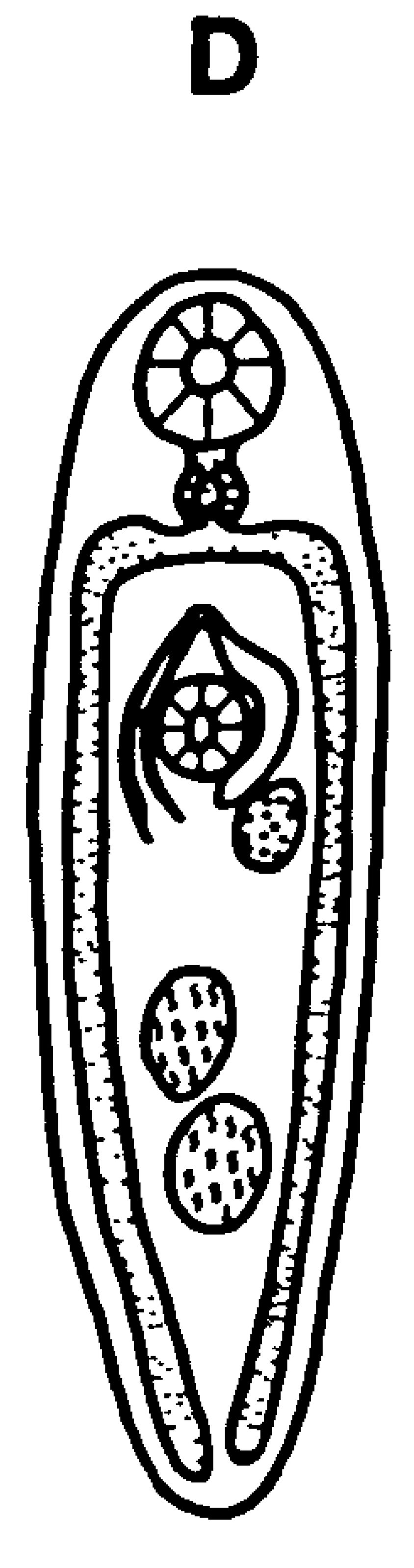

D

Fig. 5.11. Plagiorchis elegans 7 days old recovered from avian hosts. Chick (A), pigeon (B), duckling (C). Drawings of specimens approaching the mean for each group. Scale 0.40 mm.

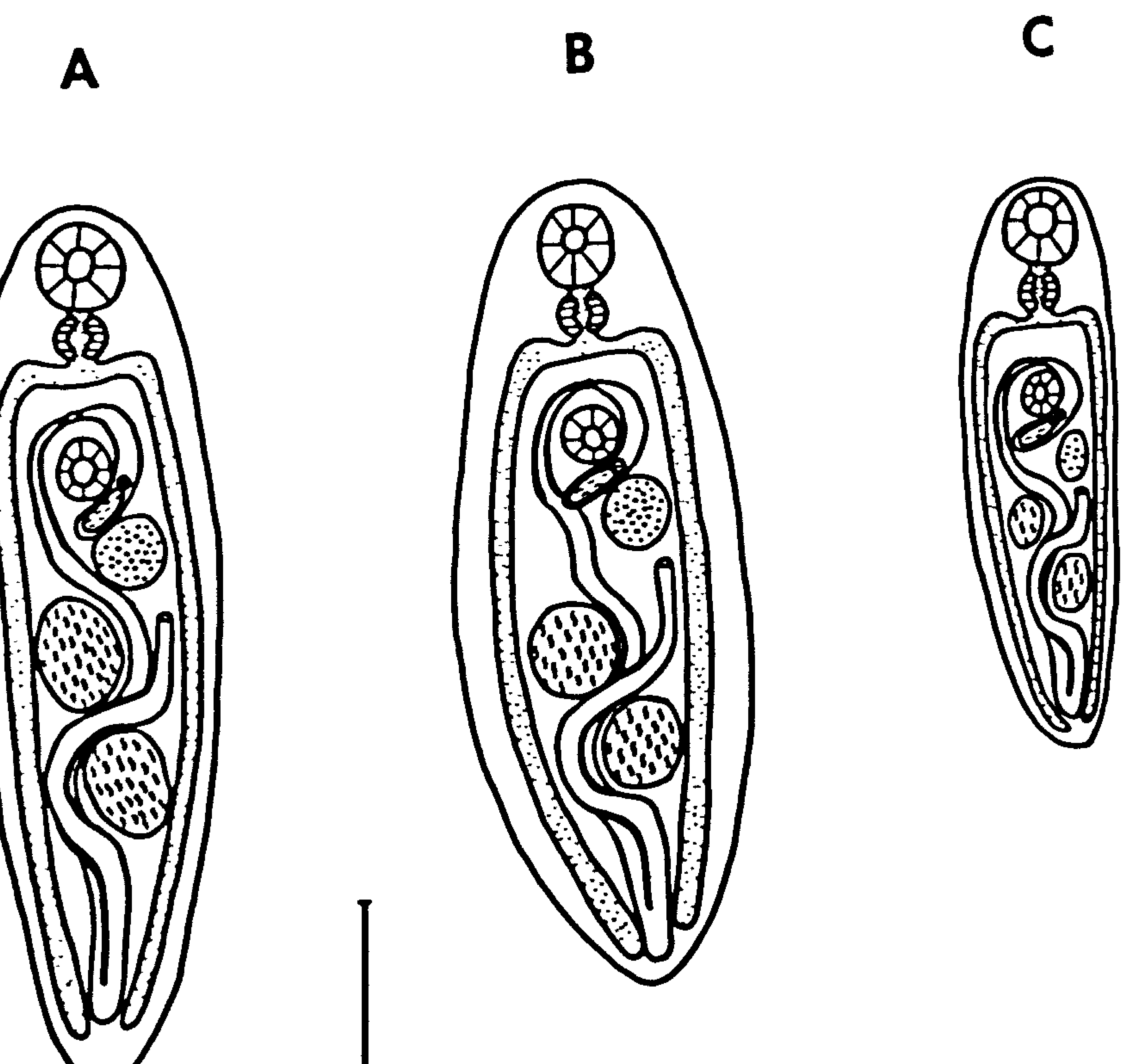

A
B
C

Fig. 5.12. Plagiorchis elegans 7 days old recovered from LACA mice; passage was completed using Lymnaea stagnalis as the first intermediate host and Asellus aquaticus (A), Gammarus pulex (B), and Lymnaea stagnalis (C) as second intermediate hosts. Lymnaea palustris (D) was used as the first intermediate host in place of L. stagnalis with Chironomus sp. as the second intermediate host. Compare with Fig. 5.10A. Specimens drawn approaching the mean for each group. Scale 0.40mm.

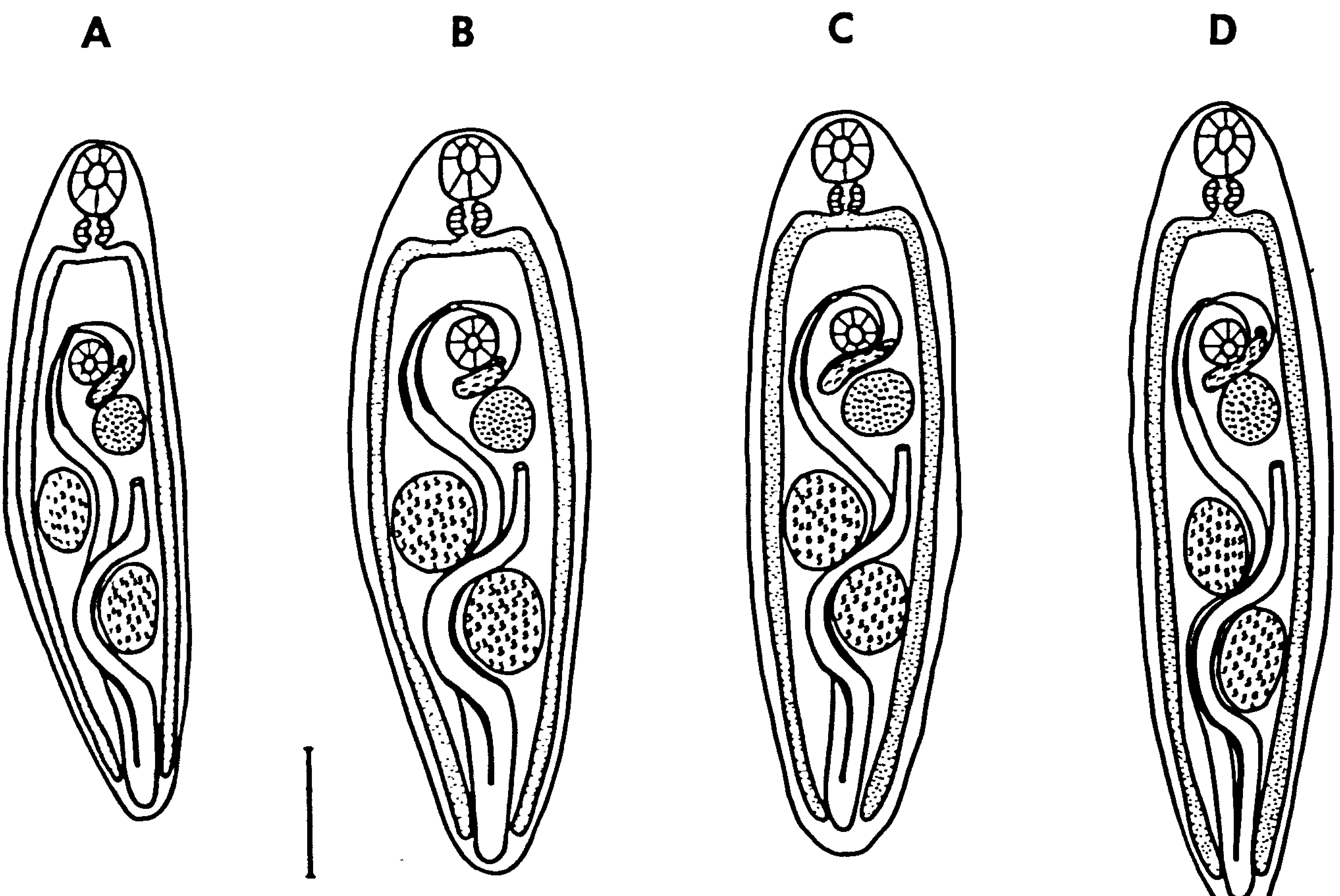

A
B
C
D

Fig. 5.13. Position of the oral sucker of _P. elegans_; (A) terminal, (B - F) subterminal and various shapes assumed by apertures of both the oral and ventral suckers.

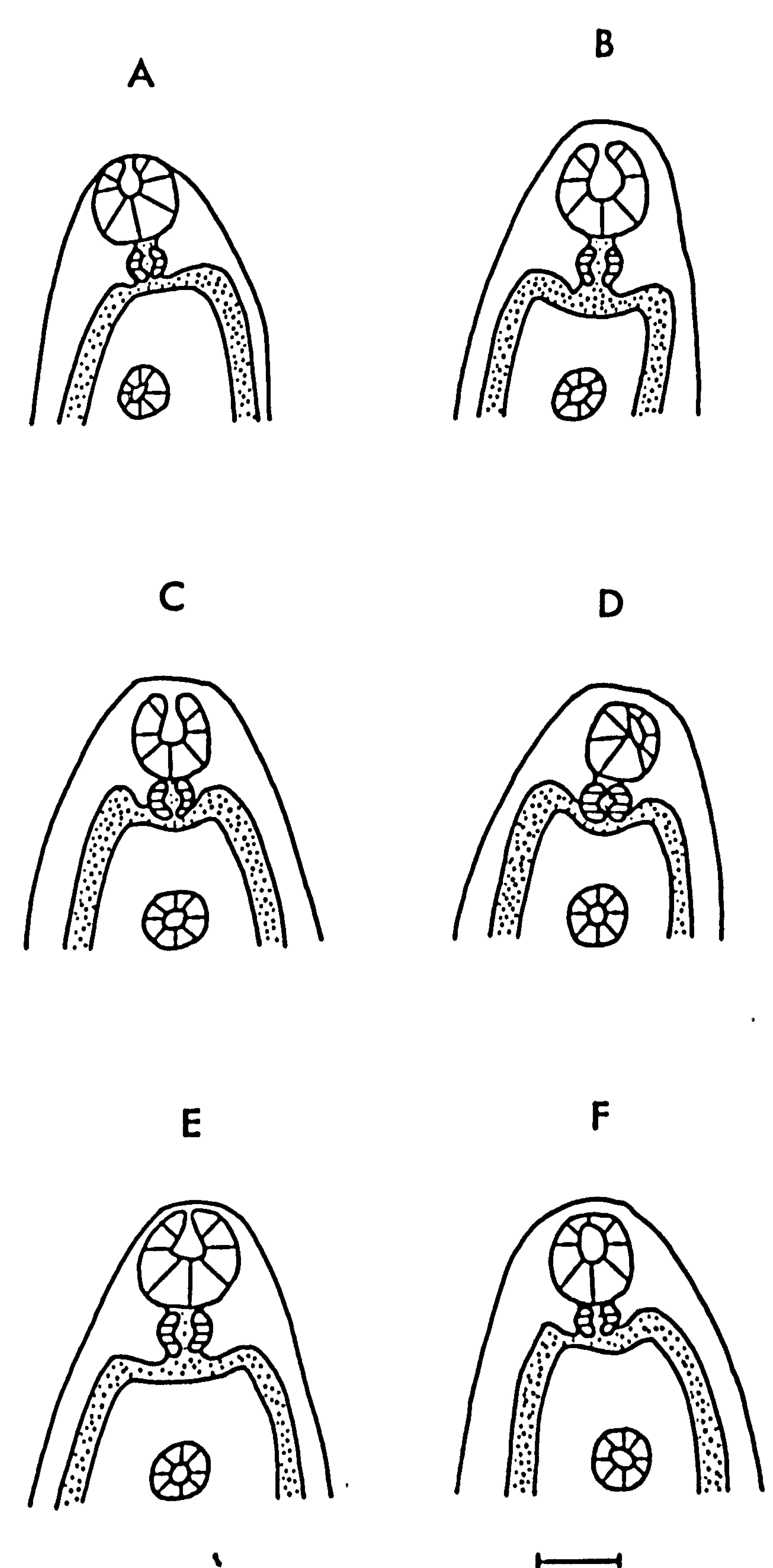
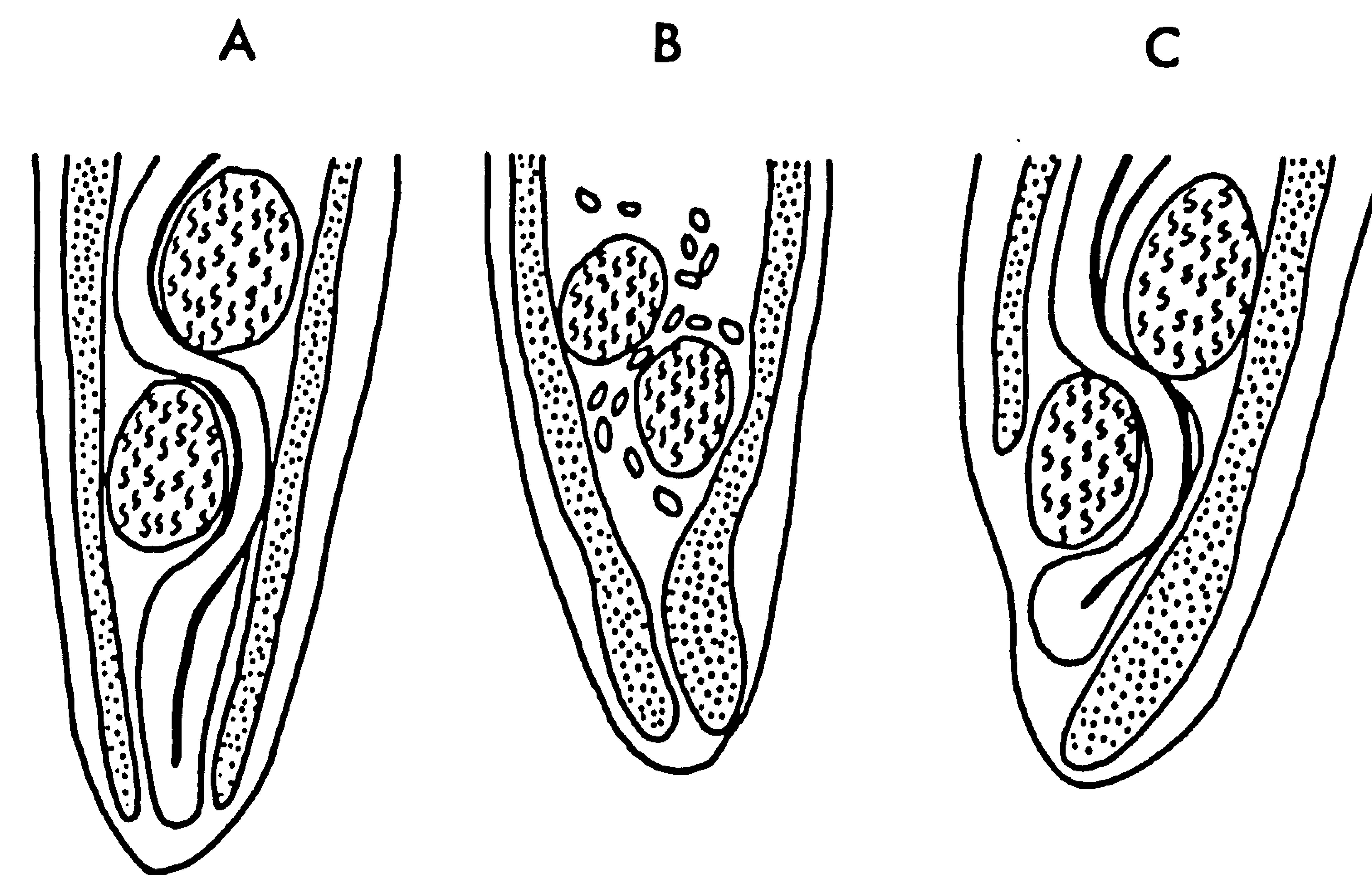

A
B
C
D
E
F
0.2 mm

Fig. 5.14. Variation noted in the posterior extent of the caeca and uterus of P. elegans.


A
B
C

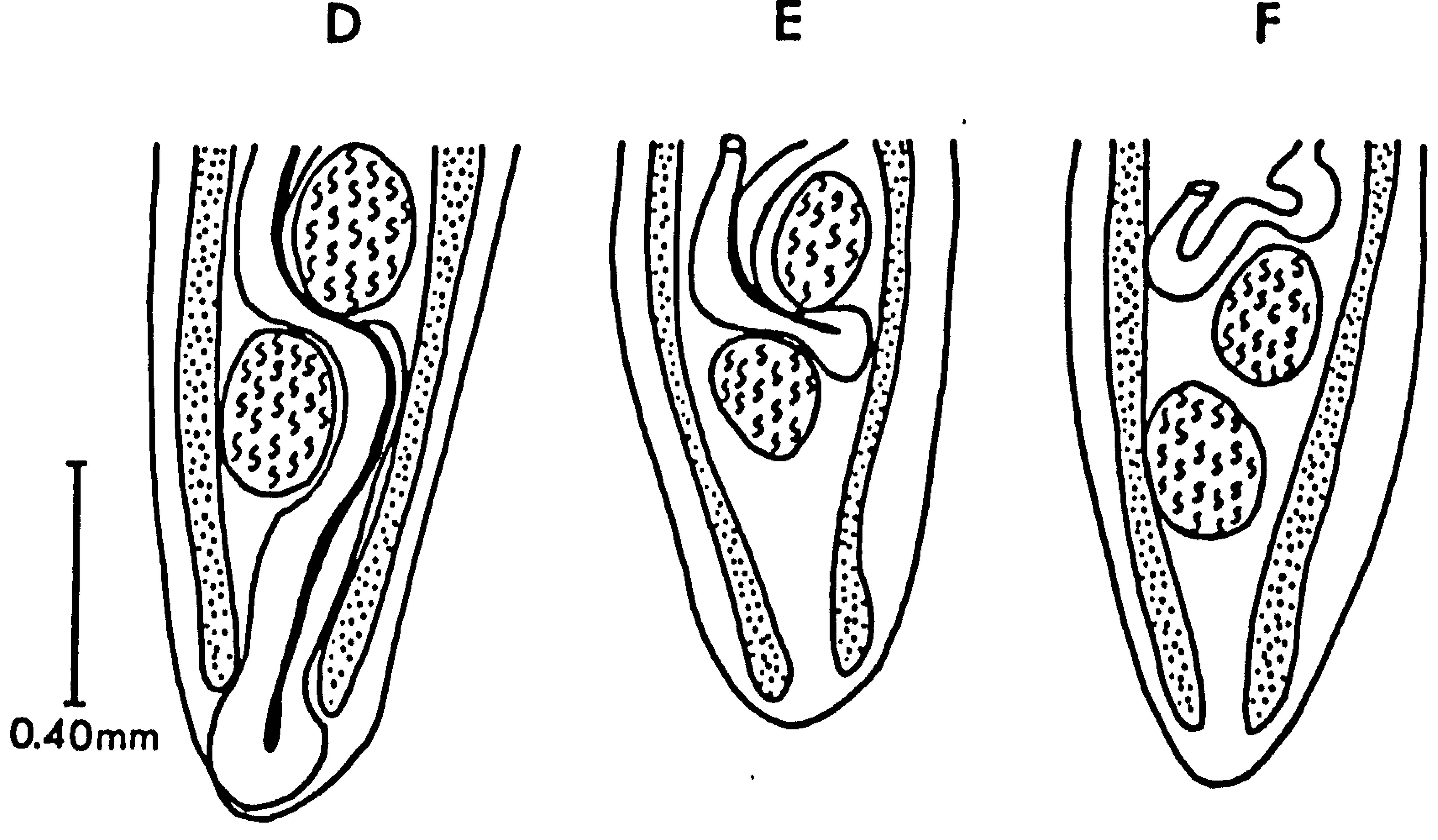

D
E
F
0.40mm

Fig. 5.15. The gonads of P. elegans are most frequently smooth in outline (A), but the ovary and more frequently the testes may be irregular in outline; in specimens such as B this is probably due to fixation and in C to senility. Variation noted in the size, shape and position of the cirrus sac are shown in figures D to I.

