STUDIES ON THE GENUS MESOCESTOIDES

(CESTODA: CYCLOPHYLLIDEA)

by

Hugo Alvin James

A Dissertation Submitted to the

Graduate Faculty in Partial Fulfillment of

The Requirements for the Degree of

DOCTOR OF PHILOSOPHY

Major Subject: Zoology (Parasitology)

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

Head of Major Department

Signature was redacted for privacy.

Dean of Graduate College

Iowa State University
Ames, Iowa

1968

TABLE OF CONTENTS

INTRODUCTION

This investigation was initiated during the early summer of 1961 when particularly heavy infections of the cestode genus, Mesocestoides, were found in raccoons in the environs of Lakeside Laboratory in northwestern Iowa. According to published reports, no substantiated life cycle of these cestodes had been published, nor was the position of the tetrathyridium, in the mesocestoidid life cycle, clear. The availability of gravid Mesocestoides provided an ample supply of eggs for feeding experiments with selected invertebrate and vertebrate hosts. Finding of tetrathyridia in local toads and frogs provided the materials for a developmental study of this larval stage.

The genus Mesocestoides Vaillant, 1863 has been an enigma since the description of its type species by Goeze in 1782. Exclusive of generic synonyms, 35 species, varieties and/or forms of species have appeared in the literature. In the absence of an experimentally proven life cycle for any member of this group, the systematic position of the genus or even family is not clearly understood. During the course of this investigation, attempts to elucidate the life cycle of Mesocestoides have proven unsuccessful. However, data obtained from natural and experimental infections of host animals coupled with those data available in the literature have resulted in a clearer understanding of the taxonomic

position of the genus and, to a lesser degree, of the family.

Witenberg (1934) and Voge (1955) have suggested that morphological features used in determining mesocestoidid species are unsuitable or at least unreliable. Witenberg has pointed out that the anatomy of the members of this genus is so unstable that even apparently distinctive characteristics overlap to such a degree that species identification is quite difficult. This study supports the contention that present criteria for defining species of Mesocestoides are useless and strongly suggests that if more than one species does exist, criteria for separation will probably be physiological and not morphological.

Numerous early investigations, well reviewed in Witenberg (1934), have shown conclusively that tetrathyridia are larvae of Mesocestoides. However, whether a tetrathyridium is required in the cycle, or is merely paratenic,[*] or is the terminal stage of oncospheral development in a cycle requiring only a single intermediate host, as suggested by Spasskii (1951), are all a matter of conjecture. Most previous investigators have felt that Mesocestoides required two intermediate hosts. This study casts doubt on the validity of the currently proposed life cycles of mesocestoidids and suggests a need for an experimentally proven cycle before any categorical statements are made. However, the primitive

_____

[*]As defined in Baer (1951).

phylogenetic position of this genus, suggested by its broad
host spectrum for both adult and larval stages, points
clearly to the potentiality of the tetrathyridium to exist
paratenically. Experimental transfers of tetrathyridia
from one class of vertebrate hosts to another conclusively
support the paratenic capabilities of this larval stage.

Because the taxonomic position and productivity of
parasitic worms is so closely associated with their morphol-
ogy, an interest in the anatomy of the Mesocestoididae could
hardly be avoided. Morphologically, the family is an ex-
ception to the general pattern of cyclophyllideans. Obser-
vations of Byrd and Ward (1943) on the segmental anatomy of
a gravid mesocestoidid from an opossum have been corroborated
during the course of this study but have not been incorporated
in it. However, the parauterine (= paruterine) organ, be-
cause of its especial role as a taxonomic criterion and its
function as a possible mechanism of egg dispersal and/or
protection, has been carefully studied in detail. Ultra-
structural and histochemical observations of the mesoces-
toidid parauterine organ are reported in Part II of this
dissertation.

The dual nature of this investigation has necessitated
a physical dichotomy of the work: Part I is comprised of
systematic problems and their possible solutions; Part II,
of morphology and histochemistry of the parauterine organ.

PART I. SYSTEMATIC CONSIDERATIONS

MATERIALS AND METHODS

Post-tetrathyridial stages of Mesocestoides used in this study were obtained from both naturally and experimentally infected hosts. Wild hosts were taken by shooting and trapping or as road kills. In many cases during the trapping season, only viscera of host animals were examined and data from these animals are hence incomplete. Experimental hosts were taken either as nursing young and were reared to infective age, or were obtained at an already infective age from the Iowa State Conservation Commission at Boone, Iowa. Infective age is used in this study to mean the age at which hosts could take solid food, since no experimental work was done to determine at what chronological age infectivity could occur. All hosts received from the Iowa Conservation Commission had been held in captivity for a minimum of 33 days, during which time they were fed on commercially prepared food. During the course of this investigation, animals in the laboratory were fed Purina Dog Chow unless an otherwise specified diet was required. Preceding all experimental exposures to parasites, animals were quarantined for several days during which time they were wormed and their faeces periodically checked for proglottids. Animals to be infected were fed tetrathyridia obtained from frogs (Rana pipiens Schreber) and/or toads (Bufo americanus Holbrook and B. cognatus Say). Frogs harboring tetrathyridia were caught

wild in northwestern Iowa or came from E. G. Steinhilber & Company, Inc. in Oskosh, Wisconsin but, according to Mr. Steinhilber,[*] were not necessarily collected in Wisconsin. Toads were collected from central and northwestern Iowa (B. americanus) and southeastern South Dakota (B. cognatus). Larvae obtained from the body cavities of the amphibian hosts were transferred to mice of moderately inbred Marker stock, maintained by the Iowa State University Genetics Department. The technique of Specht and Voge (1965) was followed but modified by omission of antibiotics. A few laboratory-reared Peromyscus, Rattus and Citellus as well as frogs (R. pipiens), toads (B. americanus) and salamanders (Ambystoma tigrinum Green) were also injected. Following injection, tetrathyridia were successfully maintained in mice for several months. Tetrathyridia obtained from naturally infected amphibian hosts or experimentally infected transfer hosts were fed to a variety of animals including amphibians, birds, and several species of mammals. These are listed specifically in the text under experimental feedings.

In addition to the vertebrate hosts, numerous invertebrate hosts, some taken from the wild and some laboratory reared, were fed whole proglottids, parauterine organs

---

[*]Steinhilber, E. G. Steinhilber & Company, Inc., Oskosh, Wisconsin. Personal communication (letter). 1967.

excised from proglottids, or crushed proglottids. Some

tenebrionid beetles were injected with oncospheres artifi-

cially removed from their eggs with pepsin-trypsin treatment.