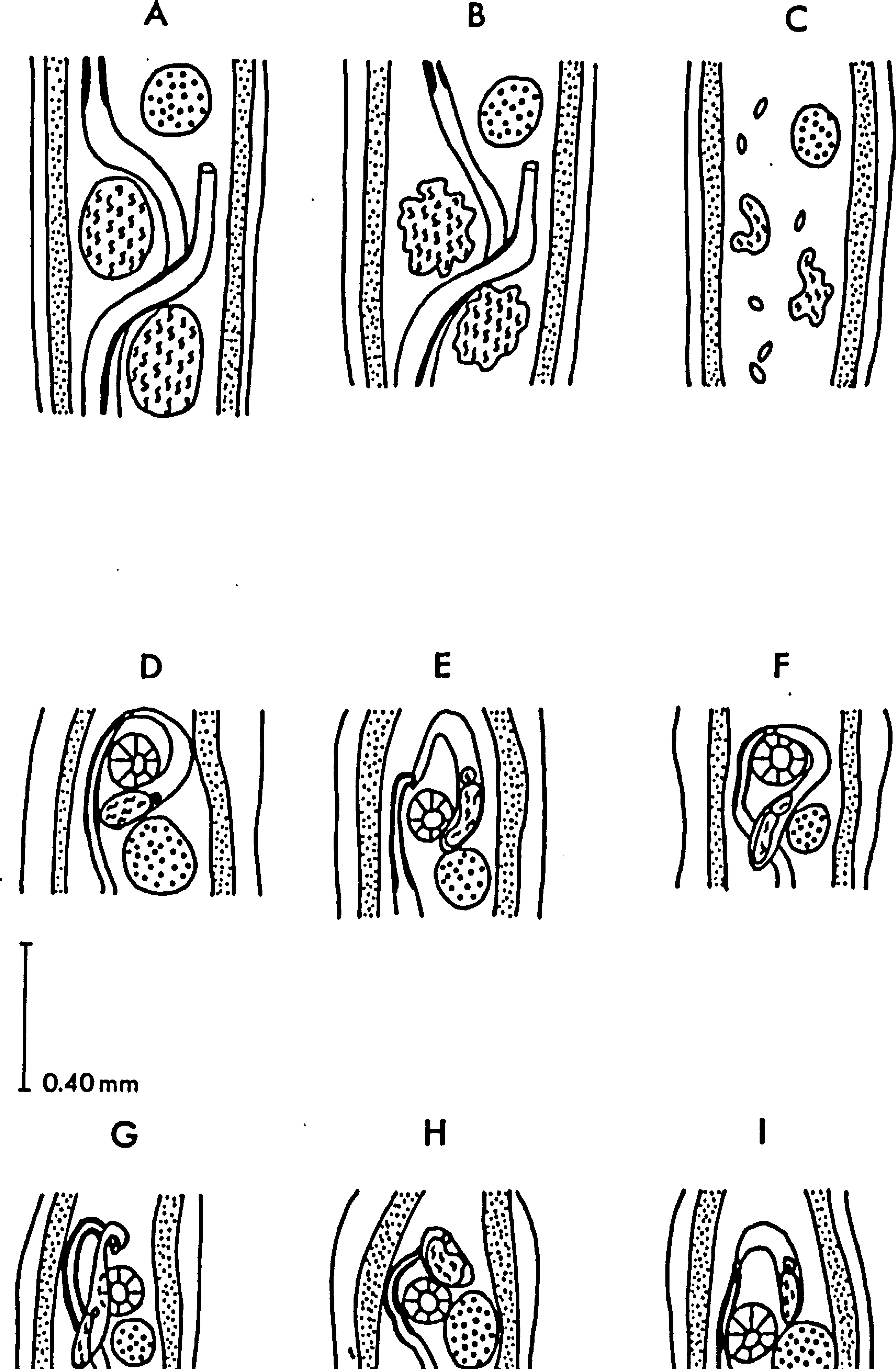
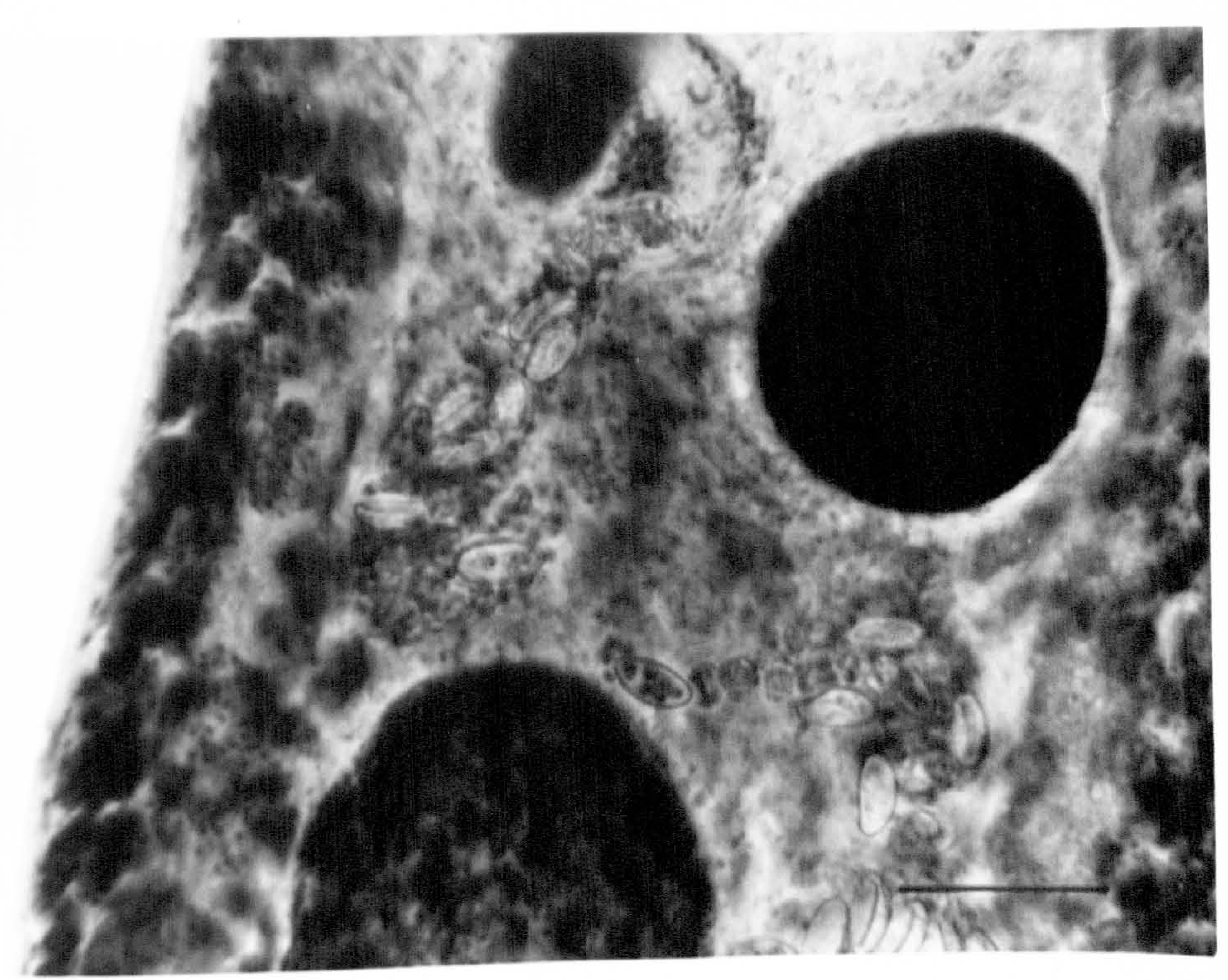

A
B
C
D
E
F
0.40 mm
G
H
I

Fig. 5.16. Mid portion of P. elegans showing the ovary, posterior testis and a short section of the uterus. Note the free yolk and ova in addition to the apparently normal eggs contained within the uterus. Scale 0.10mm.

Fig. 5.17. Sole specimen of P. elegans in which the seminal vesicle does not appear to be bipartite. Scale 0.10mm.



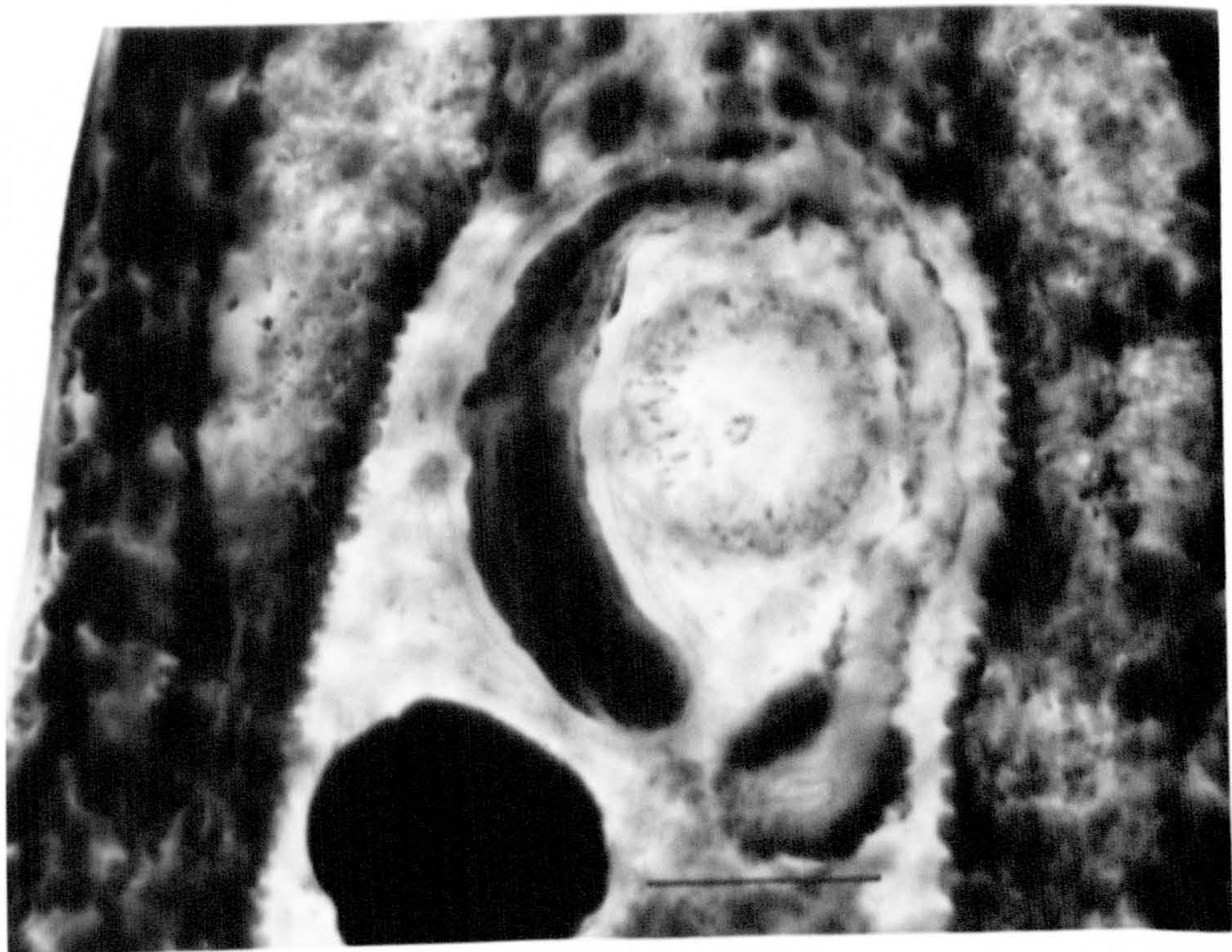


Fig. 5.18. Anterior distribution of the vitellaria in specimens of P. elegans recovered from LACA mice (M), gerbils (G), hamsters (H), rats (R), pigeons (P), ducklings (D), and chicks (C). In no specimen harvested from chicks did the vitellaria join in the anterior midline. The unequal distribution of the vitellaria noted along the lateral body margins at the level of the ovary is illustrated in 1 and the unequal posterior extent of the vitelline follicles is shown in 2.

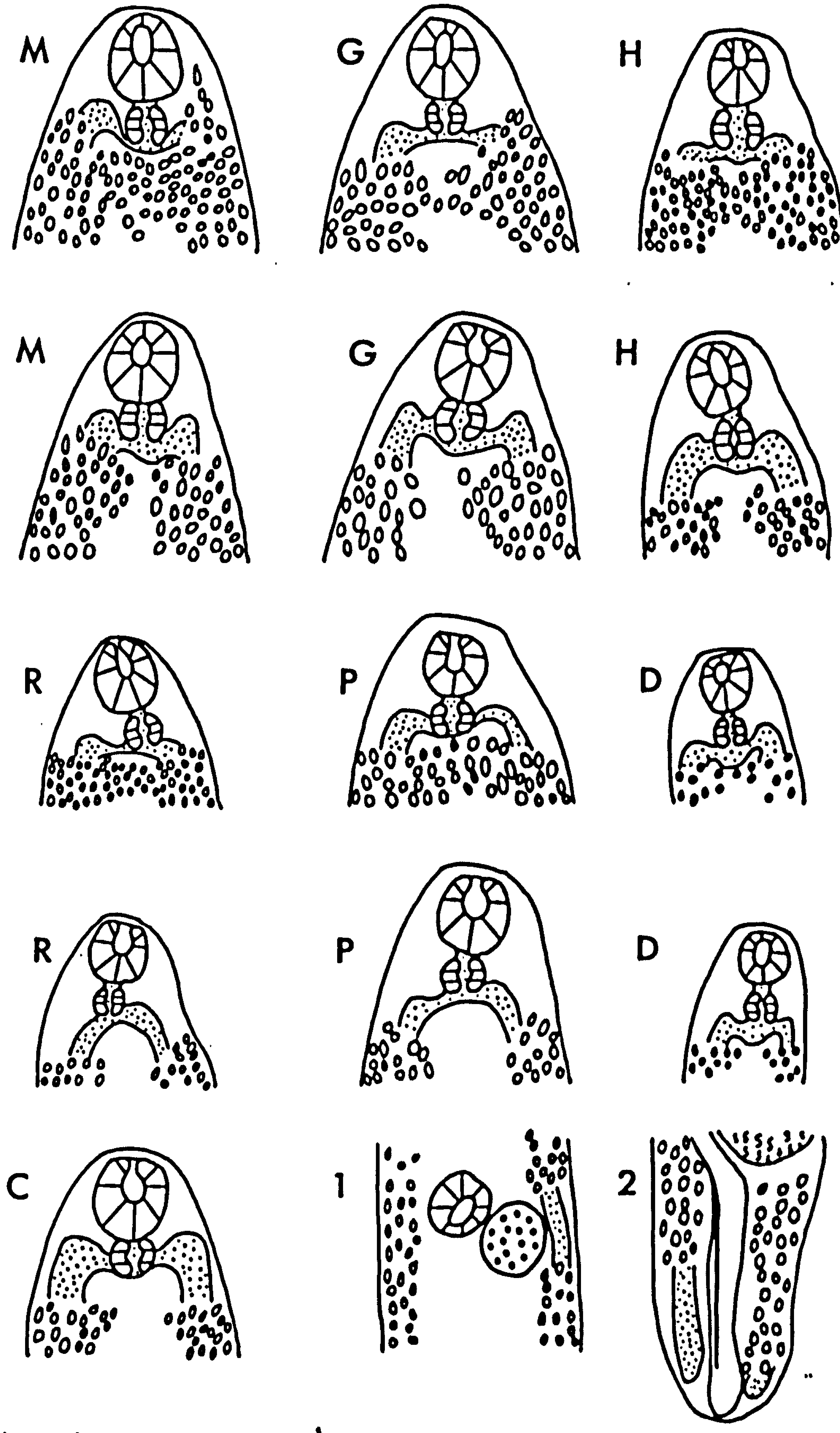

M
G
H
M
G
H
R
P
D
R
P
D
C
1
2
0.20mm

Fig. 5.19. Egg counts for individual mice each fed 10 *P. elegans* metacercarial cysts. One mouse was sacrificed on each of the following days post- infection - 5,7,9,11,14,17 and 25. Eggs per day on vertical axis; days post-infection on horizontal axis. (Days 4 and 5 for all mice in addition to day 14 for mouse no. 5 are of 24h periods only; all other points represent the average egg release for two consecutive 24h periods.)

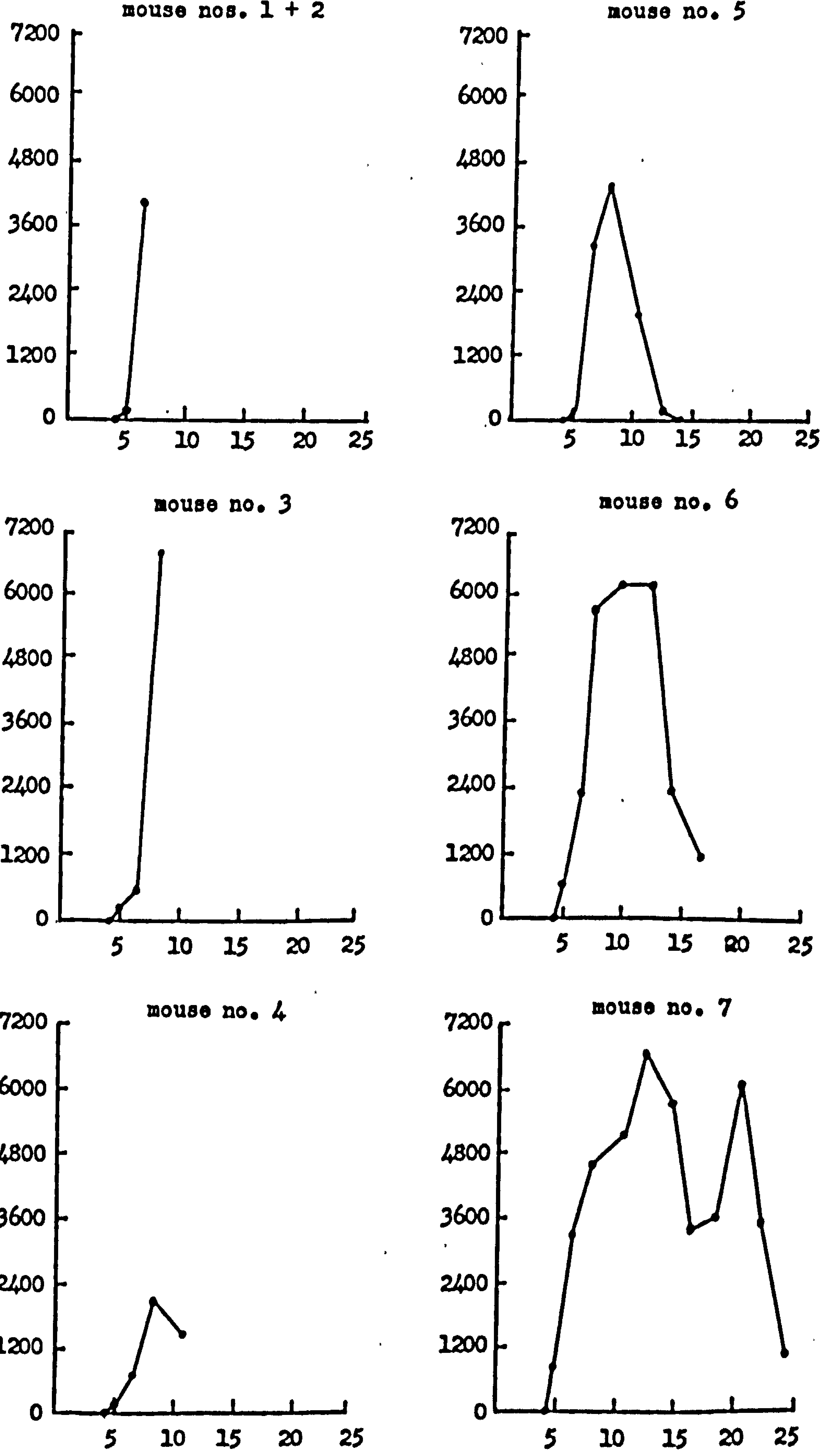
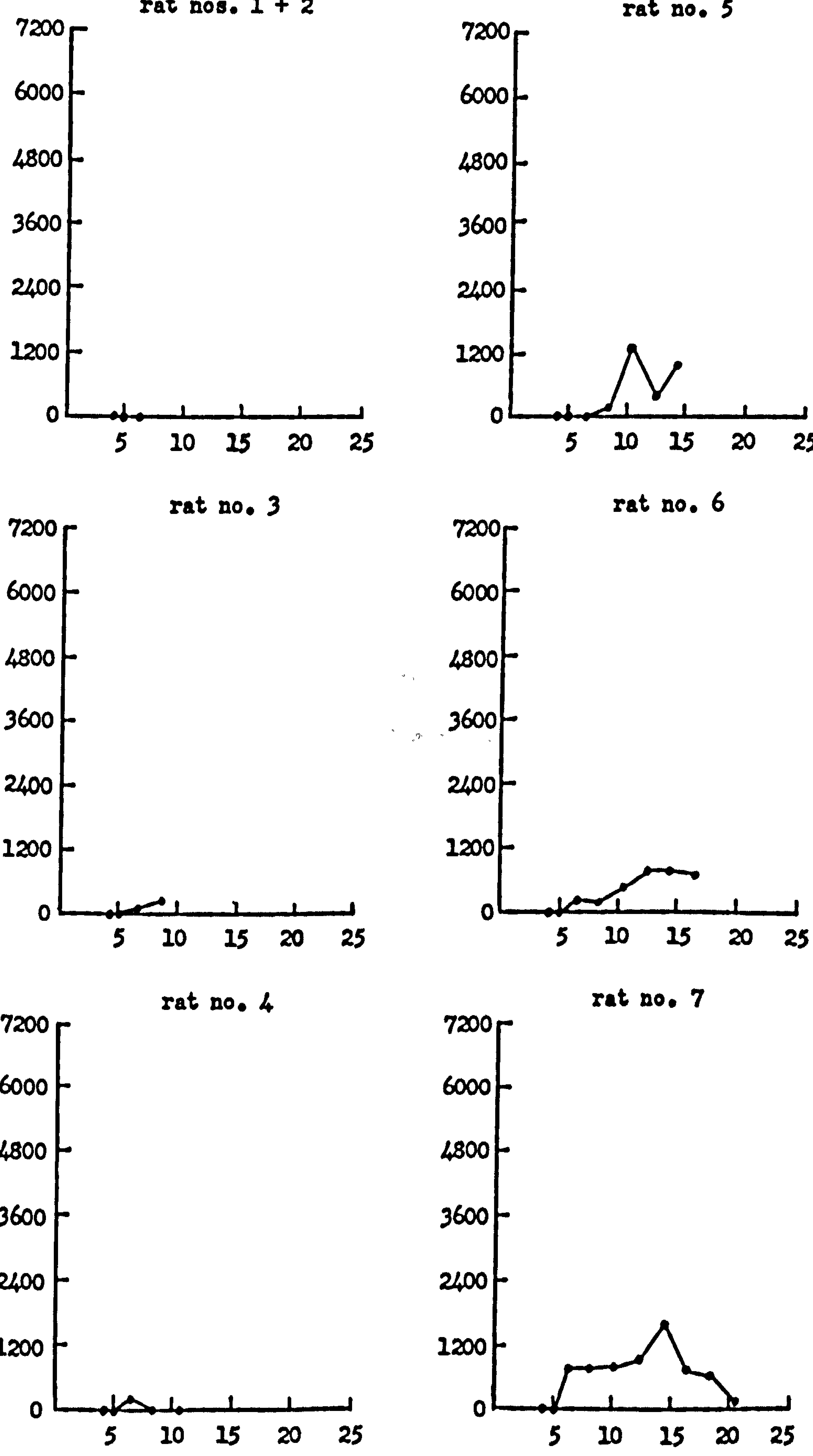

mouse nos. 1 + 2
7200
6000
4800
3600
2400
1200
0
5
10
15
20
25
mouse no. 5
mouse no. 3
mouse no. 6
mouse no. 4
mouse no. 7

Fig. 5.20. Egg counts for individual rats each fed 15 P. elegans metacercarial cysts. One rat was sacrificed on each of the following days post-infection - 5,7,9,11,14,17 and 21. (Rat no. 7 was believed to be losing its infection, so it was killed after 21 and not 25 days.) Eggs per day on vertical axis; days post-infection on horizontal axis. (Days 4 and 5 in addition to day 14 for rat no. 5 are of 24h periods only; all other points represent the average egg release for two consecutive 24h periods.)



rat nos. 1 + 2
rat no. 5
rat no. 3
rat no. 6
rat no. 4
rat no. 7
7200
6000
4800
3600
2400
1200
0
5
10
15
20
25

Section 6

Immunity.

INTRODUCTION.

In recent years numerous investigations concerning immunity to helminth parasites have been conducted. Gordon (1948) and Stewart (1950) have investigated the "self-cure" phenomenon of infections of Haemonchus contortus. This occurs when a new infection is superimposed on an existing adult worm burden of a sensitized host and results in the elimination of only the original adult population. The course of primary infections of Nippostrongylus brasiliensis has been studied by Mulligan et al. (1965). They noted an abrupt drop in egg output within two to three weeks of initial infection, with concurrent or subsequent loss of adult worms. In challenge infections N. brasiliensis is expelled before it reaches patency (Ogilvie, 1965). Panter (1969) has experimentally demonstrated that, in challenge infections of mice, worm burdens of Nematospiroides dubius are reduced, the worms themselves are stunted, and the distribution of the larvae is altered. Crompton (1973) has written an excellent review of the distribution of parasitic helminths within the alimentary tract of vertebrates. The importance of the host strain and supply company has been emphasized by Chappell and Pike (1976), who noted differences in the host-parasite relationships between infections with Hymenolepis diminuta in different strains of rats and in the same strain obtained from different suppliers. Much of the work concerning trematode infections has dealt with aspects of immunity to schistosomes. Ritchie, Garson, and Knight (1963) and Sadun and Bruce (1964) have studied the relationship between Schistosoma mansoni and the laboratory rat, while a series of investigations has been conducted by Smithers and Terry (1965 a,b,c) concerning the course of infections with S. mansoni in a variety of laboratory hosts, as well as the development of acquired resistance in the rhesus monkey and in the albino rat.

The majority of adult P. elegans harboured in LACA mice are expelled within three and often within two weeks of infection. However, other Plagiorchis species have been maintained in the experimental final hosts Sterna hirundo and Larus ridibundus by Zdarska (1966) and Mus musculus by Buttner and Vacher (1959) for six and eight weeks respectively. In the present work preliminary investigations indicated that specimens recovered from challenge infections of LACA mice with P. elegans are stunted and a smaller proportion of them is recovered than in primary infections. As a result of these observations studies

on the possible development of immunity in LACA mice to infections of *P. elegans* were carried out. The definition of "immunity" given by Michel (1968) is used in the present work and is ". . . resistance to the establishment of new worms." Déspite the large number of laboratory studies performed on species of *Plagiorchis*, the occurrence of immunity to these parasites has not previously been investigated.

MATERIALS and METHODS.

Mice: LACA laboratory-reared mice 6 to 10 weeks of age were used unless otherwise stated. Ten-month old mice were infected to test for the occurrence of age resistance; food and water were provided *ad libitum*. In all cases known numbers of metacercarial cysts aged 6 to 15 days were fed to the mice by means of a stomach tube.

Preparation of specimens: Specimens recovered were fixed and measured as described in Section 5 (p. 87). Longitudinal paraffin wax sections were cut at 5µm and stained using either Perl's method for the determination of ferric iron or Periodic Acid Schiff for demonstrating the presence of polysaccharides.

Egg counts: Infected mice were kept individually in cages provided with grated floors through which their faeces could easily fall; faecal samples were collected at 24h intervals. The McMaster Method (Ministry of Agriculture, Fisheries and Food, 1971) was used for the determination of egg output. Total egg output per 24h period/ mouse was calculated rather than eggs/g of faeces, because the daily weight of faeces per mouse did not often exceed 3.0g.

Distribution: During a period of 2 1/2 years, no matter what the primary purpose of the experiments conducted a record has been kept of the distribution of the worms in the small intestine of all mice used.

After each mouse was sacrificed by cervical dislocation, the entire small intestine from the pylorus to the caecum was immediately removed, placed in an extended though not taut position on a dissecting board, measured and cut into 6 equal sections. Each section was placed in a petri dish containing 0.9% saline, slit longitudinally and examined for the presence of worms under a low power dissecting microscope. The worms were removed and the number per segment recorded.

When food material in the small intestine obscured the view, the section was rinsed and placed in clean saline; small volumes of this solution were then examined separately.

RESULTS.

Duration of the primary infection.

A series of experiments was devised to determine when the expulsion of the infection of *P. elegans* from the small intestine occurs and although small numbers of mice were used in this particular series, the results are representative of the observations made by the author concerning the normal course of infection of LACA mice with *P. elegans*.

Groups of four or five mice were fed doses of 10 or 15 cysts and sacrificed after 7,14,21 and 25 days; the numbers of *P. elegans* present were counted. The results are given in Table 6.1. A large percentage of the infecting dose was present in the hosts sacrificed on the 7th day of infection. However after the first week of infection there was a rapid expulsion of the worm burden, so that after 14 days 40% remained and by the 25th day only 4% of the worms had not been expelled.

Table 6.1. Duration of primary infections of *P. elegans* in LACA mice.

days post-infection	7	14	21	25
no. mice	4	5	5	5
cysts/ mouse	10	10 to 15	10	10
mean no. worms recovered/mouse (SE)	7.75 (0.50)	4.00 (2.34)	1.20 (1.64)	0.40 (0.89)
total no. worms recovered (%)	31 (78)	20 (40)	6 (12)	2 (4)

Egg counts.

Egg counts were performed to elucidate the pattern of egg production during the course of initial infections and to determine whether the drop in the number of worms is accompanied by a decrease in the numbers of eggs released by surviving worms.

(i). 10 (15-day old) cysts/mouse.

In the first experiment seven mice were each fed 10 cysts and on each of the following days after infection one mouse was sacrificed:

5,7,9,11,14,17 and 25; consequently only a single mouse was maintained for the entire 25 days of the experiment. The worms present in each mouse at the time of sacrifice were fixed and measured (see Section 5; p. 101). Graphs of the egg counts for each mouse are given in Fig. 6.1. Daily egg counts and estimates of daily egg production per worm are presented in Tables 6.3 and 6.4 respectively; the latter were obtained by dividing the total egg output from each mouse by the number of worms either known or thought to be present on any one day.

No eggs were recovered in the faeces of the mice on day 4, but after day 5 the egg counts of all the mice, with the exception of mouse 4, rose sharply. Although 100% of the infecting dose of metacercariae was recovered as adults from mouse 4 on day 11, the worms were significantly shorter than the specimens more than 5 days old recovered from the other mice in the group (Table 6.2). In mice 5,6 and 7 (Fig. 6.1) the peak of egg production occurred some time between the 8th and 13th days post-infection and although in mice 5 and 6 the total egg production dropped rapidly, in mouse 7 it remained at a high level from the 7th until the 22nd day post-infection. Whether the second peak in graph mouse no. 7 (Fig. 6.1) is real or due to experimental error cannot be determined. When egg production is at its maximum (estimated 862 eggs/worm/day) in the above experiments, the rate of egg release per worm is greater than 1 egg per 2 minutes.

At this dosage there is considerable variation among the individual mice in their tolerance to infection; as illustrated in Fig. 6.1, the infection was terminated in mouse 5 by day 13, while in mouse 7 (Table 6.3), as extrapolated from the egg counts, the majority of the worms survived until approximately day 22.

(ii). 100 (15-day old) cysts/mouse.

The experiment was repeated according to the procedure described in (i) but the dose was increased from 10 to 100 cysts (15-day old)/mouse (Fig. 6.2). Random samples consisting of 10 specimens harvested from each mouse were fixed and measured; it was not possible to examine the entire worm burden present because of the time needed to fix large numbers of worms properly and to perform the egg counts.

As in (i) eggs were not present in the faeces of the mice until the 5^{th}day after infection. In mouse 4 (Table 6.1) the specimens recovered were significantly smaller than those present in the other mice more than 5 days after infection and the egg counts of mouse 4 were correspondingly low.

Egg production recorded from mice 5,6 and 7 (Fig. 6.2) peaked be-

Table 6.2. Comparison of the size of P. elegans recovered 5 to 25 days post-infection from LACA mice given light or heavy infections. Measurements in mm.

		10 cysts/ mouse			100 cysts/ mouse		
mouse no.	days post-infect.	no. measured (total recov.)	mean length (1 SE)	mean width (1 SE)	no. measured (total recov.)	mean length (1 SE)	mean width (1 SE)
1	5	8 (8)	1.93 (0.04)	0.57 (0.01)	10 (82)	2.10 (0.07)	0.53 (0.01)
2	7	8 (9)	2.76 (0.04)	0.73 (0.02)	10 (66)	2.44 (0.11)	0.61 (0.01)
3	9	9 (10)	2.90 (0.06)	0.78 (0.02)	10 (92)	2.94 (0.06)	0.72 (0.02)
4	11	9 (10)	2.58 (0.06)	0.66 (0.02)	10 (31)	2.14 (0.07)	0.59 (0.02)
5	14	0 (0)	-	-	10 (86)	2.89 (0.07)	0.71 (0.01)
6	17	2 (2)	2.78 (0.07)	0.73 (0.03)	10 (17)	2.68 (0.05)	0.67 (0.02)
7	25	1 (1)	2.73	0.60	10 (29)	2.43 (0.07)	0.68 (0.01)

Table 6.3. Total daily egg counts; each mouse fed 10 P. elegans metacercarial cysts (15 days old). Single mouse sacrificed on days 5,7,9,11,14,17 and 25 post-infection. (No. worms present when mice were killed.)

day post-infection	mouse no. 1	2	3	4	5	6	7
4	0	0	0	0	0	0	0
5	150 (8)	100	150	50	50	600	700
6		3300	3000	400	3100	1650	2850
7		4900 (9)	7100	1050	3450	3100	3750
8			5400	1350	4800	5700	5400
9			8400* (10)	3300	4050	5850	4050
10				2100	2300	6150	5250
11				1100 (10)	1500	6450	4800
12					150	6900*	6600
13					0	5700	6900*
14					0 (0)	3900	6900*
15						900	4350
16						1350	4650
17						900 (2)	2250
18							4700
19							2500
20							5925
21							6450
22							5025
23							2100
24							1875
25							300 (1)

*peak of egg release.

Table 6.4. Estimated range of P. elegans eggs released per worm per day (no. worms surviving) when the infecting dose was 10 cysts/mouse.

day post-infect.	mouse no. 1	2	3	4	5	6	7
5	19 (8)	10–11 (9–10)	15 (10)	5 (10)	5–6 (8–10)	60–75 (8–10)	70–88 (8–10)
6		330–367 (9–10)	300 (10)	40 (10)	310–388 (8–10)	165–206 (8–10)	285–356 (8–10)
7		544 (9)	710 (10)	105 (10)	345–431 (8–10)	310–388 (8–10)	375–469 (8–10)
8			540 (10)	135 (10)	480–600 (8–10)	570–712 (8–10)	540–675 (8–10)
9			840 (10)	330 (10)	405–506 (8–10)	585–731 (8–10)	405–506 (8–10)
10				210 (10)	230–288 (8–10)	615–769 (8–10)	525–656 (8–10)
11				110 (10)	150–188 (8–10)	645–806 (8–10)	480–600 (8–10)
12					15–150 (1–10)	690–862 (8–10)	660–825 (8–10)
13					0 (0–10)	570–712 (8–10)	690–862 (8–10)
14					0 (0)	390–488 (8–10)	690–862 (8–10)
15						90–450 (2–10)	435–544 (8–10)
16						135–675 (2–10)	465–581 (8–10)
17						450 (2)	250–281 (8–10)
18							470–588 (8–10)
19							250–312 (8–10)
20							592–740 (8–10)
21							645–806 (8–10)
22							502–628 (8–10)
23							350–525 (4–6)
24							312–469 (4–6)
25							300 (1)

Table 6.5. Total daily egg counts; each mouse fed 100 P. *elegans* metacercarial cysts (15 days old). Single mouse sacrificed on days 5,7,9,11,14,17 and 25 post-infection. (No. worms present when mice were killed.)

day post-infection	mouse no. 1	2	3	4	5	6	7
4	0	0	0	0	0	0	0
5	150 (82)	500	1350	300	3300	1000	2100
6		13500	7800	10200	21900	15900	17850
7		12300 (66)	15100	12150	24150	20250	17550
8			15600	10800	41100	17300	24450
9			18300 (92)	9450	56550	38850	46050
10				10200	85050	58500	69750
11				6150 (31)	72600	37500	67350
12					55950	28800	45150
13					37950	42150	43650
14					23400 (86)	16500	14550
15						7560	20850
16						1950	7650
17						1200 (17)	6000
18							4950
19							3450
20							2400
21							4650
22							4500
23							1000
24							1950
25							2400 (29)

Table 6.6. Estimated range of *P. elegans* eggs released per worm per day when the infecting dose was 100 cysts per mouse. eggs/day; no. worms surviving.

day	mouse no.						
post-inf.	1	2	3	4	5	6	7
5	2;82	5-8; 66-100	14-15; 92-100	3-10; 31-100	33-38; 86-100	10-12; 80-100	21-26; 80-100
6		135-204; 66-100	78-92; 92-100	102-330; 31-100	219-255; 86-100	159-199; 80-100	178-223; 80-100
7		186; 66	151-164; 92-100	122-392; 31-100	242-281; 86-100	202-253; 80-100	176-219; 80-100
8			156-170; 92-100	108-348; 31-100	411-478; 86-100	173-216; 80-100	244-306; 80-100
9			133; 92	94-304; 31-100	566-658; 86-100	388-486; 80-100	460-576; 80-100
10				102-329; 31-100	850-989; 86-100	585-731; 80-100	698-872; 80-100
11				198; 31	726-844; 86-100	375-469; 80-100	674-842; 80-100
12					560-651; 86-100	288-360; 80-100	452-564; 80-100
13					380-441; 86-100	422-527; 80-100	436-546; 80-100
14					272; 86	165-330; 50-100	146-182; 80-100
15						76-445; 50-100	208-261; 80-100
16						20-115; 17-100	76-264; 29-100
17						71; 17	60-207; 29-100
18							50-171; 29-100
19							34-119; 29-100
20							24-83; 29-100
21							46-160; 29-100
22							45-155; 29-100
23							10-34; 29-100
24							20-67; 29-100
25							83; 29

tween days 10 and 11 and then fell abruptly. In mouse 7 it was maintained at a low level from day 15 until the mouse was sacrificed on day 25.

Table 6.6 shows the estimates of egg production/worm/day. The rate of egg production appears to increase more slowly in heavy than in light infections; this may be due to a crowding effect. On the other hand the highest estimate of eggs released /worm/day in either (i) or (ii) is 989 from mouse 5 on day 10 (Table 6.6); in addition a larger percentage of the infecting dose survived 14 days and longer in 100 cyst infections than in 10 cyst infections. These results are considered to be a reflection of individual variation in host tolerance to infection.

Challenge infections.

In many instances adult specimens of P. elegans, recovered from freshly killed mice, contained a bright red substance within their caeca; this localized pigment made them easily visible during the search of the small intestine for worms. When Perl's reaction and Periodic acid Schiff (PAS) test for ferric iron and polysaccharides respectively were performed the results obtained were positive and demonstrated the presence of blood and mucus in the caeca of P. elegans. It was thought that the ingestion of this material could stimulate an immune response on the part of the host and as a result place the parasites in intimate contact with the host's antibodies, thus accounting for the rapid termination of the infection. In order to test this hypothesis the following series of four experiments was conducted and the results are shown in Table 6.7.

High initial dose; low challenge dose. (Table 6.7, Group 1)

Four mice 6 to 10 weeks of age were each given an immunizing dose of 100 cysts. Five weeks later they and four control mice approximately the same age were each fed 10 cysts. After one further week all the mice were sacrificed and the worms harvested.

One of the challenged mice was not infected, but 45% (or 60% for the three infected mice) of the total challenge was recovered and 44% (8) of these worms were not patent. Seventy-five% (30 worms) of the infecting dose was recovered from the control mice and all 30 specimens were mature. In addition the control worms were significantly longer ($p<0.001$) and wider ($p<0.001$) than those recovered from the challenge infections.

Low initial dose; low challenge dose. (Table 6.7, Group ii)

Fifteen of 23 naive mice (6 to 10 weeks old) were each fed 20 cysts. Four weeks later, when the initial infection should have terminated, each of the 15 previously infected mice and four of the eight naive control mice was fed 20 cysts. After one week four of the challenged mice and the four control mice were sacrificed.

Only 44% of the worms were recovered from the challenge infections in contrast to 91% of the primary infection. Student's _t_ test was used as a test of significance and shows that the specimens recovered from the primary infection were significantly larger than those of the challenge infection (Figs. 6.4, 6.5). Thirty-nine percent(14) of the specimens from the challenged mice and 100% (68) of those from the control mice were mature.

Second challenge infections. (Table 6.7, Groups iii and iv)

This series of experiments was performed to investigate the possible effect on the host response of the time interval between infections. The time intervals between the second and third infections of groups iii and iv were 4 and 15 weeks respectively. In both cases two control mice were each fed 20 cysts and the experiments were terminated a week later.

When the time interval between the second and third infections was 4 weeks (Group iii), 4 of the 6 challenged mice were refractory to infection and the 2 infected mice harboured 1 and 2 specimens, one of which contained apparently viable eggs. However when 15 weeks elapsed between the second and third infections (Group iv) all the challenged mice were infected; 24% (29) of the worms were recovered, of which 55% (16) were mature; 80% (32) of the infecting dose was present when the control mice were sacrificed and all of them were mature. In both Groups iii and iv the controls were significantly larger than the specimens recovered from the challenged mice, but it was noted that when compared with the 3 other control groups (i,ii and iii) the control specimens of Group iv were significantly smaller (length $p<0.001$; width $p<0.001$).

Since the mice in Group iv were approximately 21 weeks old when infected, the possibility of age resistance developing in the definitive host and inhibiting the development of _P. elegans_ was considered and this suggestion was tested by the following two experiments.

Age resistance.

10 cysts/ mouse (Table 6.8, Group a)

In the first experiment 4 mice (10 months old) and 2 mice (6 weeks

Table 6.7. Challenge infections of LACA mice with *P. elegans*.

	no. mice	init. inf. cysts/mouse	challenge inf. cysts/mouse 1st : 2nd	weeks between 1st & 2nd inf.: 2nd & 3rd inf.
Group i				
	4	100	10	5
Controls				
	4	10	0	-
Group ii				
	4	20	20	4
Controls				
	4	20	0	-
Group iii				
	6	20	20 : 20	4 : 4
Controls				
	2	20	0 : 0	- : -
Group iv				
	5	20	20 : 20	4 : 15
Controls				
	2	20	0 : 0	- : -

worm burden/ mouse-% rec.	mean worm burden (SD)	no. meas.	mean length (1 SE) (all worms 7 days old)	mean width (1 SE) (all worms 7 days old)
			measurements in mm	
0				
4				
6 45%	4.50 (3.42)	18	1.53 (0.05)	0.43 (0.01)
8				
tot. 18 10 (56%) of these worms were mature.				
5				
8				
8 75%	7.50 (1.73)	28	2.23 (0.03)	0.63 (0.01)
9				
tot. 30 100% mature		Student's *t* test	$p<0.001$	<0.001
1				
10				
11 44%	8.75 (5.32)	28	1.05 (0.41)	0.34 (0.02)
13				
tot. 35 14 (39%) mature				
16				
18				
17 91%	17.00 (1.41)	46	2.10 (0.04)	0.62 (0.01)
17				
tot. 68 100% mature			$p<0.001$	<0.001
0				
0				
0				
0 3%	0.50 (0.84)	2	1.38 (0.21)	0.44 (0.01)
1				
2				
tot. 3 1 mature				
19				
20 98%	19.50 (0.71)	19	2.18 (0.05)	0.66 (0.01)
tot. 39 100% mature			$p<0.001$	<0.001
3				
4				
6 24%	5.80 (2.39)	23	1.19 (0.05)	0.35 (0.01)
7				
9				
tot. 29 16 (55%) mature				
15				
17 80%	16.00 (1.41)	11	1.46 (0.06)	0.48 (0.01)
tot. 32 100% mature			$p<0.010$	<0.001

Small size here due to age resistance?

Table 6.8. Age resistance in LACA mice to *P. elegans*. All worms measured when 7 days old; measurements in mm.

	no. mice	cysts/mouse	worm burden per mouse	mean worm burden (SD)	% recov.	no. meas.	mean length (1 SE)	mean width (1 SE)
Group a (10 months old)	4	10	9 5 8 6 tot. 28	7.00 (1.83)	70	28	1.94 (0.07)	0.56 (0.02)
Controls (6 weeks old)	2	10	10 10 tot. 20	10.00 (0)	100	16	2.38 (0.05)	0.64 (0.01)
					Student's *t* test		p <0.002	<0.001
Group b (10 months old)	4	50	31 33 35 0 tot. 99	24.75 (16.58)	50	29	2.10 (0.04)	0.56 (0.01)
Controls (6 weeks old)	2	50	44 35 tot. 79	39.50 (6.36)	79	20	2.09 (0.04)	0.59 (0.01)
				Student's *t* test		no significant difference		p<0.020

old) were each fed 10 cysts and sacrificed after 7 days.

As shown in Table 6.8 a substantially larger percentage of the worms were recovered from the young mice (100%) than from the old mice (70%), while Figs. 6.6 and 6.7 clearly demonstrate that specimens harvested from the former hosts were significantly larger than those from the latter hosts.

50 cysts/mouse (Table 6.8, Group b).

The second experiment was conducted using the above procedure, but the dosage was increased from 10 to 50 cysts/mouse. Seventy-nine % (79) of the worms were recovered from the young mice and 50% of the total dosage (99 worms) were recovered from the old mice even though one of them was uninfected. The length of the worms recovered did not vary significantly according to the age of the host, but the specimens recovered from the young mice were significantly wider than those from the old mice ($p < 0.020$).

Distribution.

The mean length of the small intestine of 130 LACA mice when 7 weeks to 10 months of age was 43.0cm. Student's *t* test, performed on the data obtained when conducting studies on age resistance, demonstrates that the length of the small intestine does not vary significantly in 7-week old and 10-month old mice; the small intestine was 41.8cm in the former sample and 42.9cm in the latter sample of mice.

During the present investigation the small intestine of each mouse was divided into 6 equal sections (see p. 101) and the numbers of *P. elegans* per section were recorded separately. Figure 6.3 is a series of histograms illustrating the distribution of the worms recovered from mice sacrificed after various periods of infection.

Primary infections.

Excystment appears to occur in the duodenum. After 45 to 60 minutes just over half of the excysted juveniles were found in the second and third intestinal sections, while the remaining worms were recovered from the 4th section. Only 24% of the infecting dose was recovered from the two mice; this can probably be attributed to the difficulty in finding them, although a number of metacercariae may have failed to excyst. The excysted juveniles do not often exceed 0.20mm in length and the presence of food material in the small intestine makes their detection difficult. The excysted worms were found among or on the lumenal surface of the villi, where they were most easily discernable when they moved or when the characteristic Y-shaped excretory bladder was clearly visible.