Egg shells were easily weakened sufficiently to release

oncospheres by treating them with 1.0 percent pepsin dis-

solved in a 0.01 percent HCl solution. The HCl proved to be

toxic to the oncospheres and as soon as their activity slowed

appreciably, the acid medium was decanted and 1.0 percent

trypsin in normal saline, adjusted to pH 8-10 with NH_4OH, was

added. The basic trypsin solution increased activity of

oncospheres and further weakened the egg shell. Upon release

of the oncospheres from eggs, the trypsin solution was de-

canted and a basic normal saline (pH $8 \pm .1$) was added.

Oncospheres have been maintained alive in this way for up to

three hours. Released oncospheres freed in this manner were

easily injected into selected hosts by using a syringe and

narrow bore needle of appropriate diameter.

Standard posting techniques were used to obtain im-

mature, mature, or gravid worms from the small intestines

of definitive hosts and were then transferred to an appro-

priate saline solution. Some gravid proglottids obtained

in this manner provided eggs with oncospheres for feeding

experiments and for testing the effects of temperature,

desiccation and aging on the oncosphere. However, most

proglottids obtained for experimental purposes were taken

from naturally shed faecal masses of infected hosts. Adult

and larval worms to be used for wholemount preparations or sections were cold-relaxed, whenever possible, and were stretched over a cylindrical bottle of appropriate diameter. Wholemount specimens were generally fixed in FAAG (Turtox formula) and stained in Mayer's paracarmine; when counterstained, a variable but highly diluted fast green was used. Material for sectioning was fixed in FAAG, 70 percent ethanol, Bouin's, Carnoy's, Zenker's, osmium tetroxide, 10 percent formalin phosphate buffered to a pH of 7.2-7.5 or similarly buffered glutaraldehyde. Glutaraldehyde fixed material was usually post-fixed in osmium tetroxide and embedded in Maraglas (Marblette Corporation, Long Island City, New York) for electron microscopy. Formalin fixed material was embedded in gelatin from which frozen sections were cut to be histochemically tested for presence of lipids. All other fixed material was embedded in paraffin, sectioned at 6-10 micra, and stained with standard hematoxylin and eosin or specific histochemical stains. Most histochemical stains and procedures were taken from the Armed Forces Institute of Pathology Manual of Histologic and Special Staining Techniques (1960) and from Pearse (1960). Special materials and methods specifically concerned with histochemical and ultrastructural techniques are described in detail under Section II of this dissertation.

LITERATURE REVIEW

Since the first published account of mesocestoidid species from birds and mammals (Goeze, 1782), several hundred reports have appeared in the literature. Of these, few represent experimental studies; the majority are taxonomic, in the classical sense, and deal primarily with species, hosts, and host localities of Mesocestoides. Most of the described species (Table 1)[*] have already been reduced to synonomy but, for Mesocestoides, as for so many parasitic organisms, taxonomic status can be validated only when the life cycle of at least one member of the genus is understood. Thus far, in spite of reports to the contrary, no mesocestoidid cycle has been fully elucidated, either by experimental or natural means.

Works on Mesocestoides can be divided arbitrarily into the following four categories or groups: (1) taxonomic reports of new or previously described species, hosts, and host localities or reviews of such reports; (2) taxonomic revisions, usually based on examination of previous reports, type specimens and/or other available specimens; (3) life cycle studies, generally experimental although some conjectural reports based on natural findings have occurred; (4) anatomical or histochemical studies, usually, although not always, incorporated into papers of the above three groups.

---

[*]For all tables, see Appendix A.

Most of the significant studies in this latter category are reported in Section II of this study.

Group 1. Taxonomic papers comprise the greatest bulk of literature on Mesocestoides, are widely scattered and no single review contains even a fraction of the currently available data. Since this study is not intended to be a taxonomic revision, no attempt has been made to introduce all or even the greater part of the taxonomically oriented literature. Certainly the most important segment of this group includes those papers introducing new species noted in Table 1. Of great value are taxonomic reviews which are compilations of widely scattered and often obscure reports, yet generally contain little or no original material. Excellent in this respect are the following general works: Neumann (1905), Meggitt (1924), Meggitt (1931), Fuhrmann (1932), Sprehn (1932), Joyeux and Baer (1936), Neveu-Lemaire (1936), Lopez-Neyra (1947) and Yamaguti (1959). Reviews restricted to Mesocestoides are Cameron (1925), Mueller (1928), Joyeux and Baer (1932) and Petrov and Spasskii (1954). Although Witenberg (1934) and Voge (1955) are, in part, reviews, they more properly belong with Group 2.

Group 2. Revisions incorporate reviews, and hence belong in part to Group 1. Most revisions of Mesocestoides have resulted in reduction of species by synonomy and although several minor revisions exist, only two major works on Mesoces-

toides have been attempted. The finest and most complete work of this type was that of Witenberg (1934), who reduced all of the species then known to three, one from mammals and two from birds. In this same work, Witenberg (1934) included an extensive experimental study of his own. Disregarding Witenberg's (1934) suggestions, most American helminthologists not only continued to accept and use many of the species placed into synonomy by him but have since erected several new ones. Voge (1955), in her revision, North American Cestodes of the Genus Mesocestoides, attempted to reduce the existing taxonomic confusion and further synonomy resulted. However, in spite of the two excellent revisions cited, Chandler (1942, 1944), Ciordia (1955) and Grundman (1956) have added four new species to the long list (Table 1).

Group 3. The first successful experimental study to prove the relationship between tetrathyridia of Mesocestoides and their adult forms was that of Henry (1927). Both before and after Henry's (1927) work, experiments to clarify the life cycle were undertaken. Witenberg (1934) reviewed these experimental efforts and presented some of his original studies as well. Since Witenberg's (1934) review, Carta (1939), Srivastava (1939), Wetzel and Quittek (1940), Soldatova (1944), Webster (1949), Maskar (1953), Anantamaran (1954) and Specht and Voge (1965) have all contributed to life cycle studies on Mesocestoides. Witenberg (1934) did not report investigations of Joyeux, Baer and Martin (1933),

Markowski (1933) or Ssolonitzin (1933). Moniez (1880) suggested that tetrathyridia found in birds may be the larval stage of adult cestodes in raptorial birds, but apparently no extensive experimental studies have been made on mesocestoidids in birds.

Group 4. The first detailed anatomical and histological study of Mesocestoides to appear in the literature seems to be that of Zschokke (1885, 1889). This work is of historical interest, but more recent studies, noted in Part II of this investigation, are of greater value.