After 4 and 5 days 85% of the worms were recovered; all but a few of them were found in the posterior half of the small intestine. Seven and 8 days after infection no specimens were present in the anterior half of the small intestine. It is emphasized that these data for 7 and 8-day old infections were collected from 45 mice given doses of 1 to 100 cysts, so the pattern of distribution is not dependent upon the numbers of worms present.

On and following the 9th day post-infection an increasing proportion of the worms are present in the anterior half of the small intestine, although after day 9 there is a substantial decrease in the number of worms recovered per mouse.

On day 17 the majority of the adults was recovered from the last 2 small intestine sections. It is possible in this instance that the parasites had not migrated anteriorly; alternatively they may have migrated but when the mice were sacrificed the majority of the worms recovered were passing through the ileum before being expelled. This latter alternative is supported by the condition of the specimens recovered; the vitelline follicles were nearly spent and the gonads were significantly smaller than in 14-day old worms recovered during the same series of experiments (Figs. 6.8, 6.9). The wasted appearance of these structures indicates that the older parasites would not have survived for more than 2 or 3 days.

Twenty-one and 25 days post-infection all the surviving specimens were present in small intestine sections 1 to 4.

Challenge infections.

In mice that received challenge infections the worm burden was found scattered throughout the small intestine after 7 days and significantly fewer specimens were recovered than from primary infections of the same duration. Plagiorchis elegans does not survive as long in challenge infections as in primary infections; after 14 days only 1 stunted adult was recovered from 1 of 4 mice each fed 20 cysts after an initial infection of 20 cysts per mouse 8weeks previously.

From these data concerning the distribution of P. elegans in the small intestine it is believed that excystment occurs in the duodenum and that the excysted juveniles are carried posteriorly by peristalsis. Other factors, probably physiological, may also be involved in inducing the juveniles to become established initially in the posterior half of the small intestine. Since P. elegans does maintain an intimate histological association with the villi (Fig. 6.10) and the spines, which are more numerous on the ventral and antero-dorsal surfaces of the worm,

may abrade the mucosa, it is possible that within 9 days of infection the posterior half of the small intestine becomes a less suitable environment for P. elegans. Some specimens are unable to survive in this environment and are expelled, while others begin to migrate anteriorly.

DISCUSSION.

Schwabe and Kilejian (1968) noted that absolute acquired resistance to helminth infections is extremely rare. However instances of partial resistance are more frequently encountered and are manifested in one or more of the following ways:

1. a reduction of the percentage of infecting parasites which establish themselves as adults.
2. the stunted growth of those parasites which do attain adulthood.
3. a depression of the reproductive capacity of established adults.
4. failure of the infecting worms to reach maturity.

Initial infections of P. elegans in LACA mice are of short duration; the majority of the worms are expelled within 3 weeks of infection and egg output generally decreases rapidly 10 to 14 days after infection. Specimens of challenge infections are stunted and a large proportion of the surviving worms do not reach patency. Therefore according to the criteria of Schwabe and Kilejian (1968) noted above, partial resistance to infection by P. elegans is developed in LACA mice.

Smithers and Terry (1965b) have stated that variation in the establishment of challenge infections may reflect the individual host's immunological capacity to respond. They have also postulated that there is a threshold primary dose below which no resistance is induced. Since in the present investigation partial resistance to reinfection develops in mice initially infected with 20 metacercarial cysts, the threshold dose is less than 20 cysts.

The host response may depend upon the time interval between infections; when the interval between the second and third infections was 4 weeks, the host response was greater than when the interval was 15 weeks. Chandler (1935) found that, by increasing the time interval between primary and secondary infections of Nippostrongylus muris from 21 to 29 days, resistance decreased and substantially more worms were recovered from the latter infection. These data suggest that the time

interval between infections is an important factor in determining the intensity of the host response.

It is also possible that the variation in response noted during the present investigation was due to the metacercariae rather than the mice. Smithers and Terry (1965b) and Evans and Stirewalt (1951) working with Schistosoma mansoni have found that there is more variation in results when different batches of cercariae are used to infect monkeys than when the same batch of cercariae is used to infect all the hosts. Although a pure laboratory strain of P. elegans was used and the metacercariae were all 6 to 15 days old teased from chironomid larvae, it is possible that the results may have been affected, because different batches of cercariae were used since in some instances months elapsed between infections.

The experiments concerning age resistance did not yield conclusive evidence in support of its occurrence in LACA mice to infections of P. elegans. However there is a strong indication that age resistance develops, since heavier worm burdens are tolerated by young than by old mice; furthermore specimens from the latter hosts are in most instances significantly smaller than those from the former hosts. In 50 cyst/mouse infections the interpretation of the data, particularly the measurements of the specimens, is complicated by an additional factor, a possible crowding effect. As shown by the egg counts, although in both infections of 10 and 100 cysts/mouse eggs were recovered from the faeces starting on day 5, daily egg production increased initially more slowly in infections of 100 cysts/mouse than in infections of 10 cysts/mouse, indicating that the development of P. elegans proceeds more slowly in heavy than in light infections. On the basis of these results, a comparison of the percentage of the worms present may be of more relevance in judging the occurrence of age resistance than the size of the worms recovered.

Migration in primary infections and the altered distribution of parasites in challenge infections have been well documented in cases of acquired partial immunity. During the first 11 days of infection, Nippostrongylus brasiliensis is found in the anterior half of the rat small intestine. Some worms then migrate anteriorly within the intestine, some posteriorly and a large percentage of the infection is lost (Brambell, 1965). The expulsion of the worms at this time has been found to be in agreement with the host response to antigens produced primarily by female worms (Ogilvie, 1965). Panter (1969) working with Nematospiroides dubius, reported the results of challenging 10 immune

and 9 normal mice with 200 infective larvae. Fourteen days later the worms of the challenge infection were present throughout the small intestine, although predominently in the anterior 20cm, whereas in the normal mice no larvae were recovered more than 20cm distal to the pylosus.

Individuals of _P. elegans_ in primary infections migrate in the small intestine of LACA mice. Excystment appears to occur in the duodenum; a large percentage of the excysted metacercariae recovered after 45 to 60 minutes are found in the posterior half of the small intestine and it seems reasonable to assume that peristalsis may be largely responsible. Although after 4 to 5 days some worms are still present in the anterior half of the small intestine, after 7 to 8 days all are situated in the posterior half. It is possible that the worms are carried initially in the direction of flow (Crompton, 1973) and that the digestive physiology of the host may also be involved in the migration of the population by stimulating the juveniles to select the posterior region. After approximately 8 days an anterior migration begins, while increasing numbers of the worms are lost. On the 17th day of infection a large percentage of the specimens are present in the posterior half of the small intestine. However, judging by their spent condition, it is believed that these worms were recovered within one or two days of their being expelled. In challenge infections the distribution is altered and on the 7th day of infection the worms are scattered throughout the small intestine.

Migration within the small intestine may be stimulated by:

1. the inability of the mouse to satisfy the nutritional requirements of the worm, which may change with the age of the parasite.
2. the infection eliciting a host response which causes the environment of the small intestine to become unsuitable for the survival of _P. elegans_.

It appears unlikely that the dietary requirements of _P. elegans_ change with age. It has been possible by surgical transplantation to extend the life of a number of specimens of _P. elegans_ for up to 42 days beyond the expected 21 (see Section 7).

The second alternative seems to be more likely, especially when one takes into account the scattered distribution of the population in challenge infections.

It is suggested that to the criteria for acquired partial resistance given by Schwabe and Kilejian (1968) be added the alteration of the distribution of worm burdens during primary and challenge infections.

Fig. 6.1. Egg counts for individual mice each fed 10 _P. elegans_ metacercarial cysts. One mouse was sacrificed on each of the following days post-infection - 5,7,9,11,14,17 and 25. Eggs per day on vertical axis; days post-infection on horizontal axis. (Days 4 and 5 for all mice in addition to day 14 for mouse no. 5 are of 24h periods only; all other points represent the average egg release for two consecutive 24h periods.)

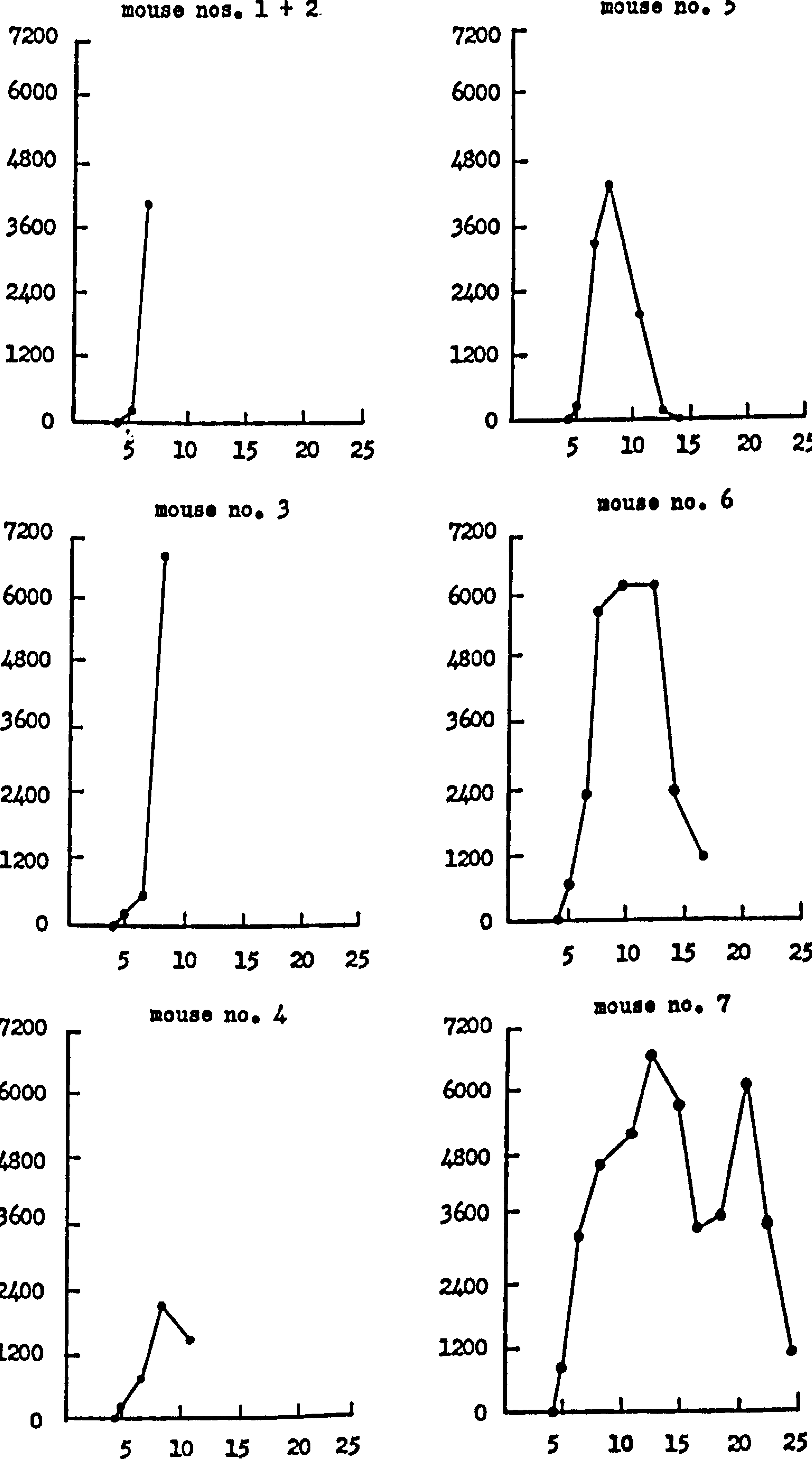
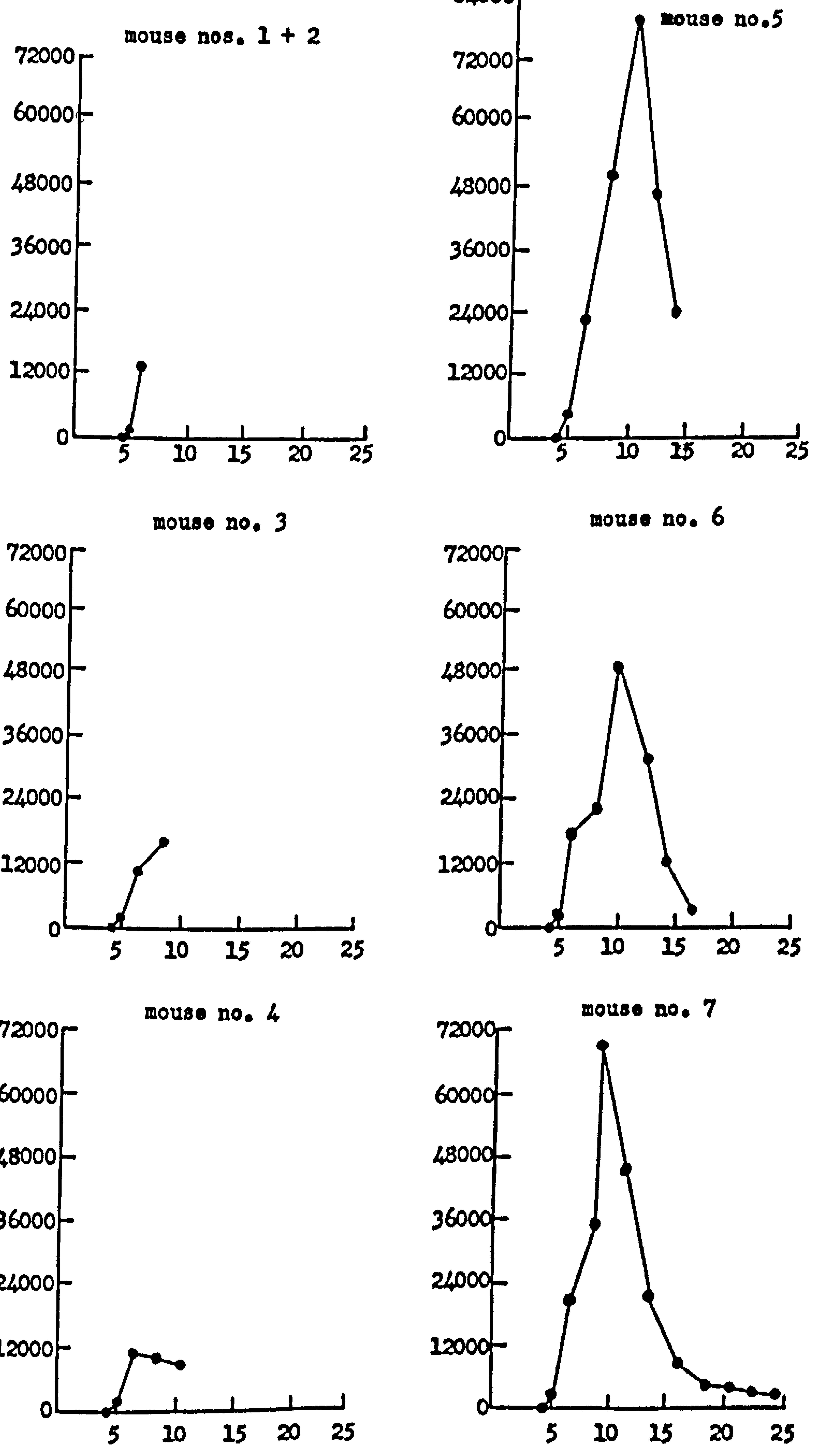

mouse nos. 1 + 2
mouse no. 5
mouse no. 3
mouse no. 6
mouse no. 4
mouse no. 7
7200
6000
4800
3600
2400
1200
0
5
10
15
20
25

Fig. 6.2. Egg counts for individual mice each fed 100 P. elegans metacercarial cysts. One mouse was sacrificed on each of the following days post-infection - 5,7,9,11,14,17 and 25. Eggs per day on vertical axis; days post-infection on horizontal axis. (Days 4 and 5 for all mice in addition to day 14 for mouse no. 5 are of 24h periods only; all other points represent the average egg release for two consecutive 24h periods.)



mouse nos. 1 + 2
72000
60000
48000
36000
24000
12000
0
5
10
15
20
25
mouse no.5
84000
72000
60000
48000
36000
24000
12000
0
5
10
15
20
25
mouse no. 3
72000
60000
48000
36000
24000
12000
0
5
10
15
20
25
mouse no. 6
72000
60000
48000
36000
24000
12000
0
5
10
15
20
25
mouse no. 4
72000
60000
48000
36000
24000
12000
0
5
10
15
20
25
mouse no. 7
72000
60000
48000
36000
24000
12000
0
5
10
15
20
25

Fig. 6.3. Distribution of P. elegans within the small intestine of LACA mice during the course of primary and challenge infections (1x - challenged once; 2x - challenged twice). The horizontal axis represents the distance along the small intestine which was divided into 6 equal sections. The anterior of the intestine is to the left. The vertical axis represents the percentage of the number of worms present per section. Each graph is based on n number of specimens of P. elegans recovered from the given number of mice.

Primary infections

% worms

45 to 60 min.
2 mice n=19

4 & 5 days
4 mice n=127

7 & 8 days
45 mice n=396

% worms

9 days
2 mice n=102

11 days
2 mice n=41

14 days
13 mice n=122

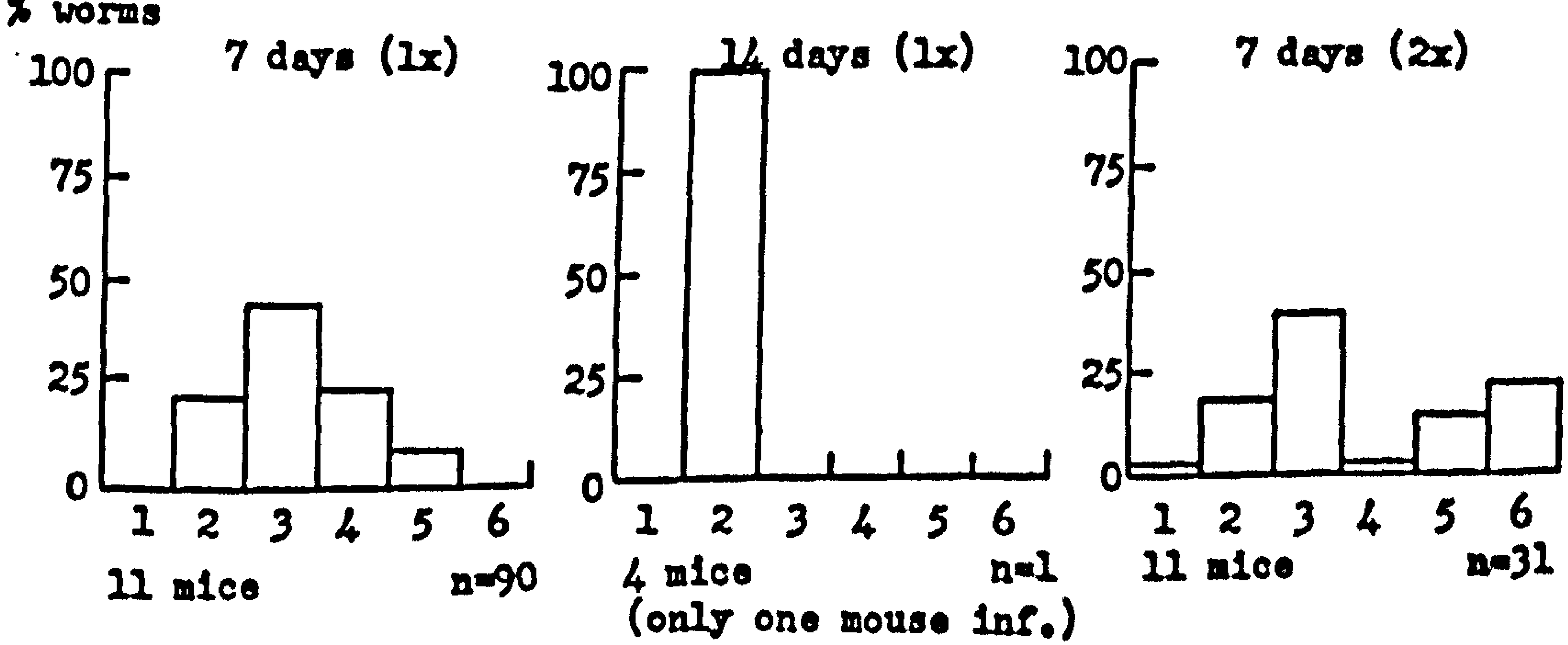
% worms

17 days
2 mice n=19

21 days
13 mice n=10

25 days
7 mice n=32

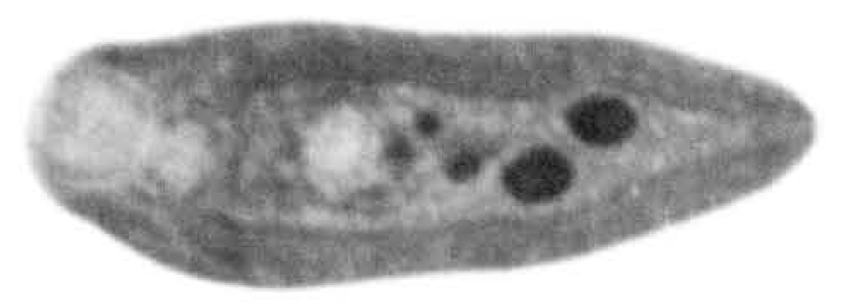
Challenge infections



<u>Plagiorchis</u> <u>elegans</u>: 7 days old. Specimens approaching the mean dimensions for groups of worms harvested from 7 to 11-week old LACA mice. Scale 0.25mm.

Fig. 6.4. Control group (primary infection of 20 cysts/ mouse administered at same time as challenge infection of Fig. 6.5).

Fig. 6.5. Mice challenged once (initial dose 20 cysts/ mouse; challenge dose of 20 cysts/mouse administered 4 weeks later).



<u>Plagiorchis elegans</u> (specimens approaching the mean) recovered from 7-day old infections (10 cysts/mouse) of

Fig. 6.6. 10-month old LACA mice

and

Fig. 6.7. 6-week old LACA mice, demonstrating the effect of host age on parasite development. Scale 0.25mm.

Plagiorchis elegans (specimens approaching the mean for each group) illustrating the difference between

Fig. 6.8. 17-day old flukes (note wasted condition of the reproductive system)

and

Fig. 6.9. 14-day old flukes recovered during the egg count experiments (100 cysts/mouse). Scale 0.25mm.

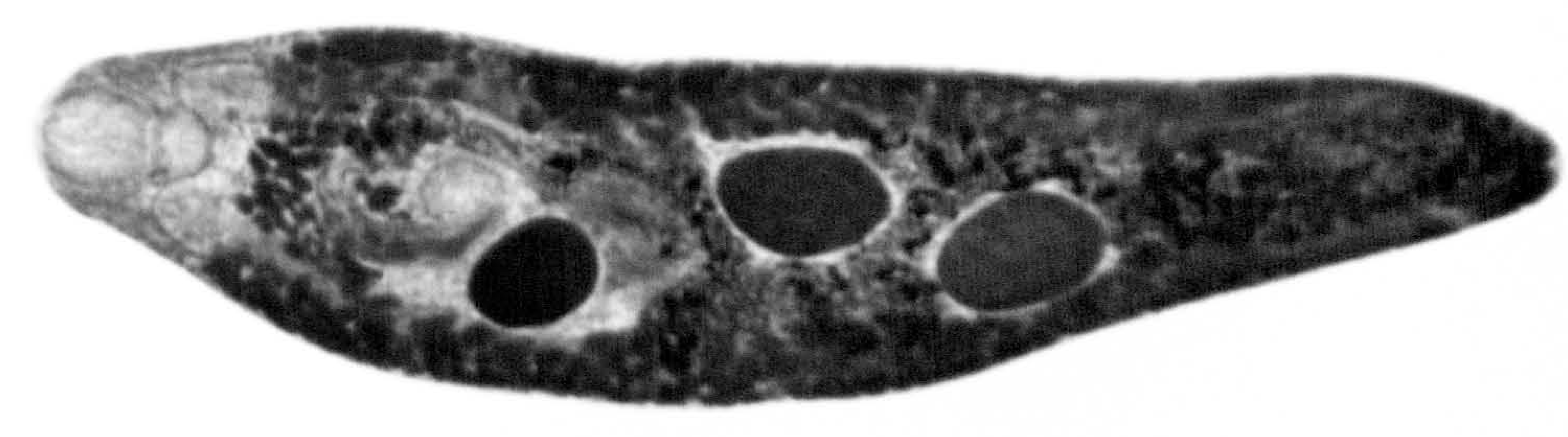
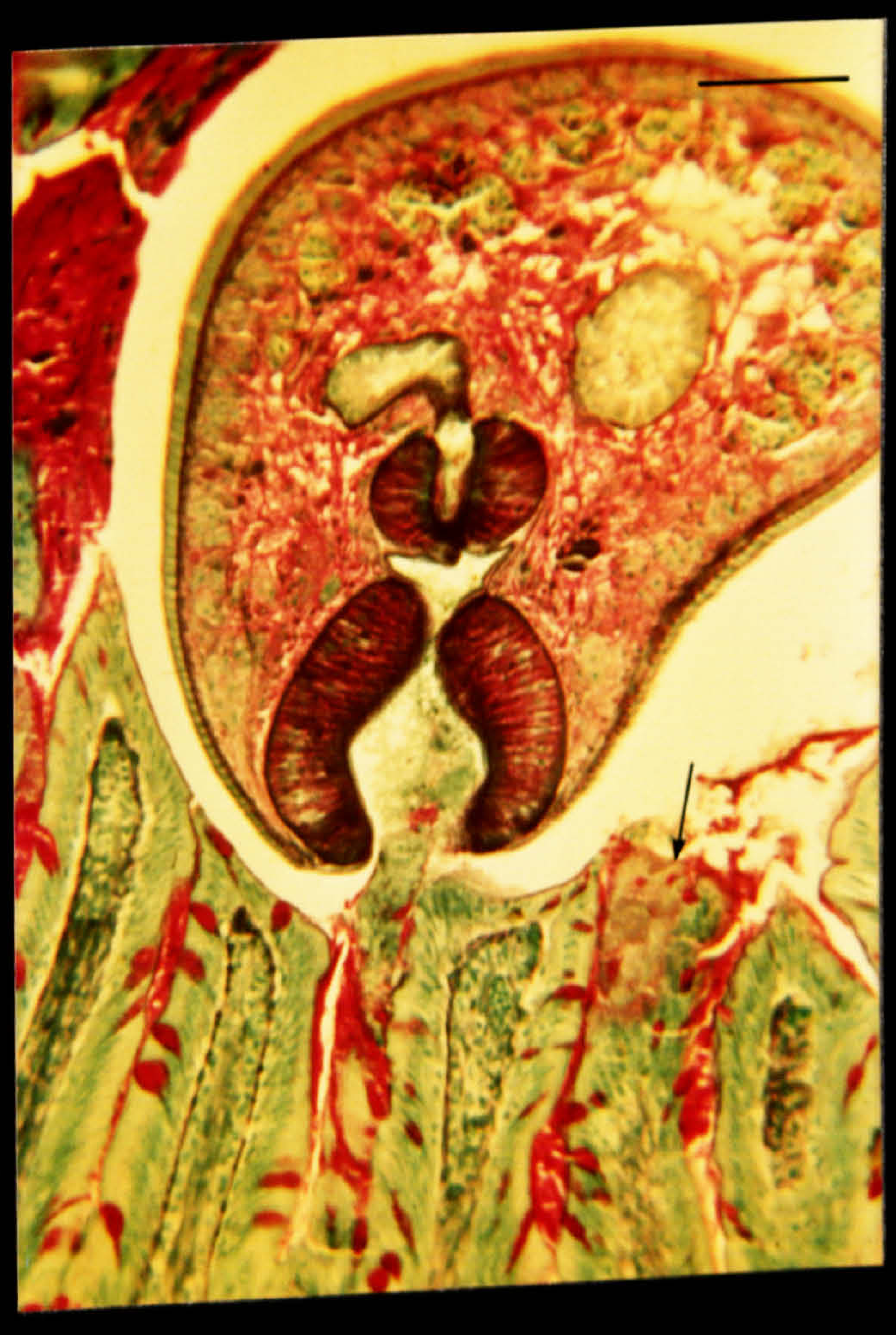


Fig. 6.10. Transverse section (5µm stained with PAS and fast green) through mouse small intestine showing P. *elegans* feeding. Arrow points to probable previous site of grazing. Scale 0.100mm.



Section 7

Transplantation.

INTRODUCTION.

The technique of surgically transplanting helminths from one host to another has been employed by a number of investigators for a variety of reasons. Transplantations have been performed to determine the role of the adult worm in immunity, particularly in instances where the presence of migrating larvae has been implicated in stimulating a host response (Ogilvie, 1965; Smithers & Terry, 1967). Bacha (1962) described a technique for transplanting Zygocotyle lunata from one rat host to another, while Goodchild (1958) after transecting the posterior strobila from specimens of the rat tapeworm Hymenolepis diminuta, successfully transplanted the anterior end into naive rats and noted rapid growth of the cestodes when compared with that of H. diminuta grown from cysticercoids in the primary host. In 1976 Hopkins and Zajac showed that the life span of H. diminuta could be increased by surgically transplanting the worms into naive recipient mice.

As described in Section 6 primary infections of LACA mice are of short duration. Only 40% of the infecting dose is present after 14 days and 12% after 21 days, yet nearly 80% survive for the first 7 days of infection in the mouse. This appears to be a remarkably short life span for a digenetic fluke since some flukes may live for years within their definitive hosts. Clonorchis sinensis is reported to survive for more than 25 years and Schistosoma haematobium for 5 to 18 years in man (Dogiel, 1962); as far as plagiorchids are concerned Plagiorchis cirratus has been reported to survive for an apparent maximum of 62 days in primary infections of white mice (Buttner & Vacher, 1959).

It was considered possible in the present instance that the infected host may exert some deleterious effect, thus bringing about the premature death of the flukes. It is also possible that the mouse is not a normal host of P. elegans and that the short life span of the latter reflects this. In order to determine whether specimens of P. elegans possessed a potential life span longer than that evident in the single mouse host it was decided to utilize a transplantation technique, that is to surgically transplant P. elegans harvested from primary infections of LACA mice into naive recipient mice. Surgical transplantation was also considered to be an appropriate method for determining whether or not -

1) the effects of exposure to the environment of the small intestine of resistant mice, manifested by the stunted growth of those specimens which do survive, are reversible and
2) further growth of specimens recovered from primary infections and transplanted into resistant mice is inhibited.

MATERIALS and METHODS.

Worms were harvested from the small intestine of the initial mouse host, placed in Hedon-fleig solution and incubated at 39°C until they were transplanted. An 18-guage needle with a 5.0cm shaft was placed on a 1.0cm^3 syringe containing 0.20cm^3 Hedon-fleig solution (Lillie, 1965). An 18 guage needle was used because it was sufficiently large for the majority of the worms to be drawn through its aperture without being damaged, but not large enough for the site of injection through the intestinal wall to require suturing. Care was taken to draw up the worms into the shaft of the needle only, not into the barrel of the syringe, where they might be damaged or lost. The mice were anaesthetized with ether. The technique employed was clean but not sterile; the instruments were washed in 70% ethyl alcohol. After swabbing the abdominal surface with 70% ethanol a short longitudinal incision was made through the skin slightly to the left and anterior to the mid point of the abdomen. It was then possible to see the blood vessels in the abdominal wall and to avoid cutting them when making the incision into the body cavity. The small intestine was then gently grasped with forceps and a small portion of it drawn through the incision. The worms were injected directly into the small intestine which was then replaced into the abdominal cavity and the incisions were sutured separately using ethicon 4/0 BP plain surgical catgut. No antibiotics were administered. Following the operation the syringe was rinsed to determine the number of specimens successfully injected. In most cases the mice revived shortly after the operation although on occasion they took longer to recover. The entire operation took approximately 15 to 20 min. per mouse. Prior to the operations the mice were not starved, because it was found to be easier to inject the worms into a small intestine containing food material than one which was empty and whose sides were closely apposed.

RESULTS.

Series I.

In a preliminary experiment 6 naive mice were each infected with 15 metacercarial cysts; 4 of the mice were sacrificed after 12 days, as a source of worms to be transplanted, and the other 2 were maintained as controls (Table 7.1). Up to 10 P. elegans were transplanted into each of 5 naive mice and when the experiment was terminated 7 days later all the worms were 19 days old.

The percentage of transplanted worms present at the end of the experiment was substantially greater than the percentage of the worms of the primary infection surviving in the control mice and the former specimens were significantly larger (by Student's t test) than the latter specimens. In addition it can be seen in Figs. 7.1 and 7.2 that in transplanted P. elegans the vitellaria and gonads are well developed and the uterus is replete with eggs while in those specimens not transplanted the reproductive system is nearly exhausted. The latter is the normal condition of flukes more than approximately 16 days old (pp. 89, 148), when harvested from primary infections of LACA mice.

Series II.

Successive transplants of adult P. elegans were performed at weekly intervals to determine whether, and to what extent, the length of life of P. elegans could be increased. There were no controls for this series of experiments because as stated above adults of primary infections of P. elegans only exceptionally survive in LACA mice beyond 3 weeks. The initial donors were 2 naive mice each infected with 40 cysts; 63 worms were recovered from these primary infections after 7 days and 47 of them were used for the first transplantation. The schedule of transplantation, including the dose/mouse and the number recovered, is given in Table 7.2.

It is apparent that throughout the experiment, except where one mouse died, the majority of worms transplanted were recovered. Three worms were harboured by the mouse that died and despite their appearing moribund they were immediately transplanted into another mouse. Unfortunately all died within 3 days.

On the 56th day after the initial infection and after 7 successive transplants 1 of the 2 remaining worms was fixed for microscopic examination. It measured 2.89mm long and 0.80mm wide. The remaining

Table 7.1. Preliminary experiments transplanting P. elegans into naive recipient LACA mice. Measurements in mm.

source of transpl. worms. no. LACA mice (inf. when 6–8 wks)	cysts/mouse	mean no. worms recov./mouse (12 days old)	total % recov.			
4	15	10.5	70			
no. control mice (inf. when 6–8 wks)		worms rec./ mouse (19 days old)		no. meas.	mean length (1 SE)	mean width (1 SE)
2	15	4 3	23	6	3.15 (0.06)	0.73 (0.02)
			reproductive system nearly exhausted (as normally the case after about 16 days).			
no. recipient mice (8–10 wks old)	worms transpl/mouse (12 days old)					
5	4 9 9 10 10	3 6 9 1 10	69	26	3.35 (0.04)*	0.84 (0.01)*
			vitellaria, gonads and uterus all fully functional.			
			(* Value significantly larger;	Student's t test	p<0.010	p<0.001

Table 7.2. Successive transplantation of P. elegans at weekly intervals into individual naive LACA mice (6 to 10 weeks of age).

age of transpl/worms (days)	recov. at (day)	worms transpl/mouse	worms recov/mouse	total % recovery
0 (40 cysts)	7	0	30 33	78.7
7	14	9 9 9 10 10	6 9 9 10 10	93.6
14	21	3 5 5 6	1 4 5 5	78.9
21	28	4 4 4	4 4 4	100.0
28	35	3 3 (3 died	3 2 -)	83.3
35	42	2 2	1 1	50.0
42	49	1 1	1 1	100.0
49	56	1 1	1 1 fixed	100.0
56	63	1	1 fixed	100.0

specimen was transplanted for the 8th time and after a further week, that is on the 63rd day after the initial infection, it too was fixed; it was 2.88mm long and 0.79mm wide.

Figures 7.3 to 7.6 clearly show that the transplanted specimens, 56 and 63 days old, are in better condition than untransplanted P. elegans even when 14 and 25 days old. Although there are relatively few eggs in the uterus of the 56 day old P. elegans the gonads and vitellaria are still well developed. The uterus of the 63 day old specimen contains numerous apparently normal eggs and it is evident that the gonads are well developed despite the fact that the testes are partially

obscured by the darkly staining vitellaria. As shown in Fig. 7.3 the vitellaria are not confined to distinct follicles along the lateral sides of the body, but rather are nearly a confluent mass of yolk glands extending from the anterior border of the ventralsucker to the posterior extremity along the left side of the fluke; the right side appears to have developed normally. It was noted by the writer that the vitellaria of this specimen were abnormal when it was recovered after 7 days from its initial mouse host. There is no evidence to support abnormal development of the vitellaria either with increased age or as a result of surgical transplantation. Because of their robust condition it is believed that the 2 transplanted worms would have survived beyond the 8 and 9 weeks for 2 or possibly more weeks within the same mouse and probably even longer if the transplants had been continued.

<u>Series III</u>.

The primary purpose of this series of experiments was to determine whether or not specimens involved in challenge infections suffer damage which is irreversible.

Nine resistant mice, previously infected twice with 20 cysts/ infection, were each fed 40 cysts 12 weeks after the initial infection. Forty-four worms (22% of the cysts administered) were recovered; 38 of them were transplanted into 4 naive mice, 8 to 10 per mouse. Seven days later the mice were sacrificed. As shown in Table 7.3, all but one (97%) of the transplanted worms were recovered as opposed to only 7% of the inoculating dose given to the resistant mice and the transplanted worms are significantly larger than the controls (Figs. 7.7, 7.8). On the basis of their general appearance it is often not possible to distinguish the transplanted specimens from 14-day old *P. elegans* recovered from mice with no previous experience of infection, although in some instances the former worms are in a more robust condition than the latter.

As a result of these experiments it can be said that at least within the first 7 days of challenge infections, even if damage is sustained by the worms, it can be reversed by transplanting them into naive recipient mice.

<u>Series IV</u>.

This series of experiments was conducted to investigate the effect of resistant recipient mice on adult specimens of *P. elegans* trans-

Table 7.3. Transplantation of 7-day old *P. elegans* from resistant donors into naive recipient mice (resistant mice previously inoculated with 2 infections, each of 20 cysts). Measurements in mm.

source of transpl worms no. mice (18-22 wks old)	cysts/mouse/inf. (wks between infec.)	worms recov./mouse	total no. worms rec.	% recov.		
5	20; 20; 40 (0; 4; 8)	8.8	44	22		
no. recipient mice (16-20 wks old)	**worms transpl/mouse (7 days old)**	**worms rec/mouse (14 days old)**	**total % recov.**	**no. meas.**	**mean length (1 SE)**	**mean width (1 SE)**
4	8 10 10 10	7 10 10 10	97	33	2.72 (0.02)*	0.69 (0.01)*
controls no. resistant mice (18-22 wks old)	**cysts/mouse**	**worms recov./mouse (14 days old)**				
4	40	0 1 1 9	7	9	1.97 (0.15)	0.54 (0.04)

* (Value significantly larger than control; Student's *t* test $p < 0.001$)

planted from primary infections.

Five adult worms, 7 days old, were transplanted into each of 3 naive and 4 resistant recipient mice. The infections were allowed to continue for a week after which the mice were sacrificed.(Table 7.4). Although a large percentage of the specimens transplanted survived in both the naive and resistant mice, P. elegans recovered from the former hosts were significantly larger and the reproductive system is better developed than in those specimens recovered from the latter hosts (Fig. 7.9, 7.10).

It is evident from these data that growth continues for a time when P. elegans is transplanted into naive mice, but further growth is inhibited when P. elegans is transplanted into resistant mice.

Table 7.4. Transplantation of 7-day old *P. elegans* recovered from primary infections of LACA mice into naive and resistant recipient mice. Measurements in mm.

source of transpl. worms no. mice (6–8 wks old)	cysts/mouse	mean no. rec/mouse	total % rec.			
4	10	9.0	90			
recipient mice (age in wks)						
no. naive	no. worms transpl/mouse	no. worms rec/mouse	total % rec.	no. meas.	mean length (1SE)	mean width (1 SE)
3 (16–18)	5	5 5 4	93	13	2.92 (0.06)*	0.80 (0.02)+
no. resistant						
4 (16–18)	5	1 5 4 5	75	14	2.57 (0.07)	0.68 (0.02)

each resistant mouse previously infected twice – 20 cysts/infection. 0; 4; 6 weeks between the infections.

Student's *t* test * and + significantly larger. * $p < 0.002$ + $p < 0.001$

DISCUSSION.

These investigations have helped to clarify several aspects of the host-parasite relationship between *P. elegans* and LACA mice. It has been demonstrated that transplanting adult *P. elegans* at weekly intervals can extend the life span of *P. elegans* to at least 63 days, while primary infections do not often exceed 21 days (p. 135) although most worms survive for about 7 days. It is therefore postulated that primary infections of *P. elegans* are terminated in LACA mice by a host response and that 7 days is not a sufficient length of time to elicit this response. It might also be suggested that initial infections are of short duration because the dietary requirements of *P. elegans* are not satisfied within the mouse small intestine. This seems unlikely however in light of the transplantation results, since all the mice had been provided with food and water *ad libitum* and were of the same laboratory-bred strain. However the role of the diet in primary infections.has yet to be investigated.

When the five resistant donor mice for Series III experiments were sacrificed only 44 worms (22%) of the inoculating dose were present, yet when 38 of them were transplanted into naive recipient mice 97% (37 worms) were recovered 7 days later. Perhaps the 44 specimens survived initially in the donor challenged mice because they themselves were more resistant to the effects of the hostile environment of the small intestine than the 156 specimens that did not survive the week. Another factor which may have contributed to their success when transplanted is their size. As explained above an 18 guage needle was used throughout these experiments because its aperture is large enough to accommodate the worms without damaging them while not producing a hole in the intestine wall large enough to require suturing. Because the worms were stunted it was very easy to draw them into the needle without their being damaged either within the shaft or on the sharpened edges of the tip.

It is interesting to note that a large percentage of the specimens harvested from primary infections and transplanted into resistant mice were present after a week, although their continued growth had been inhibited. Again it may be that be selecting week old specimens for the transplants a more resistant worm population was used and that when challenged it required several days for a response to be elicited in the mice.

A number of factors may limit the success of the transplantations. The worms themselves may be injured either when being harvested or transplanted, particularly when being drawn up into the needle shaft as mentioned above. To avoid damaging the worms when recovering them, the small intestine was divided into 6 sections and rather than immediately slitting the sections longitudinally, the intestinal contents, including most of the worms present were first squeezed into a petri dish; any specimens remaining could then be seen through the gut wall and were easily removed intact. The possibility of the worms being damaged when being drawn into the shaft of the needle increased with age because of their larger size. On a number of occasions when in the process of drawing large specimens into the needle they were observed to be trapped part in, part out, of the shaft and in danger of being caught on the sharp edges of the needle tip. It is very probable that if injuries were sustained by the worms they were incurred at this stage. Smithers and Terry (1967) successfully transplanted "half-worms" of Schistosoma mansoni which in some instances eventually produced eggs, but they also noted that "damaged worms" were not so capable of surviving as intact adults. The continuing health of the host itself is of course a prerequisite for the survival of the transplanted worm burden.

The results of these experiments support the hypothesis that infections of P. elegans in LACA mice are terminated by a host response.

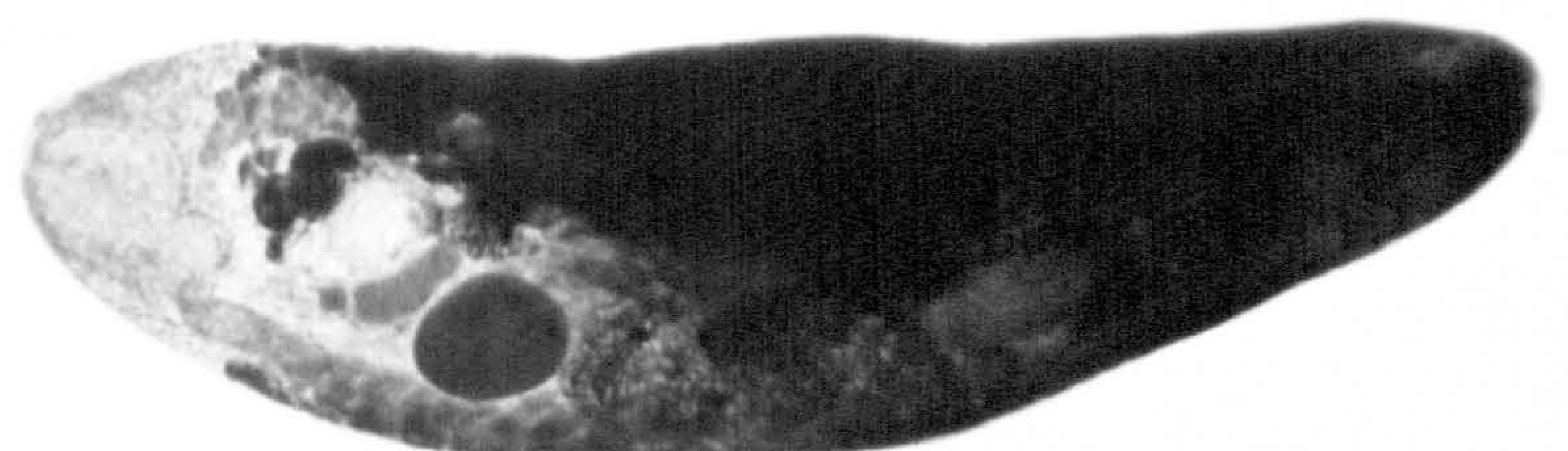
Comparison of transplanted and untransplanted specimens of *P. elegans* both 19 days old, recovered from LACA mice and approaching the mean dimensions for each group. Scale 0.5mm.