NATURAL INFECTIONS

Adults

Species and species criteria

The first known record for Mesocestoides is that of
Goeze (1782), who described Taenia candelabrarius, T.
perlatus, and T. lineata from owl, buzzard, and wild cat,
respectively. The generic diagnosis, provided by Vaillant
(1863), was based on a specimen recovered from a genet
(Genetta genetta) collected in North Africa and which he
named Mesocestoides ambiguus. The family was erected in
1897 by Perrier. Since the report of Goeze (1782), 35
species, excluding forms and varieties of species of
Mesocestoides or generic synonyms, have been recorded.
Currently, the taxonomic state of the group is highly con-
fused.

Following the introduction of the species into the
literature, numerous characteristics were employed in
erecting and/or identifying the described 35 species.
Most characteristics used seem to have been selected on the
basis of few or incomplete specimens and in most instances,
without comparative laboratory infections. Some major
taxonomic criteria used in identifying species of Mesoces-
toides are outlined below. In species delineation, some
workers have used a single characteristic, others have em-
ployed several in combination, but no attempt is made below

to identify individual workers nor to associate certain
criteria with individual taxonomists.

Taxonomic criteria previously used in identifying species
of Mesocestoides include the following:

1. Scolex: width, length

2. Suckers: diameter, shape, notch position

3. Neck: presence, absence, length when present

4. Strobila: width, length, color

5. Proglottids: number, size, shape

6. Calcareous corpuscles: presence, absence, size,
   shape, position

7. Testes: number, size, location

8. Cirrus: spination, if any

9. Cirrus pouch: size, shape, position

10. Parauterine organ: size, shape, position

11. Caudal appendage: presence, absence, shape

12. Eggs: size, number

13. Uterus: size, shape

14. Ovaries: size, shape, position relative to
    vitellaria

15. Vitellaria: size, shape, position relative to
    ovaries and to each other

16. Genital aperture: position

17. Host species.

Species identification of cestodes may be difficult with
whole specimens, but is even more so when only partial speci-

mens are available. Erection of species, however, with few
or incomplete organisms at hand is an unwise practice but
one that has undoubtedly contributed to the much confused
taxonomic status of Mesocestoides and to many other groups
of organisms as well.

During this investigation, several hundred mesoces-
toidids have been observed alive, fixed and in various
stages of relaxation. Studies on wholemount preparations
and sections as well as on living specimens have provided
not only data on these criteria but also additional informa-
tion pertaining to morphological and histochemical studies
presented in Section II.

It would appear that body length is taxonomically value-
less especially when Mesocestoides have been reported with
total lengths of 5.5 to approximately 1600 mm. (Table 2).
In this study, a single, carefully relaxed specimen of
Mesocestoides measured 60 cm. but was capable of contracting
to 30 cm. or of stretching, without breaking, to well over
120 cm. The degree of relaxation affects proglottid shape.
Proglottids which might appear trapezoidal (Figure 10) in
some preparations could easily be square or rectangular if
fixed under different conditions (Figures 66 and 67). Fur-
thermore, some adult worms derived from the same larval
source were over 100 cm. long (Figure 4), but others were
less than 50 cm.; yet, all were gravid and had been handled
as identically as possible in preparation. Strobilar width

appears to be correlated with length; exceptionally long worms may or may not be wider (at their widest point) in a relaxed state than similarly treated shorter specimens (Tables 14 and 15). Size of internal organs may vary considerably in worms from the same and different hosts and accordingly, under the same host conditions, development of worms is not always equal. A single host infected with ten larvae may produce worms of varying lengths and stages of reproductive development. Hence, so-called prepatent periods (i.e., from infection to first appearance of proglottids in faeces) ascribed to "species" of Mesocestoides seem meaningless. Worm color apparently varies from white to pink and hence should be considered a useless taxonomic criterion. Number of proglottids in strobila is deceptive since mesocestoidids shed fully gravid proglottids either singly or in chains and, ordinarily, only young or early gravid worms are found to be entire. In my studies, several worms from the same host, infected at the same time with larvae of known lineage, produced strobilae varying from 360 visible segments (+ 42 mm. of unsegmented strobila, neck and scolex) to over 430 segments. The taxonomic uselessness of sucker notches becomes apparent upon examination of drawings of scoleces (Skrjabin and Schultz 1926:71, Cameron 1925:35 and others) which illustrates the instability of this characteristic contrary to Grundmann (1956) who suggested position of such notches as one difference between M. carnivoricolis

and <u>M</u>. <u>corti</u>. It is clear from numerous representative
mesocestoidid scoleces (Figures 57 thru 61) studied during
this investigation that notching of sucker rims cannot be
employed as a valid taxonomic criterion. The considerable
morphological variation in internal organs is also directly
related to the contracted or relaxed state of the cestodes.
In stretched specimens, an oval parauterine organ and
straight caudal appendage may be present (Figure 65), but in
a more relaxed region of the same worm a round parauterine
organ and typically S-shaped caudal appendage may appear
(Figure 64).

Age of the entire worm or, more specifically, that
portion of the specimen used for descriptive purposes must
be considered. Testes of younger mature proglottids (Figure
7) may be small but discrete, and testes of early gravid
stages (Figure 12) may also be small as a result of degen-
erative processes. Unless the investigator has at hand an
entire worm or at least that portion of the specimen from
scolex to gravid proglottids, organ size and/or form may
be considered taxonomically weak and useless for comparative
purposes. In those mesocestoidids examined during the course
of this study, fully developed testes of maximum size appear
in only several proglottids, often comprising only one or
two centimeters of a 50 cm. worm. It is doubtful that most
investigators prepare entire specimens of the larger cestodes

for observation; hence, testes measurements from the litera-
ture may be of little comparative value.

Other reproductive elements such as vitellaria, ovaries,
parauterine organs, cirrus pouch position etc. are also
affected by age and, as with testes, morphological variations
occur and similar taxonomic problems are introduced. It is
suspected that the difficulty in preparing a good wholemount
specimen of an entire 50 cm. cestode (common length for
mesocestoidids), a procedure absolutely necessary for any
comparative study, has probably resulted in the use of
partial specimens and hence reduces the accuracy and useful-
ness of resultant descriptions. However, age does not appear
to affect the average number of testes per proglottid in a
single specimen of Mesocestoides. Immature proglottids have
exhibited the same average number of testes primordia as
fully developed testes of late mature proglottids. Since
eggs for all described mesocestoidids are generally consistent
in form and size (Figure 62), egg number appears to vary in
direct proportion to parauterine organ size. Numbers of
testes or proglottids and measurements of various structures
used as taxonomic criteria for species of Mesocestoides have
been compared (Table 2) and have been found to be unacceptable.
The quantitative and qualitative overlap (Figures 2 and 3) of
all criteria precludes their usefulness in the designation
and/or identification of species of Mesocestoides.