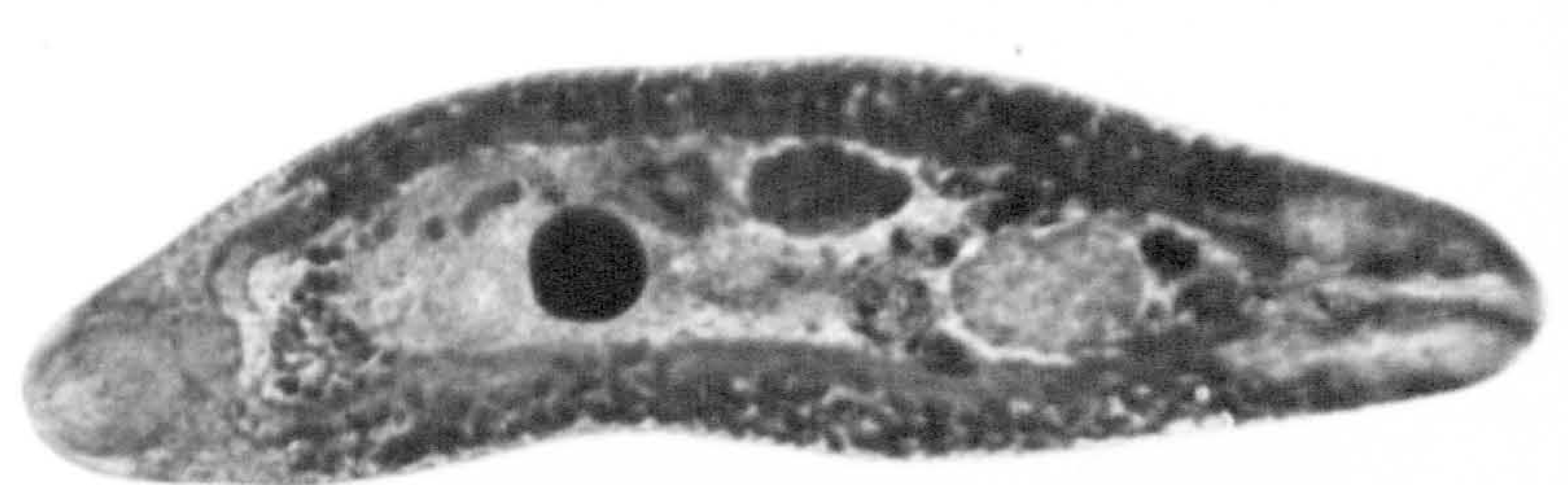
Fig. 7.1. Specimen not transplanted.

Fig. 7.2. Transplanted from a 12-day old primary infection into a naive recipient mouse.

Fig. 7.3. *Plagiorchis elegans* 63 days old surgically transplanted at weekly intervals for 8 weeks into naive recipient mice. (The vitellaria of this specimen developed irregularly during the first 7 days in the initial host.) Scale 0.5mm.

Fig. 7.4. *Plagiorchis elegans* surgically transplanted at weekly intervals for 7 weeks into naive recipient mice. Recovered and fixed when 56 days old. Scale 0.5mm.

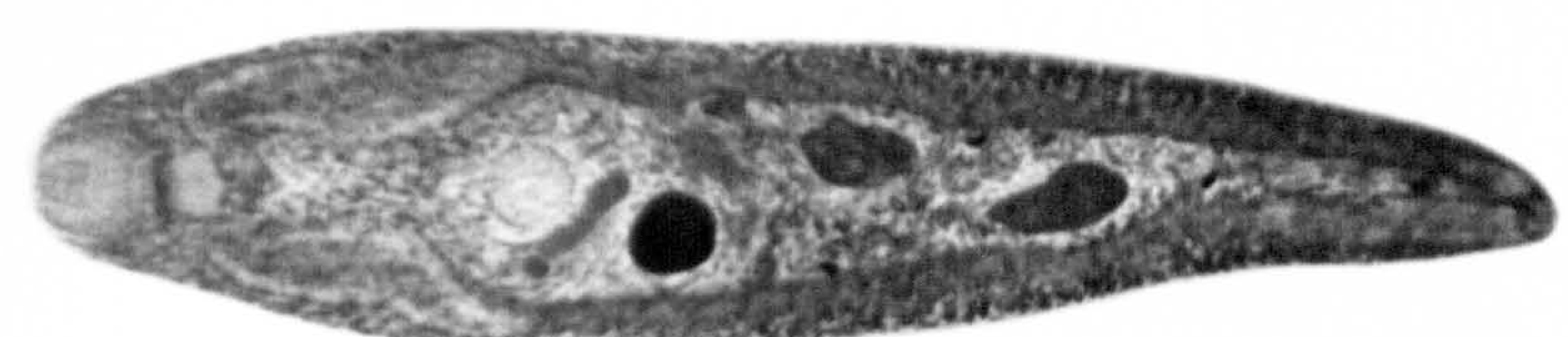




Specimens of P. elegans recovered from primary infections of LACA mice illustrating the atrophied condition of worms when only 14 and 25 days old. (Compare with Figs. 7.3 and 7.4). Scale 0.5mm.

Fig. 7.5. Plagiorchis elegans 25 days old.

Fig. 7.6. Plagiorchis elegans 14 days old.



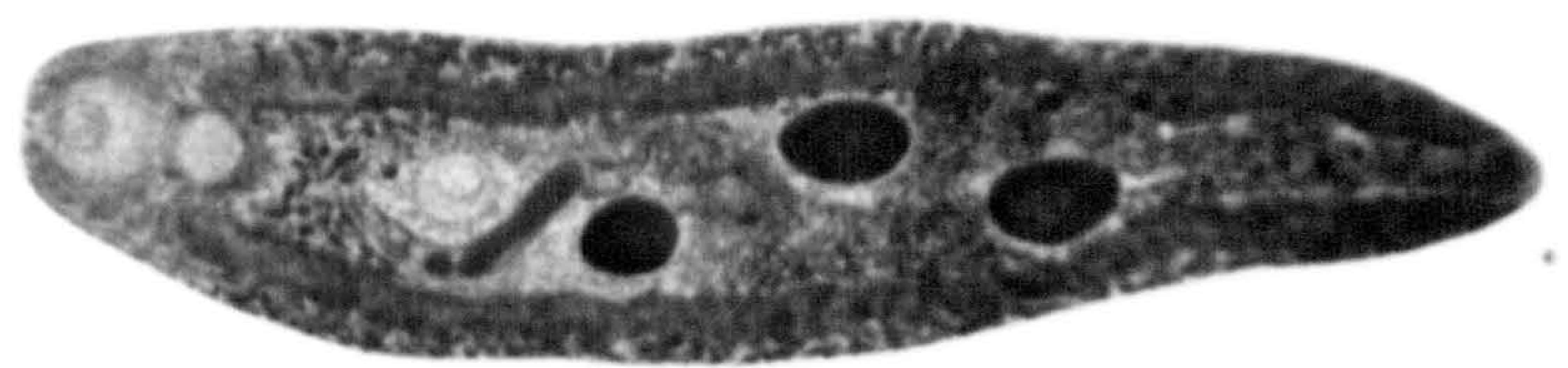
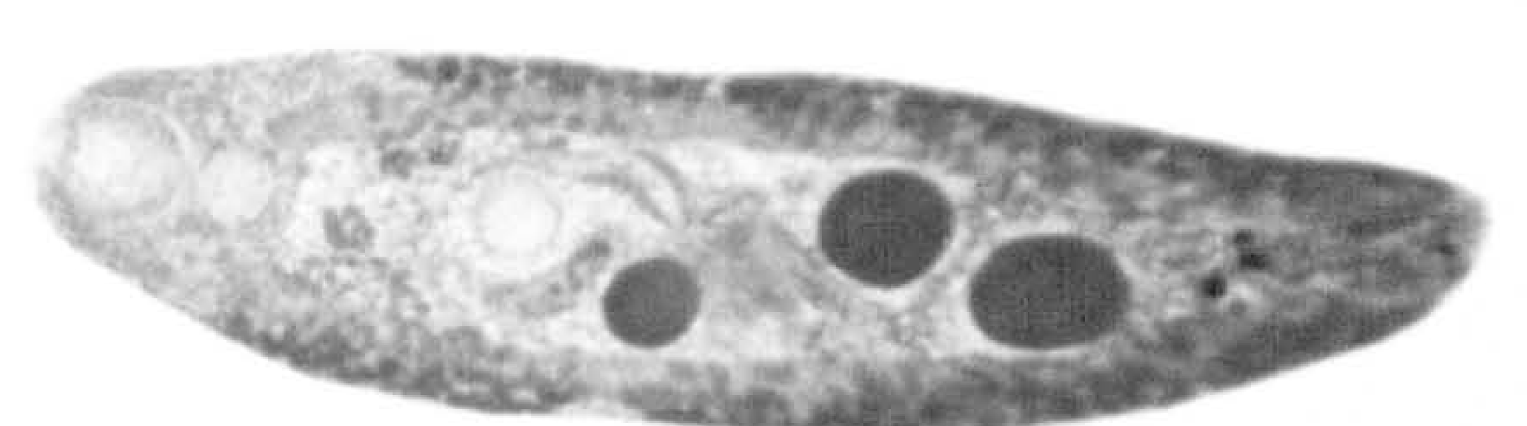


Fig. 7.7. *Plagiorchis elegans* maintained for 14 days in resistant mice (previously infected twice with 20 cysts per infection). Scale 0.5mm.

Fig. 7.8. *Plagiorchis elegans* 14 days old recovered from resistant mice (previously infected twice with 20 cysts per infection) after 7 days and transplanted into naive recipient mice. Scale 0.5mm.

Specimens of both Figs. 7.7 and 7.8 approach the mean dimensions for each group.



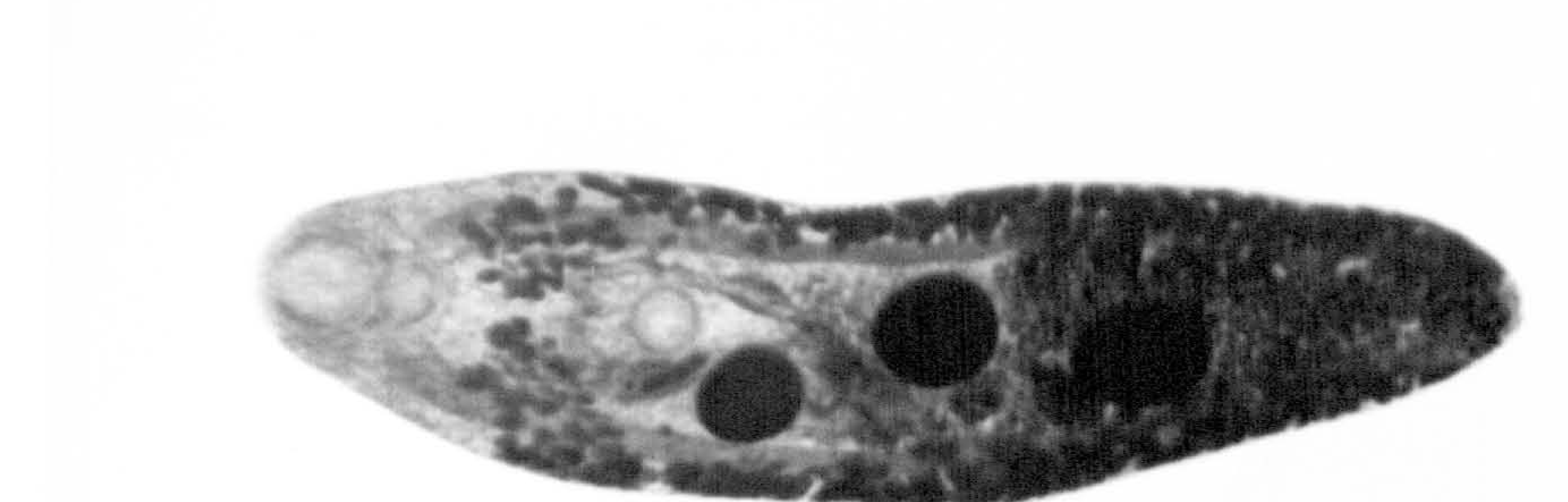
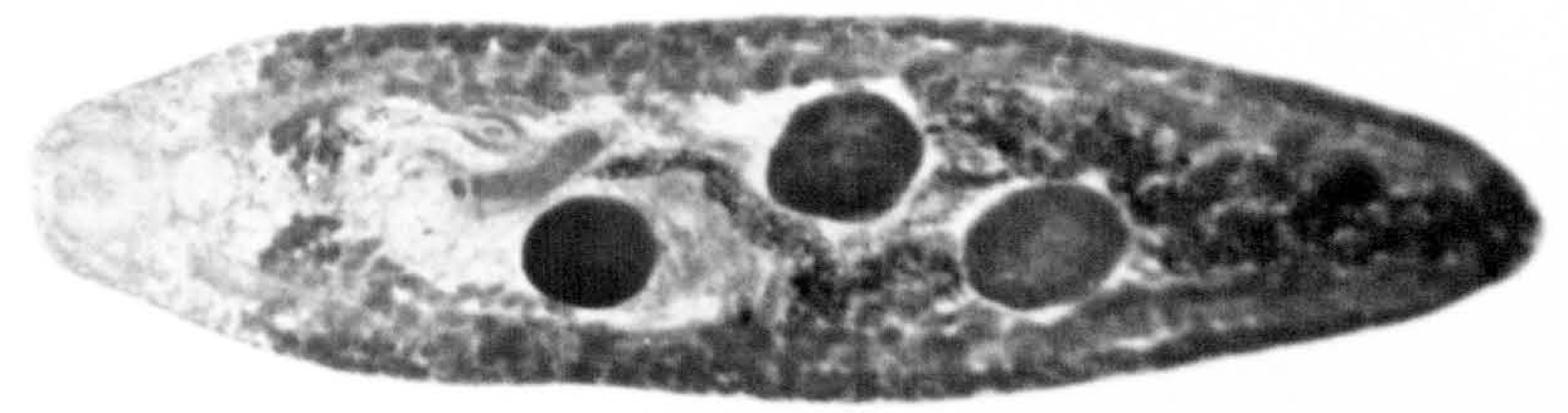


Fig. 7.9. Plagiorchis elegans transplanted from 7-day old primary infections of LACA mice into resistant recipient mice and sacrificed after a week; illustrating the inhibitory effects of resistant mice on the further development of the parasite. Specimen approaches the mean dimensions for the group. Scale 0.5mm.

Fig. 7.10. Plagiorchis elegans transplanted from 7-day old primary infections of LACA mice into naive recipient mice and sacrificed after a week; illustrating the continued development of the flukes. Specimen approaches the mean dimensions for the group. Scale 0.5mm.



Section 8

Statistical analysis of the affect of the host species on the morphology of *Plagiorchis elegans*.

INTRODUCTION.

Few investigators of the genus *Plagiorchis* have either suggested or demonstrated that variation in adult morphology is host induced. Blankespoor (1974), working with an experimental strain of *P. noblei*, showed that changes in body, gonad size and the posterior extent of the uterus occurred regularly depending upon the species of second intermediate host; he also stated that the final host may affect the rate of parasite development. Gupta (1963) and Blankespoor (1974) attributed morphological differences to the definitive hosts in which specimens of *P. proximus* and *P. noblei* respectively developed. In contrast McMullen (1937b) found no apparent differences in specimens of *P. muris* harvested from experimentally infected rats, mice and pigeons; however adults of the same age from pigeons were on average 0.5mm longer than those from the rodent hosts. While Groschaft and Tenora (1974) provided experimental evidence implicating neither the second intermediate nor final host in morphological variation of *P. vespertilionis* and *P. koreanus*, they did observe that the dimensions of the gonads varied directly with the degree of maturity of the flukes.

A pure strain of *P. elegans* was established by the author and the life cycle was completed using various intermediate and final hosts. The specimens recovered from the definitive hosts are compared, according to their gross morphology, in Sections 5 and 6, while in this section they are compared statistically. The method chosen for this comparison is canonical variate (or discriminant) analysis (Seal, 1964; Sadler, 1977). This technique has been employed for studies concerning, for example, incipient speciation (Delany and Healy, 1964) and the influence of the host species on parasite morphology (Sadler, 1977).

MATERIALS and METHODS.

Specimens of the laboratory established strain of *P. elegans* which were harvested from the definitive host are compared in this analysis. They were obtained by completing the life cycle using various combinations of first, second and final hosts and by giving LACA mice one or two challenge infections.

The dose and age of all metacercarial cysts used were known except for those where *Lymnaea stagnalis* served as the second intermedi-

ate host, and here their age could not be determined. For the comparison of specimens derived from different hosts the duration of infection was standardized at seven days. Additional specimens from mice (4,14 and 21 days old) and from rats (14,21 and 28 days old) were included to determine the affect of age on the morphology of P. elegans.

All parasites were prepared by the same method. They were individually flattened under the slight pressure of a cover-slip, heat-killed and fixed in 10% formol saline, stained with alum carmine and mounted in XAM (Gurr's). Half of the specimens were orientated to show the ventral side and the remainder the dorsal surface.

Canonical variate analysis is a multivariate technique which is based on the measurements of a number of morphological characters of the groups being studied. From the data a series of eigenvectors is calculated; these combine and transform the original variables, which may be correlated, to produce a new set of uncorrelated canonical variates. These variates define axes in multidimensional space and are derived so that the first transformed axis is inclined in the direction of greatest variability between group means, the next axis is inclined in the direction of next greatest variability and subsequent axes continue in like manner. The transformation matrix maximizes the between variance of the groups, which are then as distinct as possible considering the original variables. Because the end result of such a maximization would be an infinite set of components, the variates must be uncorrelated (have zero covariance) and each be of unit variance. So when the groups are plotted on any of the canonical axes, each group has unit standard deviation.

The I.C.L. 1900 Series Statistical Analysis, Mark 2 programme was used for this discriminant analysis. This programme produced the following data: group centroid vectors, eigenvalues, eigenvectors and group covariance matrices on the canonical axes. The group centroid vectors are the mean discriminant scores for each group on the respective canonical axes. To transform each of them to canonical form, that is having unit standard deviation, they were divided by the pooled estimate of standard deviation of the groups in discriminant space. The eigenvalues provide a measure of the relative importance of each canonical variate. Taken together they measure the total variance present in the canonical variates, so when a single eigenvalue is considered as a percentage of the whole it is possible to judge the importance of the corresponding variate. Eigenvectors on the other

hand provide a measure of the relative contribution of each variable to its associated canonical variate.

Measurements made for this analysis and which are considered important in the erection of new species of *Plagiorchis* by most authors are (see Fig. 8.1):

1. body length
2. body width
3. oral sucker length
4. oral sucker width
5. ventral sucker length
6. ventral sucker width
7. ovary length
8. ovary width
9. anterior testis length
10. anterior testis width
11. posterior testis length
12. posterior testis width
13. pharynx length
14. pharynx width
15. preacetabular distance

The preacetabular distance was measured from the mid point of the oral sucker to the mid point of the ventral sucker because the oral sucker may be terminal or subterminal as a result of fixation. Occasionally it is difficult to measure the oral sucker when it is terminal because its anterior margin is not clearly defined. Although the dimensions of the cirrus sac would provide valuable comparative measurements, it is too frequently distorted by fixation (p. 97, 127) to be included in this analysis. Egg dimensions were also omitted because a number of specimens were immature.

To determine the reproducibility of the results two groups of seven-day old specimens from LACA mice, which had been infected three months apart according to the same method, are compared.

RESULTS.

Inspection of the eigenvalues given in Table 8.1 shows that the first two canonical variates absorb most of the variation (80%) and as a result provide significant discrimination of the groups. Table 8.2 lists the eigenvectors of the variates. While examining the im-

portance of each of the variables to the canonical variates, the fact that body length (BL) is considerably larger than the other variables should be taken into account.

Table 8.1. Eigenvalues and percentage of total variation absorbed by each.

canonical variate	eigenvalue	percentage of total variation
1	8.2005	49.63
2	5.0279	30.43
3	0.8379	5.07
4	0.6833	4.17
5	0.4943	2.99
6	0.3843	2.32
7	0.2666	1.61
8	0.2204	1.33
9	0.1283	0.78
10	0.1044	0.63
11	0.0599	0.36

In producing maximum separation of the groups (see Fig. 8.2 and Table 8.2), the first variate contrasts ventral sucker width (VSW) [oral sucker length (OSL), ventral sucker length (VSL) and ovary width (OVW) contributing to a lesser extent] with oral sucker width (OSW), pharynx length (PHL) and pharynx width (PHW). Variate 2 discriminates among the groups largely on the basis of the non-reproductive and reproductive structures, thus contrasting body length (BL), body width (BW), oral sucker length and width (OSL and OSW respectively) and ventral sucker length (VSL) with ovary length (OVL), anterior testis width (ATW), posterior testis width (PTW), pharynx width (PHW) and preacetabular distance (PRE) [ovary width (OVW), posterior testis length (PTL) and pharynx length (PHL) also contribute to a lesser extent].

The standard deviations of most of the groups (Table 8.3) on the canonical variates approach unity. However, on the first variate groups 11 (specimens recovered from LACA mice challenged once) and

21 (pigeons) displayed considerable variation (s = 1.82 and 1.42 respectively), while on the same variate, groups 2 (LACA mice), 18 (gerbils) and 20 (ducklings), all comprising seven-day old specimens, exhibited very little scattering (s = 0.33, 0.36 and 0.33 respectively) as did group 7 (four-day old parasites from mice) on the second variate (s = 0.58).

Table 8.2. The eigenvectors on the first two canonical variates.

variable	canonical variate 1	canonical variate 2
BL	0.0323	-0.1012
BW	0.0035	-0.3818
OSL	0.4261	-1.9990
OSW	-0.5612	-1.9502
VSL	0.8765	-1.1239
VSW	2.3520	-0.1636
OVL	0.1591	0.5534
OVW	0.6881	0.2324
ATL	0.0564	0.1035
ATW	0.0487	0.9470
PTL	0.1175	0.3442
PTW	-0.0500	0.7460
PHL	-0.3278	0.3522
PHW	-0.2702	0.5983
PRE	-0.0577	0.3217

Table 8.3. The standard deviations of the group centroids in discriminant space.

group	canonical variate 1	canonical variate 2
1	0.79	1.15
2	0.33	0.70
3	0.74	0.94
4	0.75	1.02
5	0.93	1.06
6	0.56	0.79
7	0.87	0.58
8	1.08	1.35
9	0.97	1.27
10	1.06	1.01
11	1.82	1.09
12	0.69	0.68
13	1.09	0.73
14	1.11	0.77
15	0.72	0.94
16	1.07	0.92
17	1.11	1.24
18	0.36	1.24
19	1.17	0.80
20	0.33	0.72
21	1.42	1.06
22	1.13	1.06

DISCUSSION.

It is apparent that although a single species was involved in this analysis considerable and frequently complete separation of the groups was accomplished. While it may be expected that samples of a single *Plagiorchis* species recovered from different final hosts are morphologically distinct (Rees, 1952; Gupta, 1963; Blankespoor, 1974), it is of particular interest that those seven-day old specimens recovered from LACA mice (groups 1, 2, 3, 4, 5, 6 and 10), regardless of the intermediate host species, form a definite cluster. These find-

ings contrast with those of Blankespoor (1974), who reported that the species of second intermediate host affects the morphology of adult P. noblei in a regular manner. A notable exception however is group 12; as discussed in Section 6 (pp. 143-150) these specimens were harvested from old LACA mice and the age of the host is believed to have contributed to their slow development. The seven-day old control groups 1 and 2, although separated by 1.1 units on the second variate are only 0.3 units apart on the first variate. Several factors may have affected these results, including the age of the infection in the first intermediate host (Lymnaea stagnalis - a single individual being the source of cercariae), the batch of chironomid larvae, generation of LACA mice and technique of the experimenter. Considering these possible influences, the differences between the groups are not thought to be substantial.