Witenberg (1934) in Europe, and Voge (1955) in North America, realized the enigmatic state of this cestode group and attempted clarification. The species of Mesocestoides, however, remain poorly delineated if, indeed, more than one species exists. Witenberg (1934) has shown that length of neck, width of scolex, size and shape of cirrus pouch or of female organs as well as number of testes are all morphologically unstable and therefore unsuitable as criteria for classification.

Observations made in this study and those of Voge (1955) support Witenberg's (1934) rejection of several taxonomic characteristics already stated. He did, however, retain as valid species criteria, shape of segments and uterus as well as distribution of testes. But, as he wisely cautioned, the anatomy of this genus is so unstable that even the most distinctive characteristics overlap to such a degree that species identification, if possible, is most difficult. On the basis of his studies, Witenberg (1934) reduced to three the 25 then recognized species; he retained M. lineatus (morphae: caesta, lineata and litterata) from mammals as well as M. perlatus and M. charadrii from rapacious birds. He separated these species as follows:

	Testes mostly or entirely medial to excretory vessels	Testes mostly external to the excretory vessels
Segments longer than broad	M. lineatus (forma lineata) (forma litterata)	M. charadrii
Segments broader than long	M. lineatus (forma caesta)	M. perlatus

Even if retention of morphae or varieties were desirable, length and width of segments do not, as discussed above, appear to be acceptable criteria. My own studies of proglottid form (Tables 2, 14, 15) as well as those by Voge (1955) have indicated that because of such variability in length and width of proglottids from both identical and/or different host species, this taxonomic characteristic should be discarded. If the uselessness of proglottid shape is accepted, there remains as a distinguishing characteristic only the position of the testes relative to excretory canals. Again, in those mesocestoidids examined by me and in those figured by Voge (1955), placement of testes relative to the excretory canals appears to be ambiguous and its weakness as a taxonomic criterion is clear. Witenberg (1934) has indicated that no sharp boundary exists between M. lineatus and M. perlatus and that the anatomy of the two species is substantially similar. The slightly pink appearance of M. perlatus in its living state is not restricted to Mesocestoides from birds, for I have noted on several occasions color variations from white through yellow to pink in living specimens taken from mammals.

The trapezoidal shape of the proglottids of M. perlatus used also by Voge (1955) as a species characteristic does not hold. Observations in this study have indicated that proglottids of Mesocestoides, laboratory reared from infective tetrathyridia of known lineage, may be trapezoidal, square or horizontally or vertically rectangular (Figures 9, 66, 67). M. charadrii, retained by Witenberg (1934) as a valid species, is reportedly rare, and Witenberg (1934) himself noted that M. tenuis Meggitt, 1931, (= M. lineatus) from a puppy is identical to M. charadrii. Hence, it would appear that those species of Mesocestoides described between 1782 and 1934 are most probably all M. lineatus (Goeze, 1782).

Voge (1955) has not attempted a study of European forms but has concentrated on revising those species of Mesocestoides described from mammals in North America. She has, however, examined some original material from Europe and her descriptions of these specimens do not differ significantly from details presented by Witenberg (1934) for M. lineatus. Voge (1955), in an attempt to lessen the existing systematic confusion, compared numerous specimens of the then six known species (currently eight) of Mesocestoides described from mammals in North America; her results may have compounded the problem. She has shown that specific characteristics now in use do overlap, but nonetheless, has retained, although questionably, three North American species: M. corti, M.

kirbyi and M. latus. She has not chosen to refute Witenberg's (1934) three valid species. M. latus, she has pointed out, does not have trapezoidal proglottids common to the other species and M. kirbyi possesses a distinctive "hairy" or "spiny" cirrus. It is interesting that Muehling (1898) described spiny cirri in some of his mesocestoidid specimens from mammals. In this study, cirri with hair-like processes have been observed. Such processes as Voge (1955) has noted may be of muscular origin, but contrary to Voge (1955) are apparently not limited to M. kirbyi. Voge (1955) further states that the high number of testes in M. kirbyi is another valid diagnostic feature for this species, yet her own comparative tables show considerable overlap of this characteristic with that of other species (see Table 2). Testes number according to Voge (1955) for M. kirbyi ranges from 54 - 128 (average, less than 100), and numbers of testes in other so-called valid species range from a low of 20 (average, 30) in M. manteri to a high of 117 (average, 105) in M. latus from opossum in Texas. It would seem that testes number, using Voge's (1955) data, is not an acceptable species characteristic and that Mesocestoides latus, corti or kirbyi cannot be distinguished from one another on the basis of any currently employed diagnostic features. Unfortunately, since Voge (1955) published her revision, two new mesocestoidid species, M. jonesi Ciordia, 1955 and M. carnivoricolis Grundmann, 1956, for which there seems to be no valid grounds

(see Table 2), have entered the American literature.

In summary, no characteristics used in defining the so-called species of Mesocestoides have been found which do not overlap. Further experimental evidence, pending a known life cycle, is needed in order to show conclusively that criteria used in differentiating the various "species" of Mesocestoides are useless. If more than one species does exist, the criteria for separation will probably be physiological and not morphological.

It has been of interest to note during the literature survey for this work that nearly all European parasitologists have heeded the suggested reduction of species and have restricted the number to only two: M. lineatus from mammals and M. perlatus from birds. When and where M. charadrii was dropped from the European listing of mesocestoidid species is not clear, but it does not seem to have appeared in the literature more recently than 1936 (Joyeux and Baer). It is hoped that North American helminthologists will follow this European trend and restrict their species designations for this genus until such time as clear, undisputed distinguishing characteristics are available.

Whether Mesocestoides from mammals will develop in birds or vice versa is not known, but the evident weakness of available taxonomic criteria necessitates a further reduction of species. It is here suggested that Mesocestoides is a

monotypic genus and that all forms of the genus from both birds and mammals be relegated to the single species, M. lineatus (Goeze, 1782).

Geographic distribution

A thorough search of the literature indicates Mesoces-toides to be a surprisingly ubiquitous cestode genus. Hosts for both adult and larval stages, their general distribution and specific collection localities (when known) are represented in Table 3. In addition, Figure 1 presents habital zones denoting general distribution and/or collection sites. Each zone encompasses the following arbitrarily defined geographic regions: A) most of North America; B) North Africa, Europe and eastern Asia; C) most of Asia and the Orient; D) Arctic, north of 70° N. latitude; E) Central and South America south of the Tropic of Cancer; F) Madagascar, Central of Southern Africa and southern Arabia, south of the Tropic of Cancer; G) Australasia and adjacent islands south of the Tropic of Cancer; H) Antarctica south of 60° S. latitude. Exact zonal boundaries (Table 3) are presented on and coordinated with zones of Figure 1.

Examination of Table 3 indicates a large number and variety of both definitive and intermediate hosts reported as having been infected with some stage of Mesocestoides. Host distribution is so widespread (as shown on Figure 1) that no attempt to plot every recorded host locality has

been made. Any given symbol (Figure 1) may refer to one or more species of infected hosts or to varying numbers of infected individuals. It would appear that the greatest number of infected host species has been reported from the more temperate regions of the world. No Mesocestoides have been reported from continental Australia or nearby islands. This and the fact that Australia and associated islands have not been connected with the Asian mainland since the late Cretaceous (as evidenced by fossil land fauna), have provided strong bases for postulating a host and geographic origin for Mesocestoides. This is discussed in the following section, and, in addition, some indication of possible phylogenetic position of the genus will be noted.

Hosts

It has long been known that Mesocestoides infects a large number and variety of host species. A listing of naturally infected hosts appears in Table 3. Because Mesocestoides is an obligate parasite, a review of its recorded, naturally infected hosts may provide valuable information relative to phylogeny, taxonomy, ecology and subsequent experimental studies. An attempt has been made to correlate previously published information with data accumulated in this study from the examination of 365 potential hosts which have embraced 14 North American species (Table 4). Potential hosts are here considered to be those species or related

groups from which Mesocestoides have previously been reported.

Of hosts examined for adult stages, seven species harbored this cestode and 81 or 28.4 percent of 285 individuals of these seven species were infected with various developmental stages of adult Mesocestoides. Regrettably, only viscera of many hosts were obtained for examination (from the Iowa State Veterinary Research Division), and in such instances, only cestodes were prepared for identification. Refrigerated or frozen viscera, often removed from the host for three to five days previous to examination, contained living helminths, including Mesocestoides. Potential hosts which proved negative for adult mesocestoidids have not been tabulated but include 13 Felis catus L. (all but two from Iowa), 7 Taxidea taxus Waterhouse, 18 Mustela vison Schreber, 1 Mustela erminea L., 17 Canis latrans Say, approximately 20 Mus musculus L. and 3 Bubo virginianus (Gmelin). Numbers of other hosts besides those potential host species listed have been examined and also found to be negative for adults of Mesocestoides. Collections made by the Iowa State University Parasite Research Unit from 1952 to 1967 (unpublished records) have provided additional supporting data corroborating the mesocestoidid host record herein reported. No hosts, other than anurans, were found to be naturally infected with larval stages.

It is a matter of record that Mesocestoides is primarily

a cestode of carnivorous birds and mammals, although it has been reported from the common house mouse (Mus musculus) by Krabbe (1865), Hoeppli (1925) and others. Equally well known is the fact that carnivore hosts ordinarily obtain their infections from a larval stage, the tetrathyridium, which may also be found in a great variety of hosts (Table 3).

How Mus become infected is still unknown. Experiments by Henry (1927), Witenberg (1934) and Srivastava (1939) have shown that mice exposed to mesocestoidid eggs do not become infected with either adult or larval stages. In this study, mice similarly fed, proved negative upon examination, and these results have supported the findings of earlier investigators. Mice are often insectivorous as are, to some degree, most of the definitive mesocestoidid hosts. This is suggestive of the generally assumed presence of a third and probably invertebrate intermediate host. Whether or not an invertebrate primary host is required in the enigmatic life cycle of this cestode group is a point of conjecture for future discussion.

The domestic mouse is known to act both as the definitive and intermediate host for Mesocestoides, and adults from this host do not appear to differ significantly from other mesocestoidid species (Voge, 1955). Several other naturally infected definitive hosts of Mesocestoides have also been reported to harbor both larval and adult worms. Among these are the following mammals:

domestic cats. . . . . . .Ejsmont (1928b), Henry (1927),
Petrov (1940), Blumberg (1882),
Cadeac (1909)

domestic dogs. . . . . . .Petrov (1940), Fagasinski and
Piusinski (1960), Blumberg
(1882), Neumann (1905), Railliet
(1893), Riley (1921), Setti
(1897), Ward (1895), Baumann
and Böhm (1941) and Ssolonitzin
(1933)

wolves (Canis
lupus) . . . . .Neumann (1896)

jackal (Canis
aureus). . . . . .Neumann (1896)

genet (Genetta
genetta) . . . . .Buck and Buckley (1959)

raccoon dog
(Nyctereutes
procyonides). . . . . .Penrose (1882)

mustelids:
(Martes foina) . . . . .Petrov (1940)
(Martes martes). . . . .Petrov (1940), Diesing (1850),
Alessandrini (1907), Railliet
(1893)
(Mustela erminea). . . .Petrov (1940).

In addition, both Diesing (1850) and Rudolphi (1819) have

reported larval forms in species of falcons known to harbor

adult worms. Infections of definitive hosts with both

larval and adult Mesocestoides are interesting but not sur-

prising. It has been shown repeatedly (Henry, 1927, Witen-

berg 1934, Ssolonitzin 1933, and others) that tetrathyridia

under unfavorable conditions (not necessarily in unsuitable

hosts as suggested by Witenberg (1934)) may penetrate a

host's intestinal wall and remain as larval forms within

the body cavity.

Of all definitive hosts examined in this study, the raccoon (Procyon lotor) proved to be most commonly infected; of 71, 45.1 percent proved positive for adult Mesocestoides. The opossum (Didelphis marsupialis L.) was next in abundance, 33.3 percent of 93 examined harbored the parasite. Similar and even higher percentages of infection have been reported for other species in other parts of the world. Intermediate hosts, a discussion of which follows, appear to be so varied that one or more intermediate hosts would presumably be available in most localities throughout the world. With an initial infection present and assuming the availability of an intermediate host, it is proposed that the percentage of infection of any one definitive host species is probably closely related to and is dependent upon its food habits. For example, Srivastava (1939) found that nearly all Tropidonotus platyceps, a non-poisonous snake of India, were infected with tetrathyridia; however, dogs, the local major definitive hosts, did not readily eat these snakes. Upon further investigation, local lizards and rats, common prey of local dogs, were found to be infected almost as heavily as Tropidonotus.