Because the group centroids are scattered across Figure 8.2, it appears that the morphology of P. elegans has been affected by the species of final host. However, nearly as much, or more, variation is exhibited by parasites of different ages within the same host species (variate 1: 6.5 units between mouse groups 7 and 9; 3.9 units between rat groups 14 and 17; variate 2: 8.3 and 6.4 units respectively) as between those from different species of final host when the parasites are the same age (variate 1: 6.6 units between groups 20 and 18; variate 2: 3.8 units between groups 22 and 3). It is however evident that four-day old specimens (group 7) contribute most of the variation among the mouse groups of different ages on the first canonical variate (6.0 units), while on the second canonical variate three of the four age groups are quite separate, reflecting variation between the condition of the reproductive and non-reproductive structures. Yet it is of note that mouse group 7 (four-days old) is morphologically indistinct from group 1 (seven-days old) on variate 2, being separated by only 0.7 units, although none of the former specimens were mature. The outcome changes when parasite age is again the factor being considered but the species of final host is altered. Seven, 14, 21 and 28-day old specimens from rats (groups 14, 15, 16 and 17 respectively) differ from each other on both canonical variates, more especially on the second (a total of 6.4 units) than on the first (3.9 units). But variation on the latter variate is in the same direction as in groups from the mouse final host.

Whether the mouse host receives one or two challenge infections has little effect on the morphology of the flukes recovered, as evi-

denced by the proximity of groups 11 and 13 respectively on both canonical variates. However these groups are substantially different from all those of primary infections of mice on variate 1 except group 7 (1.2 units) and on variate 2 group 11 overlaps group 4 (G. pulex = second intermediate host) and group 13 overlaps group 12 (immunity control, old mice).

Examination of the spread of the group centroids from different final hosts (intermediate hosts and age of parasites being constant) reveals that most of the non-mouse groups bear a close morphological similarity to one or more mouse groups. The group from ducklings (group 20) is separated from that of second challenge infections (group 13) by only 1.1 units on the first variate and is not distinct from it on the second; that from rats (group 14) is similar to the immunity controls (group 12) on both variates, while the group from gerbils (group 18) is found in the midst of the cluster of seven-day old mouse groups. The samples from hamsters, pigeons and chicks (groups 19, 21 and 22 respectively) are intermediate in position between the immunity control groups 10 and 12 on both variates.

In conclusion it is considered probable that the final host, rather than exerting an effect on the morphology of P. elegans, influences its rate of development, thereby producing worms that may appear to be different because they have attained various degrees of maturity. The question of the final host inducing morphological variation in the adult parasite is not entirely resolved however, since groups more than seven days old from rats compared with groups of comparable age from mice remain distinct from mouse worms along the first canonical variate.

Fig. 8.1. Measurements of Plagiorchis elegans included in the analysis:

BL	body length
BW	body width (widest part of specimen)
OSL	oral sucker length
OSW	oral sucker width
VSL	ventral sucker length
VSW	ventral sucker width
OVL	ovary length
OVW	ovary width
ATL	anterior testis length
ATW	anterior testis width
PTL	posterior testis length
PTW	posterior testis width
PHL	pharynx length
PHW	pharynx width
PRE	preacetabular distance

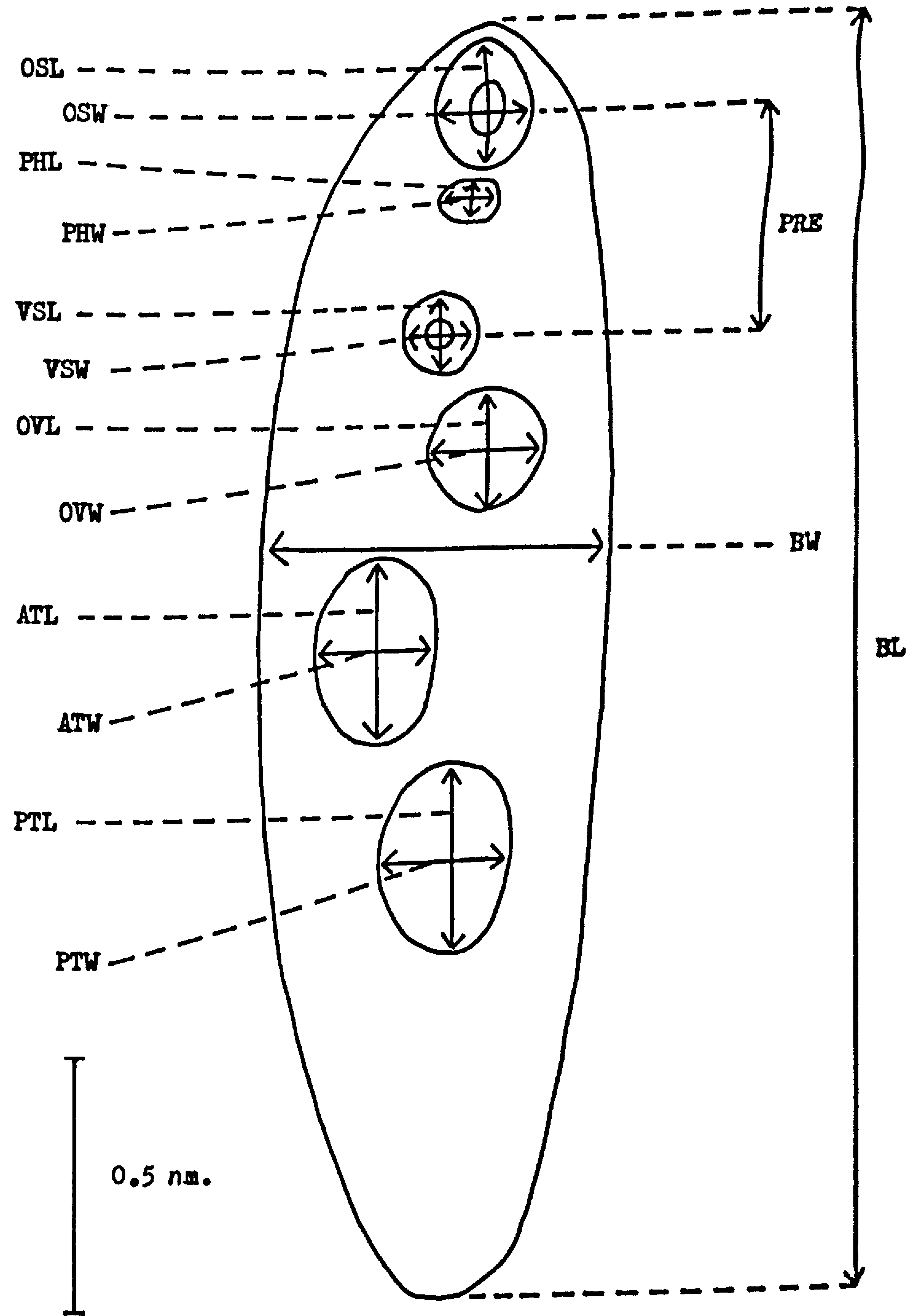

OSL
OSW
PHL
PHW
VSL
VSW
OVL
OVW
ATL
ATW
PTL
PTW
PRE
BW
BL
0.5 nm.

Fig. 8.2. Group centroids projected onto the first two canonical axes. The radius of each circle surrounding the group centroids is one standard error. Numbering of the groups and a summary of their respective life cycles are as follows:

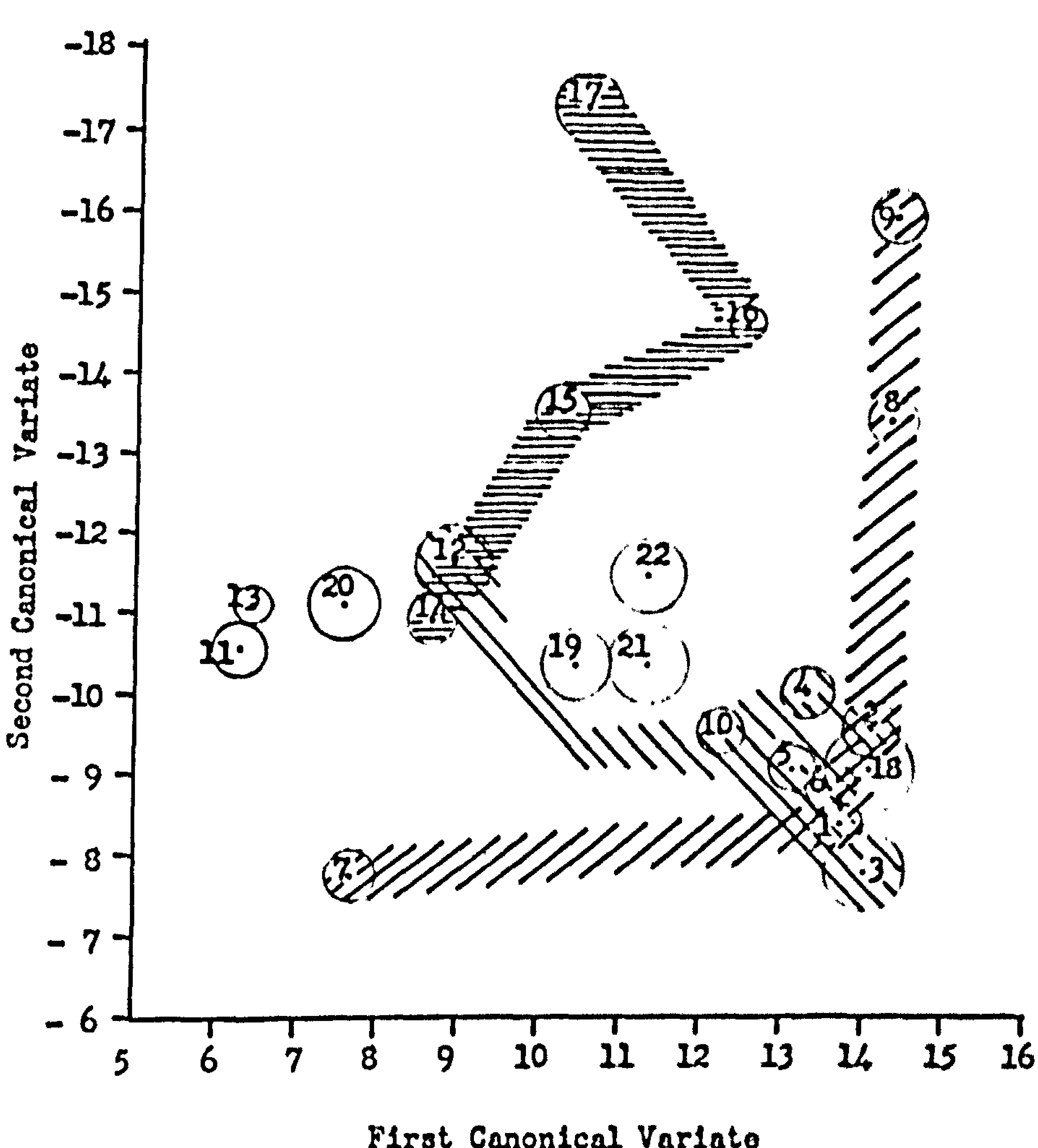
group	n	age in days	hosts: final	hosts: intermediate: second	hosts: intermediate: first
1	28	7	LACA mice	Chironomus sp.	L. stagnalis
2	16	7	LACA mice	Chironomus sp.	L. stagnalis
3	9	7	LACA mice	L. stagnalis	L. stagnalis
4	9	7	LACA mice	G. pulex	L. stagnalis
5	27	7	LACA mice	A. aquaticus	L. stagnalis
6	29	7	LACA mice	Chironomus sp.	L. palustris
7	19	4	LACA mice	Chironomus sp.	L. stagnalis
8	40	14	LACA mice	Chironomus sp.	L. stagnalis
9	8	21	LACA mice	Chironomus sp.	L. stagnalis
10	46	7	" (control)*	Chironomus sp.	L. stagnalis
11	28	7	" challenged*	Chironomus sp.	L. stagnalis
12	11	7	" (control)+	Chironomus sp.	L. stagnalis
13	23	7	" challenged+	Chironomus sp.	L. stagnalis
14	27	7	rat	Chironomus sp.	L. stagnalis
15	14	14	rat	Chironomus sp.	L. stagnalis
16	19	21	rat	Chironomus sp.	L. stagnalis
17	6	28	rat	Chironomus sp.	L. stagnalis
18	7	7	gerbils	Chironomus sp.	L. stagnalis
19	10	7	hamsters	Chironomus sp.	L. stagnalis
20	7	7	ducklings	Chironomus sp.	L. stagnalis
21	7	7	pigeons	Chironomus sp.	L. stagnalis
22	10	7	chicks	Chironomus sp.	L. stagnalis

* Group 10 = control group for LACA mice challenged once.

* Group 11 = LACA mice challenged once.

+ Group 12 = control group for LACA mice challenged twice.

+ Group 13 = LACA mice challenged twice.



Shading connects:

\\\\\ groups of seven-day old specimens of primary infections of LACA mice.

//// groups of specimens recovered from LACA mice after various periods of infection. (intermediate hosts remaining constant)

═══ groups of specimens from laboratory rats after various periods of infection. (intermediate hosts remaining constant)

Section 9

Synonymy and geographical distribution.

Synonymy.

In a previous study concerning the taxonomy of P. elegans Styczynska-Jurewicz (1962) found considerable variation in such morphoanatomical features as the shape and position of the internal organs, their size and the rate at which the body is covered with spines. Moreover she noted that some apparent taxonomic characters were artifacts, for example, the apparent lack of a prepharynx in fixed preparations; occasionally the prepharynx contracts during fixation and is thus no longer visible after fixation. As a result of her investigations she added to the existing list of synonyms of P. elegans (p. 8) the following species:

P. cirratus (Rud. 1802)
P. blumbergi Massino, 1927
P. brauni Massino, 1927
P. loossi Massino, 1927
P. massino Petrov & Tichonoff, 1927
P. uhlwormi Massino, 1927
P. potanini Skrjabin, 1928
P. casarci Mehra, 1937
P. strictus Strom, 1940
P. blatnensis Chalupsky, 1954
P. raabei Furmaga, 1956

Of these the descriptions of P. casarci Mehra, 1937, P. strictus Strom, 1940 and P. raabei Furmaga, 1956 were based on single adult specimens and are therefore of little value taxonomically. Styczynska-Jurewicz (1962) also noted that Buttner and Vacher (1959) described larval forms of P. cirratus which were significantly different from those of P. elegans. In particular the metacercarial cysts of the former species were substantially larger than those of the latter species, but more importantly they were progenetic. This led Styczynska-Jurewicz (1962) to believe that Buttner and Vacher had erroneously identified the metacercaria of Opisthioglyphe megastomus Timon-David, 1961 as that of P. cirratus. However, because investigations conducted by Brendow (1970), Theron (1976) and Krasnoloboba (1973) have demonstrated that the metacercariae of at least two other species of Plagiorchis are progenetic, it is probable that Buttner and Vacher were correct in their identification. As a result the P. elegans of Styczynska-Jurewicz (1962) and P. cirratus (Buttner and Vacher, 1959) are not synonymous, although the adults of the two species are morphologically indistinguishable.

It is of note that there is a substantial difference between adult P. cirratus Dollfus, 1960 and P. cirratus (Rud. 1802) Braun, 1902. The sucker ratio of the former species is greater than 1/1 and of the latter species is equal to 1/1; therefore according to the criteria established earlier they are not synonymous.

Accepting the limited variation presented in Tables 9.1 and 9.2, both P. noblei Williams, 1963 and P. kirkstallensis Diaz, 1976 share with P. elegans such taxonomically strong and valid characteristics that one must hold them to be synonymous.

The following species are also considered to be synonyms of P. elegans because they closely resemble P. elegans and their descriptions are inadequate to justify their separate specific status (Table 9.1). The data upon which they have been established are either incomplete or of questionable taxonomic validity.

P. muris (Tanabe, 1922) after Dollfus, 1925
P. muris McMullen, 1937
P. jaenschi Johnston & Angel, 1951
P. elegans Styczynska-Jurewicz, 1962
P. laricola Zdarska, 1966
P. peterborensis Kavelaars & Bourns, 1968

A number of species erected solely on adult features bear a close similarity to P. elegans and the descriptions as they stand are insufficient to retain them as distinct species. To complete the list of synonyms these are included in Table 9.1 (B).

Geographical distribution.

An examination of Table 9.2 reveals that the geographical distribution of P. elegans is extensive, encompassing much of the Paleartic and Nearctic regions of the world. Such a far-reaching distribution may be attributed to three primary factors:

1) The broad spectrum of suitable final hosts.
2) Common or overlapping and widespread distribution of the intermediate and final hosts.
3) Ease of parasite dispersal.

Natural infections of P. elegans have been recorded in birds and mammals; if experimental infections are taken into account, then the list of potential definitive hosts becomes both greater and more diverse (Table 9.2). Furthermore it is apparent from Table 9.2 that a number

of hosts, first (Lymnaea stagnalis and L. palustris), second (chironomids, mosquitoes and snails) and final (sparrows, gulls, pigeons, rats and mice) share a common and widespread geographical distribution. Thus the first two prerequisites for the establishment of the parasite over its observed range are satisfied. However, because species of Plagiorchis are confined to first intermediate hosts of the genus Lymnaea and are only broadly specific towards either the second intermediate or final hosts, the greatest limiting factor to the distribution of P. elegans, or any member of the genus, is the distribution of its gastropod host. The genus Lymnaea however is cosmopolitan (Hubendick, 1951).

Conceivably the utilization of migratory hosts such as Sterna hirundo, Actitis macularia, Turdus migratorius, Larus argentatus and Irodoprocne bicolor has contributed to the dispersal of P. elegans. In addition flight enables the regular but not necessarily frequent exchange between Europe (Heinzel et al., 1972) and North America (Robbins et al., 1966) of natural hosts of P. elegans such as Larus ridibundus and Xanthocephalus xanthocephalus. Man may also have been instrumental in disseminating P. elegans by introducing natural hosts such as Passer domesticus and Lymnaea stagnalis into new geographical areas (North America and New Zealand respectively) and thus increasing the host range.

Kennedy (1975) has emphasized the heterogenous distribution of parasites and particularly the focal nature of parasites having aquatic stages in their life cycles. Clearly the geographical distribution of P. elegans is extensive and isolated populations centre around freshwater habitats. Most records of P. elegans are found in Europe and North America with theioccasional description in Asia and a single record in Australia. This does not however rule out the possibility that P. elegans is more wide spread in the southern hemisphere than has been demonstrated.

Table 9.1 A. The dimensions and some taxonomically significant anatomical features of Plagiorchis elegans and its synonyms.

species	adult (mm) length by width	sucker ratio OS:VS	eggs (μm) length by width
P. elegans present study	1.04 to 3.89 by 0.34 to 0.96	1.16:1 to 1.63:1	36 to 45 by 22 to 24
P. noblei Williams, 1963	1.89 to 2.66 by 0.66 to 0.82	1.17:1 to 1.29:1 by 1.12:1 to 1.13:1	35 to 40 by 19 to 20
P. kirkstallensis Diaz, 1976	2.34 to 2.64 by 0.59 to 0.74	1.37:1	43 to 49 by 22 to 24
P. muris (Tanabe, 1922) after Dollfus, 1925	0.80 to 2.20 by 0.24 to 0.80	1.10:1 to 1.11:1	30 to 37 by 20 to 23
P. muris McMullen, 1937	2.67 by 0.52	1.48:1	38 by 19
P. jaenschi Johnston & Angel, 1951	0.85 to 1.43 by 0.23 to 0.37	1.13:1 to 1.28:1	30 to 37 by 17 to 22
P. elegans Styczynska-Jurewicz, 1962	1.49 to 2.07 by 0.36 to 0.47	1.43:1 to 1.44:1 by 1.32:1 to 1.37:1	32 to 41 by 17 to 24
P. laricola Zdarska, 1966	1.23 to 1.26 by 0.39 to 0.41	1.58:1 to 1.70:1 by 1.58:1 to 1.64:1	33 by 18
P. peterborensis Kavelaars & Bourns, 1968	1.30 to 1.70 by 0.50 to 0.56	1.17:1 to 1.29:1 by 1.06:1 to 1.10:1	38 to 41 by 20 to 24

S.R. +:-	V.D. or V.E.	C.G.A. + : -	sporocyst birthpore + : -	cercaria (µm) length by width	tail (µm) length by width
+	V.E.	+	-	160 to 330 by 80 to 140	110 to 220 by 20
+	V.E. (drawn)	+	not mentioned	212 to 246 by 102 to 136	not mentioned
+	V.E.	+	-	266 to 285 by 98 to 129	201 to 228 by 27 to 30
+	n.m.	n.m.	not mentioned	100 to 220 by 100 to 120	100 (con) 200 to 280 (ex)
n.m.	n.m.	n.m.	not mentioned	240 by 92	190 by 27
n.m.	n.m.	+	not mentioned	133 to 285 by 82 to 148	102 to 180 by 26 to 36
n.m.	n.m.	+	not mentioned	240 to 288 by 108 to 122	171 to 187 by 28
n.m.	n.m.	+	+	190 to 240 by 122 to 140	90 to 147 by 30 to 36
+	not seen	+	not mentioned	260 by 90	210 by 25

Table 9.1 A. cont.

species	cercaria: caeca posterior extent	number pairs penetration gland cells	stylet (µm) length by shaft width
P. elegans present study	to posterior	8	27 to 31 by 4 to 5
P. noblei Williams, 1963	not visible	8	32 to 33 by 5
P. kirkstallensis Diaz, 1976	near posterior	8	28 to 34 by 4 to 9
P. muris (Tanabe, 1922) after Dollfus, 1925	to posterior	4	33 by 5
P. muris McMullen, 1937	to posterior	7 or 8	33
P. jaenschi Johnston & Angel, 1951	to posterior	approx. 10	34 by 6.5
P. elegans Styczynska-Jurewicz, 1962	to posterior	6	28 to 30 by 4 to 6
P. laricola Zdarska, 1966	to posterior	7	33 by 5
P. peterborensis Kavelaars & Bourns, 1968	not mentioned	7	30 to 31 by 4.5 to 5.0

stylet (μm) width at shoulders	metacercarial cysts (μm)
6 to 8	125 to 176 by 120 to 158
8	127 to 145
8 to 10	167 to 171 by 167 to 171
8	117 to 140
not mentioned	200
9.5	120 to 173 by 98 to 158
not mentioned	120 to 150
not mentioned	170 to 180 by 156
7	110 to 130

Key

con	contracted
C.G.A.	common genital atrium
ex	extended
n.m.	not mentioned
OS	oral sucker
S.R.	seminal receptacle
V.D.	vas deferens
V.E.	vas efferens
VS	ventral sucker

192a

Table 9.1 B.

species	adult (mm) length by width	sucker ratio OS:VS	eggs (μm) length by width
P. elegans Braun, 1902	2.0 to 2.3 by 0.66	1.70:1 by 1.50:1	32.0 to 36.4 by 18.2 to 22.8
P. nanus Braun, 1902	1.0 to 1.3 by 0.17 to 0.20	1.30:1 to 1.43:1 by 1.13:1 to 1.30:1	27.3 by 18.2
P. triangularis Braun, 1902	1.40 by 0.50	1.46:1 by 1.39:1	40.9 by 22.8
P. vitellatus Braun, 1902	1.30 to 2.0 by 0.28 to 0.46	1.56:1 to 1.95:1 by 1.35:1 to 1.69:1	27.0 to 36.4 by 22.8
P. micromaculosus Skrjabin & Massino, 1925	0.90 to 1.40 by 0.46 to 0.51	1.07:1 by 1.36:1	28.9 to 38.6 by 19.3
P. obtusa Strom, 1940	0.95 by 0.35	1.50:1 by 1.36:1	30.0 to 32.0 by 17.0 to 19.0
P. blatnensis Chalupsky, 1953	2.00 to 2.60 by 0.60 to 0.80	1.29:1 by 1.42:1	38.0 to 40.0 by 22.0 to 24.0
P. proximus Grabda, 1954	2.04 to 3.62 by 0.40 to 0.89	1.10:1 to 1.31:1 by 1.24:1 to 1.25:1	35.0 to 38.0 by 18.0 to 23.0
P. stefanski Furmaga, 1956	1.99 to 2.45 by 0.65 to 0.84	1.20:1 to 1.34:1	35.0 to 37.0 by 22.0 to 24.0

Table 9.1 B. cont.

species	adult (mm) length by width	sucker ratio OS:VS	eggs (µm) length by width
P. praevitellaris Matskasi, 1973	1.69 to 2.01 by 0.80 to 0.96	1.12:1 to 1.29:1 by 1.03:1 to 1.19:1	35.0 by 18.0
*P. noblei** Blankespoor, 1974	1.81 by 0.56	1.17:1 by 1.11:1	36.0 to 40.0 by 19.0 to 21.0
P. cirratus Matskasi, 1974	1.18 to 1.45 by 0.38	1.67:1 to 1.87:1 by 1.64:1 to 1.67:1	28.0 to 34.0 by 15.0 to 18.0
P. vitellatus Fraser, 1974	1.10 to 2.60 by 0.19 to 0.37	1.63:1 by 1.22:1	32.0 to 35.0 by 21.0 to 23.0
Plagiorchoides+ *rhinolophi* Park, 1939	3.03 by 0.83	1.65:1 by 1.71:1	28.0 to 34.0 by 17.0 to 19.0

* 7-day old specimens recovered from house sparrows.