The large number and variety of definitive hosts strongly indicates that the genus Mesocestoides is a highly adaptable parasite, showing little host-specificity and presumably, on this basis, is phylogenetically primitive. Its high percentage of infection in a marsupial (the opossum)

would tend to suggest an ancient origin for members of the genus, but geographic evidence indicates otherwise. As indicated above, <u>Mesocestoides</u> is absent from Australia, Tasmania and surrounding islands. The early (presumably late Cretaceous) separation of this region, as implied by its relict marsupial population and by its fossil record, prevented the invasion of the more ecologically successful and more recently evolved Carnivora. If <u>Mesocestoides</u> were a primitive helminth, it should (assuming the presence of requisite intermediate hosts) be in the Australian region. Its absence from the Australian continent and associated islands, when correlated with its infection among the Carnivora elsewhere, implies a parasitic origin of <u>Mesocestoides</u> in the Carnivora. A single record from an Australian-Tasmanian marsupial was reported by Ransom (1907) who described a larval form from the Tasmanian "wolf", <u>Thalicynus cynocephalus</u>, which had died in a Washington, D.C. zoo. This single record, often repeated in the literature (see Imperial Bureau of Agricultural Parasitology, 1933) cannot, with certainty, be accepted as a record from Tasmania and probably is not. If mesocestoidids originated in carnivorous birds, as might be suspected, the distribution of this cestode group would not have been so sharply restricted by the oceanic separation of the Australian region proper from Asia; there are, from this latter region, several species of raptorial birds which are known to harbor

adult Mesocestoides. Marsupials other than those of Australia and adjacent areas apparently have succumbed to the amazing adaptability of this cestode, and it is therefore proposed that Mesocestoides as a group has evolved recently, together with the Carnivora and is, most likely, a highly adaptable group of primitive cyclophyllidean cestodes rather than a highly specialized (phylogenetically advanced) conglomerate of morphologically indistinguishable species.

Support for the cyclophyllidean nature of the genus is offered below as well as in Section II. Morphological, geographic, and host-correlated data lend support to Spasskii (1951) who has suggested an early anoplocephalid origin for Mesocestoides. He has based his contention on the resemblance of early larval stages ("larvacyst") of Mesocestoides to the cysticercoids of some anoplocephalans. Whether mesocestoidids arose parallel with or shortly following the appearance of taeniid cestodes, as proposed by Spasskii (1951), is a con- troversial point; findings in this study may tend to support, rather than to refute, his interesting viewpoint.

The family Mesocestoididae contained but one genus, Mesocestoides, until Voge (1952) described Mesogyna (genotype: M. hepatica Voge, 1952) from the liver of Vulpes macrotis arsipus collected in California (U.S.A.). It is suspected that this new genus is, in actuality, an aberrant form of Mesocestoides which has developed abnormally in the liver of its host. Tetrathyridia have been known to develop

strangely under various conditions. Teratogenic specimens of Mesocestoides have been reported by Ciordia (1955) and Voge (1955) and are not uncommon among those mesocestoidids collected during this investigation (Figures 69, 70, 71). If members of Mesocestoides were highly specialized, reports of their ability to develop in an abnormal host or organ would be less acceptable, but the primitive, highly adaptable nature of this cestode group makes the possibility of recovering such anomalous specimens seem plausible. The fact that members of Mesocestoides have been known to survive in an abnormal host implies that a specimen might survive equally well, though rarely, in an abnormal location within a normal host. Evidence to this effect has been reported more recently by Hart (1967) who has described strobilate tetrathyridia in experimental in vivo and in vitro studies.

The report of Mesocestoides from a fish, Anguila vulgaris, by Creplin (1825) was in error and has been shown by Riggenbach (1896) and Lühe (1910) to have been the genus Proteocephalus. A full discussion of this mistake may be found in Witenberg (1934).

There have been seven reported cases of mesocestoidid infections of man. All reports occurring in the literature have been thoroughly reviewed by Gleason and Healy (1967) who have noted the need for a detailed study of Mesocestoides from all known host species. It is hoped that this investigation has fulfilled that need. Both qualitative and quan-

titative characteristics of those mesocestoidids described from humans are all within the bounds of similar taxonomic criteria noted for other so-called species of the genus. Hence, species criteria listed for those Mesocestoides from man appear to add further support to the one species concept herein proposed.

Tetrathyridia

Relationship to adult

The tetrathyridium larva of Mesocestoides, like the adult, has had a long and complex history. Although the tetrathyridium has been known since 1819, when it was first described by Rudolphi, its relationship remained enigmatic until Leuckart (1874) and Moniez (1880) suspected and proposed respectively that the tetrathyridium is a developmental stage of Mesocestoides. This view has been repeatedly supported by numerous investigations including those of Neumann (1896), Skrjabin and Shul'ts (1926), Henry (1927), Schwartz (1927), Joyeux and Baer (1932), Joyeux, Baer and Martin (1933), Markowski (1933), Witenberg (1934), Srivastava (1939), Carta (1939), Wetzel and Quittek (1940), Soldatova (1944), Maskar (1953) and others. Although Tetrathyridium Rudolphi, 1819 was originally proposed as a generic designation, it is currently considered a type of larva, a viewpoint universally accepted by helminthologists.

This study supplied further support for the mesocestoidid

relationship of tetrathyridia, although little has been added to the overall understanding of this relationship since Neumann (1896) experimentally obtained gravid Mesocestoides from dogs to which he had fed tetrathyridia. More complete information on earlier studies may be found in reports of experimental investigations as well as in general reviews. In this study, tetrathyridia obtained from naturally infected frogs and toads, first recorded as hosts for mesocestoidid larvae in North America by James and Ulmer (1967), when fed to several species of experimental hosts, resulted in the recovery of adult Mesocestoides. Details of these results are considered under "Experimental Studies".

Forty years have passed since experiments of Henry (1927) and Schwartz (1927) conclusively supported the relationship of the tetrathyridium to Mesocestoides, yet the status of this larval form in the mesocestoidid life cycle remains dubious. One, two or three-host life cycles have been suggested for Mesocestoides. Neumann (1896) considered tetrathyridia to be an erratic, immature worm which, instead of following a typical course to adult development, penetrated the intestine and, having reached an abnormal site, eventually perished without further development. He postulated that Mesocestoides exhibits a direct life cycle and that the oncosphere, in a suitable host and location, could develop directly into an adult cestode. However, Neumann (1896) was aware that his experiments were poorly executed

and his results, inconclusive. Skrjabin and Shul'ts (1926)
suggested that tetrathyridia are not aberrant forms destined
to degenerate, but are potentially infective larvae occurring
as a normal part of the mesocestoidid life cycle. In 1927,
Henry and Schwartz, working independently, experimentally
showed Skrjabin and Shul'ts (1926) to be correct. Witenberg
(1934) indicated that tetrathyridia were able to reestablish
themselves as tetrathyridia in normal definitive or in ab-
normal hosts which he termed "facultative hosts". Might not,
he questioned, the tetrathyridial stage be entirely unnec-
essary to the mesocestoidid life cycle? Although experi-
mentally unable to establish infections of tetrathyridia by
feeding eggs to laboratory hosts, Witenberg (1934) concluded,
as had Henry (1927), that the tetrathyridium is a second
larval stage and that a primary stage, yet undiscovered,
must exist. Schwartz (1927) has suggested that the
tetrathyridium is to Mesocestoides as sparganum or cysti-
cercus is to Spirometra or Taenia, respectively. However,
the fact that tetrathyridia will penetrate a host's intes-
tine and continue to exist as larvae is one of their dis-
tinctive features.

Carta (1939) with little or no substantiating evidence
has proposed a typical cyclophyllidean cycle for Mesoces-
toides, stating that tetrathyridia are undoubtedly parasites
in paratenic hosts and that only a single, undiscovered,
first intermediate host is required. His views must be

relegated to the ranks of conjecture. In an equally dogmatic

manner, Antamaran (1954) stated, "It is beyond doubt now that

the tetrathyridium [his underscoring] carried by the verte-

brate intermediate host is the second of two larval stages

of [Mesocestoides]." Although logical, this proposal is

suppositional and must be considered as such. Hence, it is

evident that the status of tetrathyridia in the life cycle

of Mesocestoides is still questionable. That these larvae

can and do become Mesocestoides is clear, but whether or not

tetrathyridia are an obligatory part of the life cycle is

still not known. Only when the complete mesocestoidid cycle

has been made clear can the number and status of its hosts

be ascertained. At present, it is impossible to state that

Mesocestoides requires one, two or even three hosts in its

development, and these suppositions made as statements of

fact must be categorically rejected.

If the tetrathyridium is an unncessary stage in the

life cycle, or if it is a necessary stage requiring re-

establishment in a new host, the use of "paratenic host"

suggested by Carta (1939), and assumed to have the meaning

given it by Baer (1951), is preferred to that of "facultative

host" used in the same sense by Witenberg (1934) and

Srivastava (1939). However, it is possible that tetrathy-

ridia are required in the life cycle, and under such con-

ditions, only those hosts infected with reinvasive tetrathy-

ridia would be paratenic. The first host to carry tetrathy-

ridia should be designated as "second intermediate" if it is
shown that an obligatory, non-tetrathyridial stage precedes
it; on the other hand, such a host should be referred to as
an "intermediate host" if tetrathyridia develop directly
from the oncosphere without an intervening, invertebrate
host. However, like the status of the tetrathyridium itself,
proper host terminology, for which some alternatives have
been proposed, must await a completed life cycle.

Geographic distribution

Geographically, tetrathyridia are as widely distributed
as are adults of the genus. Table 3 includes both general
distribution of potential host genera and specific localities
from which these larval forms have been reported. Localities
from which the heaviest tetrathyridial infections have been
reported occur, as expected, in those areas most heavily
infected with adults. The greatest number of infected host
species has been reported from Asia (Figure 1, Zone C), but
it is entirely possible that the high number of reports from
Asia is a direct reflection on the extensive parasite surveys
undertaken in that area. It is suspected that similarly
organized surveys in other parts of the world (Australian
region excepted), would yield considerably more host species
infected with tetrathyridia than are presently known. As
previously discussed, the absence of tetrathyridia from
Australia does not preclude the presence of potential hosts,

and hence the possible extension of the infection to that
continent.

On the basis of number and variety of hosts which have
been shown to be either naturally or experimentally suscep-
tible to infection with tetrathyridia, it is doubtful that
any world locality can continue to remain mesocestoidid free.
Man, it would seem, with his domestic animals is a prime
disseminating agent for this cestode and has possibly
accounted for much of its widespread distribution.

Hosts

Rudolphi (1819) described larvae (now known to be
tetrathyridia) from reptiles, birds, and mammals. Amphibians
were first implicated in the mesocestoidid host complex by
Joyeux and Baer (1933), who reported tetrathyridia in an
experimentally infected European tree frog (Hyla arborea).
The second report, and first natural infection, of this
larval stage in an amphibian host was that of Dubinina
(1950), who recovered tetrathyridia from the edible Euro-
pean bullfrog (Rana esculenta). Subsequently, Vojtkova
(1963) also described tetrathyridia from this species of
bullfrog and added the fire bellied toad (Bombina bombina)
collected in Czechoslovakia as an additional host. Dollfus
(1965) considered the presence of tetrathyridia which he
found in R. esculenta to be accidental. From these records
and from evidence obtained during this investigation, it

would appear that amphibians are, without question, additional hosts of tetrathyridia. Thus the complex of hosts capable of harboring tetrathyridia now includes amphibians, reptiles, birds, and mammals. The wide range of phyletically diverse hosts for larval mesocestoidids is even greater than that exhibited by adults. It is parasitologically unusual for larval cestodes to be so adaptable. Perhaps the phyletic diversity exhibited by tetrathyridia is another indication of the presumably primitive and unspecialized nature of Mesocestoides. Tetrathyridia have been reported from 8 orders of mammals, 11 orders of birds, and 2 each of reptiles and amphibians (Table 3). Reptiles and amphibians have never been reported to harbor adults; however, 14 species in 10 genera of lizards, 18 species in 14 genera of snakes, 3 species each in 2 genera of frogs and 2 genera of toads have been recorded as hosts of tetrathyridia. Among mammals, 61 genera are represented as harboring Mesocestoides, 42 of these incorporating 74 species infected with the larval stage. The 11 orders of birds listed as mesocestoidid hosts include 34 genera, of which 18 (22 species) were reported to have contained tetrathyridia. Among the mammals, 4 orders have species known to harbor both adult and larval stages although not necessarily at the same time. Similarly, among birds, only 2 orders have produced both developmental stages (Table 3).

Maskar (1953), referring to Neveu-Lemaire (1936), re-
ported that Schwartz (1927) has indicated the presence of
tetrathyridia in the kangaroo. This report is apparently in
error for no reference to this host could be found in either
of the two references Maskar cited, and the error may have
been due to Maskar's interpretation of the word "marsupial",
which does occur in Neveu-Lemaire (1936).

During the five years of this investigation, numerous
hosts, representative of potential intermediate hosts for
Mesocestoides, have been thoroughly examined for both larval
and adult stages. In addition, since 1953, the Parasite
Research Unit of Iowa State University and the Iowa Lakeside
Laboratory have contributed its records spanning 15 years
of parasite-host surveys of Iowa, and these have added con-
siderable support to the collection records of the writer.
From the large numbers of hosts examined, only anuran
amphibians were found to be naturally infected with tetrathy-
ridia (James and Ulmer, 1967). Amphibian host species
found in this study to be naturally infected with tetrathy-
ridia were Bufo americanus, B. cognatus and Rana pipiens.
All of the toads and some of the frogs were caught wild in cen-
tral, southeastern and northwestern Iowa and southeastern South
Dakota; most of the R. pipiens came from supply houses in
Wisconsin and Minnesota. Mr. E. G. Steinhilber*, whose supply

---

*Steinhilber, E. G. Steinhilber & Company, Inc.,
Oskosh, Wisconsin. Personal communication (letter). 1967.

house provided most of the frogs, has indicated that those frogs obtained from him could have come from Mexico, Canada, Minnesota, or the Dakotas.