\+ by definition a seminal receptacle is present in species of this genus.

Table 9.2. Geographical distribution and host lists of P. elegans and its synonyms.

species	geographical location	definitive natural	definitive experimental
P. elegans present study	England (Leeds)		mice rats hamsters gerbils pigeons chicks ducklings
P. noblei Williams, 1963	U.S.A. (Ohio)	Agelaius phoeniceus	chicks
P. kirkstallensis Diaz, 1976	England (Leeds)		rats mice
P. muris (Tanabe, 1922) after Dollfus, 1925	Japan	rats	
P. muris McMullen, 1937	U.S.A. (Michigan)	robin (American) herring gull night hawk spotted sandpiper	man rats mice pigeons
P. jaenschi Johnston & Angel, 1951	Australia	Hydromys chryogaster	
P. laricola Zdarska, 1966	Czechoslovakia	Larus ridibundus	Larus ridibundus Sterna hirundo

HOSTS			
second intermediate		first intermediate	
natural	experimental	natural	experimental
	chironomids *Gammarus pulex* *Asellus aquaticus* *Lymnaea stagnalis*	*Lymnaea stagnalis*	*L. stagnalis* *L. palustris*
	mayflies caddisflies damselflies mosquitoes midges	*L. (Stagnicola) reflexa* Say	
Chironomus plumosus *Leptocerus* sp. *Dytiscus marginalis* *Coenagrion* sp.	*Chironomus plumosus* *Aedes aegypti*	*L. stagnalis*	
Chironomus sp. *Lymnaea pervia* *Calibaetes* sp.	*Chironomus* sp. *L. pervia*	*L. pervia*	
	dragonfly naiads mosquito larvae snails	*Stagnicola emarginata angulata*	
Cherax destructor *Lymnaea lessoni*	mosquito larvae *Daphnia* sp. *Chiltonia subtenus* *Cherax destructor*	*Lymnaea lessoni*	*L. lessoni*
	Culex pipiens *Aedes aegypti*	*Lymnaea stagnalis*	

HOSTS			
second intermediate		first intermediate	
natural	experimental	natural	experimental
Aeschna grandis Corethra sp.	damselflies mosquito larvae dragonflies mayflies Asellus aquaticus Gammarus pulex	L. stagnalis	
	Aedes aegypti	L. stagnalis	
	Aeschna sp. Coenagrion sp. Chironomus tetans	L. stagnalis Stagnicola reflexa	L. stagnalis Stagnicola reflexa

Table 9.2. cont.

species	geographical location	definitive natural	definitive experimental
P. elegans Styczynska-Jurewicz, 1962	France & Poland	*Muscicapa striata*	mice
P. peterborensis Kavelaars & Bourns, 1968	Canada (Ontario)		mice
P. noblei * Blankespoor, 1974; 1977	U.S.A. (Iowa)	*Agelaius phoeniceus* *Xanthocephalus xanthocephalus*	*Gallus gallus* *Meleagris gallopavo* *Phasianus colchicus* *Porzana carolina* *Chlidonias niger* *Spinus tristis* *Cyanocitta cristata* *Tyrannus tyrannus* *Passer domesticus* *Troglodytes aedon* *Agelaius phoeniceus* *Turdus migratorius* *Iridoprocne bicolor* *Sturnella neglecta* *Xanthocephalus xanthocephalus* *Mus musculus* *Rattus norvegicus*

* Blankespoor (1974) states in his discussion that "the single adult recovered from a yellow-headed blackbird which had been fed metacercariae experimentally developed from eggs obtained from a bat, suggests that there may be no difference between certain plagiorchiids known to occur in both avian and mammalian hosts" - but he reveals neither the species of bat nor the source of its infection.

Table 9.2. cont. Species described from natural infections of the final host only.

species	geographical location	definitive host natural
P. elegans Braun, 1902	Germany Austria	*Passer domesticus* *Glareola austriaca* *Strix scops* *Falco subbuteo*
P. nanus Braun, 1902	Germany	*Glareola austriaca*
P. triangularis Braun, 1902	Germany	*Merops apiaster*
P. vitellatus Braun, 1902	Austria	*Actitis hypoleucus*
P. micromaculosus Skrjabin & Massino, 1925	U.S.S.R.	*Sylvia atricopilla* *S. hortensis* *Muscicapa grisola* *M. atricopilla*
P. obtusa Strom, 1940	Turkestan, S.S.R.	*Rhyacophilus ochropus*
P. blatnensis Chalupsky, 1953	Czechoslovakia	*Microtus arvalis* Pallus
P. proximus Grabda, 1954	Poland	*Ondatra zibethica*
P. stefanski Furmaga, 1956	Poland	*Apodemus sylvaticus* *A. agrarius* Pallus
P. praevitellaris Matskasi, 1973	N. Vietnam	*Hipposideras armiger*
P. cirratus Matskasi, 1974	Hungary	*Glareola practincola*
P. vitellatus Fraser, 1974	Scotland (Loch Leven Kinross)	*Larus argentatus* *L. ridibundus* *L. fuscus*
Plagiorchoides rhinolophi Park, 1939	Korea	*Rhinolophus ferrum-equinum*

DISCUSSION

Work on the digenetic trematodes has concentrated largely on flukes capable of causing disease in either man or agricultural livestock, particularly those in the Schistosomatidae and Fasciolidae (Smyth, 1966; Dawes, 1968). Since these families constitute only a small portion of the digenetic fauna, our knowledge of the vast majority is limited.

This study has shown that Plagiorchis elegans follows essentially the same regime as the more well known flukes and that it is subject to similar limiting parameters. Although conflicting views concerning molluscan taxonomy have for some time clouded the issue of specificity at the first intermediate host level (Wright, 1960), it is now generally accepted that most digeneans exhibit considerably greater specificity toward the first intermediate gastropod host than the definitive host (Wright, 1960; Dawes, 1968; Erasmus, 1972). For example, Schistosoma mansoni is limited to snails of the genus Biomphalaria and while it was formerly held to be strictly parasitic in man, it has since been demonstrated to parasitize and attain maturity in a wide variety of animals representing four mammalian orders (Wright, 1960; Kennedy, 1975). Fasciola hepatica employs only members of the genus Lymnaea as its first intermediate host (Wright, 1960; Boray, 1969; Kendall, 1950) but employs a wide range of mammalian definitive hosts (Chandler & Read, 1961; Dawes, 1962). In like manner P. elegans is confined to species of Lymnaea and occurs naturally not only in mammals but also in avian final hosts. Furthermore the rate of development and establishment of P. elegans is, like S. mansoni (Bruce, Llewellyn & Sadun, 1961) and F. hepatica (Dawes, 1962), affected by both the species of final host and its immunological disposition towards the parasite. The rate of development is important because the size, shape and proportions of the body and internal organs change throughout maturation and continue to change after the onset of egg-production, which results in marked morphological modifications of the parasite (Stunkard, 1957; Dawes, 1962; Thomas, 1965). This is relevant from a taxonomic standpoint because of the emphasis which has been placed by taxonomists on adult morphology (Stunkard, 1957; Yamaguti, 1971).

However, P. elegans deviates from the regime followed by schistosomes and fasciolids in several ways. The embryonated eggs of P. elegans, like other members of the family (Noble & Noble, 1971), must be

ingested by the snail host before they hatch, while the miracidia of both Schistosoma spp. and Fasciola spp. are free-swimming. It is therefore extremely difficult either to observe penetration of the first intermediate host by P. elegans or to quantify the infections, unless of course one is dealing with single egg infections. On the other hand, penetration of the first intermediate host by F. hepatica and S. mansoni has been investigated by Dawes (1960) and Wajdi (1966) respectively. In addition the cercariae of P. elegans employ a second intermediate host, while those of the fasciolids encyst on vegetation and the schistosomes have no metacercarial stage but rather penetrate the final host directly.

Because the cercariae of P. elegans may encyst precociously in the molluscan host as well as in a wide variety of arthropods, and are viable throughout the metamorphosis of the insect host, they may become established not only in animals having diverse food preferences but also in those somewhat removed from the aquatic habitat, in contrast to the fasciolids and schistosomes whose life cycles focus more closely around water. Perhaps the most striking difference between adult members of the Schistosomatidae and Plagiorchiidae or for that matter all other digenetic trematode families but one - the Didymozoidae, is that adult schistosomes are dioecious (Dawes, 1968).

Investigations concerning members of the Plagiorchiidae and more specifically the genus Plagiorchis have with few exceptions depended upon the chance occurrence of the parasites. As a result these studies have generated a considerable literature of a superficial nature which is reflected in the taxonomy of the family (McMullen, 1937a; Mehra, 1931; Olsen, 1937; Dawes, 1968). The need for detailed species studies is therefore readily apparent and P. elegans provides an exceptionally good laboratory model for such an investigation. Its generation time of approximately 50 days is shorter than that of either F. hepatica or S. mansoni, the former being 84 days (Dawes, 1962; Boray, 1967; 1969) and the latter being 64 days (Meuleman, 1972; Smithers & Terry, 1965b). It is not pathogenic toward man (McMullen, 1937c) and is easily maintained under experimental conditions, as opposed to F. hepatica and S. mansoni whose gastropod hosts require more rigorous conditions than Lymnaea stagnalis and L. palustris (Boray, 1969; Meuleman, 1972). Most importantly however it is a member of the central genus in the largest family of digenetic trematodes, the Plagiorchiidae (Mehra, 1931; McMullen, 1937a). This investigation has demonstrated the necessity for conducting an indepth study of the life cycle and intra-spe-

cific variation of a pure strain of a non-pathological but nonetheless important digenetic trematode.

Appendix I. Egg dimensions (in µm) of some Plagiorchis species.

A. Falling within or overlapping the range of P. elegans eggs.

Species	Egg dimensions
P. elegans Braun (1902)	32 to 36 by 18 to 23
P. triangularis Braun (1902)	41 by 23
P. proximus Barker (1915)	32 to 38 by 20 to 24
P. muris Dollfus (1925)	30 to 37 by 20 to 23
P. arvicolae Schulz & Skworzow (1931)	36 to 56 by 20 to 36
P. maculosus Yamaguti (1935)	32 to 36 by 20 to 24
P. javensis Sandground (1940)	36 by 22 to 24
P. strictus Strom (1940)	35 to 41 by 17 to 22
P. upupae Strom (1940)	31 to 37 by 17 to 22
P. multiglandularis Chang-Tung Ku (1940-1941)	28 to 36 by 20 to 22
P. gonzalchavenzi Zercecero (1949)	40 by 23
P. jaenschi Johnston & Angel (1951)	30 to 37 by 17 to 22
P. blatnensis Chalupsky (1954)	38 to 40 by 22 to 24
P. proximus Grabda (1954)	35 to 38 by 18 to 23
P. stefanski Furmaga (1956)	35 to 37 by 22 to 24
P. cirratus Dollfus (1960)	40 by 22
P. vespertilionis parorchis Macy (1960)	33 to 38 by 17 to 20
P. laurenti Vercammen-Grandjean (1960)	40 by 23
P. elegans Styczynska-Jurewicz (1962)	32 to 41 by 17 to 24
P. peterborensis Kavelaars & Bourns (1963)	38 to 41 by 20 to 24
P. kirkstallensis Diaz (1976)	43 to 49 by 22 to 24
Plagiorchoides potamonades Tubangui (1946)	35 to 42 by 24 to 36

B. Smaller than P. elegans eggs:

Species	Egg dimensions
P. cirratus Braun (1902)	24 to 35 by 18 to 23
P. nanus Braun (1902)	27 by 18
P. permixtus Braun (1902)	27 to 32 by 19
P. notabilis Nicoll (1909)	31 by 19 to 21
P. micromaculosus Skrjabin & Massino (1925)	29 to 37 by 19
P. micracanthos Macy (1931)	37 by 19
P. ramlianum Azim (1935)	35 by 22
P. muris McMullen (1937b)	38 by 19
P. koreanus Ogata (1938)	28 to 30 by 18 to 19

Appendix I B. cont.

Species	Egg dimensions
P. noblei Park (1936)	33 by 20
P. laricola Foggie (1937)	28 by 19
P. orientalis Park (1939a)	28 to 34 by 14 to 19
P. magnacotylus Park (1939b)	28 to 34 by 14 to 17
P. obtusus Strom (1940)	30 to 32 by 17 to 19
P. linkuolangui Tang (1941)	22 to 40 by 16 to 18
P. megalorchis Rees (1952)	30 to 33 by 21 to 23
P. lutrae Fahmy (1954)	29 to 32 by 17 to 19
P. raabei Furmaga (1956)	31 to 33 by 18 to 20
P. vespertilionis Sogandares-Bernal (1956)	37 by 18
P. maculosus Angel (1959)	29 to 32 by 17 to 20
P. berghei Vercammen-Grandjean (1960)	42 by 21
P. noblei Williams (1963)	35 to 40 by 19 to 20
P. dilimanensis Velasquez (1964)	27 to 30 by 15 to 19
P. limnogale Richard (1965/66)	34 by 21
P. laricola Zdarska (1966)	33 by 18
P. neomidis Brendow (1970)	29 to 33 by 18 to 21
P. praevitellaris Matskasi (1973)	35 by 18
P. noblei Blankespoor (1972)	36 to 41 by 19 to 21
P. vitellatus Fraser (1974)	32 to 35 by 21 to 23
P. cirratus Matskasi (1974)	28 to 34 by 15 to 18
P. taiwanensis Fischthal & Kuntz (1975)	29 to 35 by 17 to 21
P. farnleyensis Diaz (1976)	26 to 34 by 15 to 19
Plagiorchoides rhinolophi Park (1939a)	28 to 34 by 17 to 19
C. Larger than *P. elegans* eggs:	
P. goodmani Najarian (1961)	47 by 25
P. momplei Richard *et al.* (1968)	48 by 25

Appendix II. Summary of the general size relationships of the suckers, of the laboratory-established strain of P. elegans at different ages definitive hosts. (See key to Groups p. 204)

Group	oral sucker	pharynx	ventral sucker
1	> Ph,VS,OV OS >< AT,PT	< OS,VS,AT,PT Ph >< OV	< OS,AT,PT VS ≥ OV > Ph
2	> Ph,VS OS < OV < AT,PT	< OS,VS,OV,AT,PT Ph	< OS,OV,AT,PT VS > Ph
3	> Ph,VS OS >< OV,AT,PT	< OS,VS,AT,PT,OV Ph	< OS,AT,PT VS > Ph >< OV
4	> Ph,VS OS > OV >< AT,PT	< OS,VS,OV,AT,PT Ph	< OS VS > Ph >< OV >< AT < PT
5	> Ph,VS,OV OS ≥ AT,PT	< OS,VS,AT,PT Ph >< OV	< OS,AT,PT VS > Ph >< OV
6	> Ph,VS,OV,AT,PT OS	< OS,VS,AT,PT Ph < OV	< OS VS > Ph >< OV >< AT,PT
7	> Ph,VS OS >< OV,AT,PT	< OS,VS,OV,AT,PT Ph	< OS VS > Ph >< OV < AT,PT
8	< Ph,VS,OV,AT,PT OS	< OS,VS Ph >< OV,AT,PT	< OS VS > Ph ≥ OV,AT >< PT

pharynx and gonads to one another; observations were made on specimens and when the life cycle was completed using various first, second and

Ovary	Anterior Testis	Posterior Testis
< OS,AT,PT OV: ≮ VS >≮ Ph	>≮ OS,PT AT > Ph,VS,OV	>≮ OS,AT PT > Ph,VS,OV
≯ OS OV: > Ph,VS < AT,PT	> OS,Ph,VS,OV AT >≮ PT	> OS,Ph,VS,OV PT >≮ AT
>< OS OV > Ph >≮ VS < AT,PT	>< OS AT > Ph,VS,OV >≮ PT	>< OS PT > Ph,VS,OV >≮ AT
≮ OS,AT OV > Ph >≮ VS < PT	>≮ OS AT > Ph >< VS,PT ≯ OV	>≮ OS PT > Ph,VS,OV >< AT
< OS,AT,PT OV >≮ Ph,VS	≮ OS AT > Ph,VS,OV >≮ PT	≮ OS PT > Ph,VS,OV >≮ AT
< OS OV ≯ Ph >≮ VS >< AT,PT	< OS AT > Ph >< VS,OV ≮ PT	< OS PT > Ph >< VS,OV ≯ AT
>≮ OS OV > Ph >< VS < AT,PT	>≮ OS,PT AT > Ph,VS,OV	>≮ OS,AT PT > Ph,VS,OV
< OS OV: >≮ Ph,AT,PT ≮ VS	< OS AT >≮ Ph,OV,PT ≮ VS	< OS PT >≮ Ph,OV,AT >< VS

202a

Appendix II. Cont.

Group	Oral Sucker	Pharynx	Ventral Sucker
9	> Ph,VS OS ≤ OV < AT,PT	< OS,VS,OV,AT,PT Ph	< OS,OV,AT,PT VS > Ph
10	> Ph,VS OS >< OV < AT,PT	< OS,VS,OV,AT,PT Ph	< OS VS > Ph >< OV < AT,PT
11	> Ph,VS,OV OS >≤ AT,PT	< OS,VS,OV,AT,PT Ph	< OS,AT,PT VS > Ph >≤ OV
12	> Ph,VS OS >< OV < AT,PT	< OS,VS,OV,AT,PT Ph	< OS,OV,AT,PT VS > Ph
13	> Ph,VS,OV OS >≤ AT,PT	< OS,VS,OV,AT,PT Ph	< OS,OV,AT,PT VS > Ph
14	> Ph,VS,OV,AT,PT OS	< OS,VS,AT,PT Ph ≥ OV	< OS,OV,AT;PT VS > Ph
15	> Ph,VS OS >≤ OV ≤ AT,PT	< OS,VS,OV,AT,PT Ph	< OS,OV;AT,PT VS > Ph
16	> Ph,VS OS >< OV < AT,PT	< OS,VS,OV,AT,PT Ph	< OS,OV,AT,PT VS > Ph
17	> Ph,VS OS >≤ OV < AT,PT	< OS,VS,OV,AT,PT Ph	< OS VS > Ph >< OV < AT,PT

Ovary	Anterior Testis	Posterior Testis
> OS OV > Ph,VS < AT,PT	> OS,Ph,VS,OV AT >< PT	> OS,Ph,VS,OV PT >< AT
>< OS,VS OV > Ph < AT,PT	> OS,Ph,VS,OV AT >< PT	> OS,Ph,VS,OV PT >< AT
< OS,AT,PT OV > Ph >< VS	>< OS,PT AT > Ph,VS,OV	>< OS,AT PT > Ph,VS,OV
>< OS OV > Ph,VS < AT,PT	> OS,Ph,VS,OV AT < PT	> OS,Ph,VS,OV,AT PT
< OS,AT,PT OV > Ph,VS	> Ph,VS,OV AT >< OS,PT	> Ph,VS,OV PT >< AT
< OS,VS,AT,PT OV < Ph	< OS AT > Ph,VS,OV >< PT	< OS PT > Ph,VS,OV >< AT
>< OS OV > Ph,VS < AT,PT	> OS AT > Ph,VS,OV >< PT	> OS PT > Ph,VS,OV >< AT
>< OS OV > Ph,VS < AT,PT	> OS,Ph,VS,OV AT >< PT	> OS,Ph,VS,OV PT >< AT
>< OS OV > Ph >< VS < AT,PT	> OS,Ph,VS,OV AT >< PT	> OS,Ph,VS,OV PT >< AT

Key to groups in Appendix II.

Group age in *days*	HOSTS *final*	intermediate *second*	*first*
1 (4)	LACA mice	*Chironomus* sp.	*Lymnaea stagnalis*
2 (7)	LACA mice	*Chironomus* sp.	*Lymnaea stagnalis*
3 (14)	LACA mice	*Chironomus* sp.	*Lymnaea stagnalis*
4 (21)	LACA mice	*Chironomus* sp.	*Lymnaea stagnalis*
5 (7)	rats	*Chironomus* sp.	*Lymnaea stagnalis*
6 (14)	rats	*Chironomus* sp.	*Lymnaea stagnalis*
7 (21)	rats	*Chironomus* sp.	*Lymnaea stagnalis*
8 (28)	rats	*Chironomus* sp.	*Lymnaea stagnalis*
9 (7)	LACA mice	*Lymnaea stagnalis*	*Lymnaea stagnalis*
10 (7)	LACA mice	*Chironomus* sp.	*Lymnaea palustris*
11 (7)	hamsters	*Chironomus* sp.	*Lymnaea stagnalis*
12 (7)	gerbils	*Chironomus* sp.	*Lymnaea stagnalis*
13 (7)	chicks	*Chironomus* sp.	*Lymnaea stagnalis*
14 (7)	ducklings	*Chironomus* sp.	*Lymnaea stagnalis*
15 (7)	pigeons	*Chironomus* sp.	*Lymnaea stagnalis*
16 (7)	LACA mice	*Gammarus pulex*	*Lymnaea stagnalis*
17 (7)	LACA mice	*Asellus aquaticus*	*Lymnaea stagnalis*

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