The report by James and Ulmer (1967), representing the first North American record of tetrathyridia from Amphibia, includes three new host species and the first world report for tetrathyridia in the family Bufonidae. Tadpoles as well as immature and adult hosts were examined, but only the larger and presumably older animals harbored tetrathyridia. The percentage of infection was low: 2.5 percent of 433 B. americanus, 5.9 percent of 17 B. cognatus and 1.7 percent of 1,568 R. pipiens. In addition, other amphibians were all negative for tetrathyridia. These included Rana clamitans, R. catesbeiana, Acris gryllus and the tiger salamander, Ambystoma tigrina. Vojtkova (1963) has reported that in Czecheslovakia 9.6 percent of Bombina bombina and 25 percent of R. esculanta were infected with tetrathyridia. From her description, tetrathyridia taken from the European amphibian hosts are identical to those described from North American species.

Of those R. pipiens and B. americanus whose sex was recorded, the following was noted: Among 428 R. pipiens (166 male, 262 female) only 1 of 4 hosts (25 percent) infected was male; of 275 B. americanus (249 male, 26 female) 5 of 6 (83 percent) infected hosts were male. Because the sex showing the highest percentage of infection is also the

sex making up the greatest part of the total number of in-
dividuals examined, it is doubtful that frequency of tetrathy-
ridial infections is related to sex of the host. It is pre-
sumed that an even distribution of host sexes would result
in a relatively similar even distribution of infected animals.

Tetrathyridia, varying in length from 0.32 to 1.40 mm.,
were usually scattered in numbers of about 30 to 4,000
throughout the bodies of their frog and/or toad hosts (Fig-
ures 110 and 111). Tetrathyridia generally occur encysted
in single or multiple cysts of apparent host origin (Figures
73, 74, 75). On rare occasions, larvae were found unencysted
in the abdominal cavity. Large numbers of tetrathyridia in
hosts are not uncommon; Witenberg (1934) reported, from
various sources, over 1,000 from a cat, 950 in a mouse, and
300 from a magpie. Since no pathological change, other
than the formation of the cyst wall, seems to occur in
amphibian hosts, there appears to be no reason why these
hosts cannot support the heavy infections which have been
reported. A section of a large cyst (Figure 74) from a
frog was found to be very similar to a section of a cyst
taken from a hedgehog and illustrated by Maskar (1953:192).
That extremely large numbers of tetrathyridia are found in
hosts without any visible indication that asexual multipli-
cation has occurred (either in frogs, toads or mammalian
experimental hosts), coupled with the fact that nearly all
these parasites are encased in cysts of host origin, tends

to add support to the plausibility of a single intermediate
host cycle. Even though tetrathyridia vary in size, their
associated cyst walls are complete and in a single host are
invariably at the same stage of development. This combina-
tion of factors (i.e., large numbers of tetrathyridia,
similarity of cyst wall development, and lack of asexual
reproduction) implies a single rather than multiple infec-
tion. Accordingly, it is difficult to imagine any inverte-
brate host available to hosts of tetrathyridia, which is
large enough to support the thousands of parasites known
to occur in a single amphibian host. Furthermore, it is
reasonable to assume that oncospheres may, in some manner,
develop directly into tetrathyridia. Under such conditions,
a one intermediate host, typically cyclophyllidean, life
cycle is implied. As yet, however, no experimental evidence
had provided support for this suggestion.

Almost all organs of amphibian hosts may harbor
tetrathyridia, but most of the parasites are usually found
embedded dorsally in the kidney tissue, within the intes-
tinal wall, or attached to or surrounded by mesentery of
the brachial region (Figure 74). No indication of asexual
reproduction of tetrathyridia was observed. Morphology of
those tetrathyridia removed from amphibian hosts is similar
to that described in numerous previous reports dealing with
larvae as well as those from various hosts examined during

this study (Figures 22 thru 29 and 73 thru 76). The
majority of reported tetrathyridia are non-vesicular, solid
larvae. No cystic forms as described by Carta (1939) or
Markowski (1933) have been seen in this study although a
few acephalous individuals, perhaps similar to those
described by Witenberg (1934), were observed. The smallest
tetrathyridium recovered (Figure 25) was poorly developed
internally and in general appearance, including its outer
cyst of host origin, resembled the larval stages described
by Soldatova (1944) as cysticercoids which she recovered
from mites experimentally fed with eggs of Mesocestoides.
The very close resemblance of these two larval forms, one
in an invertebrate, the other in a host known to harbor
tetrathyridia, lends some support to Carta (1939) who has
proposed a life cycle for Mesocestoides requiring but one
intermediate host.

The tetrathyridium, as a larval stage, has been
variously described. Freeman (1957) considers it to be
cyclophyllidean plerocercoid, not unlike the sparganum;
Markowski (1933) has referred to the encysted and free forms
as cycticerci and plerocerci, respectively. Voge (1967),
from her in vitro studies of mesocestoidid development,
has suggested, on the basis of an apparent apical organ
observed on the developing "tetrathyridium" (the early
development of which she has shown to be procercoid-like),
that this larval form possesses both proteocephalan and

pseudophyllidean affinities. Although the early stages of tetrathyridial development appear to be procercoid-like, as indicated by Voge (1967a, b), and hence are suggestive of a proteocephalan-pseudophyllidean relationship for Mesoces-toides, subsequent developmental stages of the tetrathy-ridium are distinctly cyclophyllidean and add considerably to evidence supporting the affinity of mesocestoidids with this group. Tetrathyridia recovered from naturally infected frogs and toads were used in subsequent experimental studies in order to understand better the developmental and systematic affinities of this enigmatic genus. These are discussed in detail below.

EXPERIMENTAL STUDIES

Eggs and Oncospheres

Feeding experiments

The following experimental investigation was undertaken
to understand better the biological affinities of Mesoces-
toides already implied above in studies of natural infections.
Although the life cycle, initially planned as a part of this
study, was not completed, accumulated experimental data have
contributed to a better understanding of the phylogenetic
and systematic relationships of both larval and adult stages.
These studies have involved feeding eggs or tetrathyridia
to potential hosts and injecting eggs or tetrathyridia into
various organisms.

Invertebrate hosts   A variety of invertebrate and
vertebrate hosts were exposed to whole or crushed proglottids,
to parauterine organs, or to eggs pressed from or crushed
within proglottids. Normally shed proglottids were obtained
throughout the course of this investigation from naturally
or experimentally infected hosts. Since a large number of
proglottids was required, several hosts and host species
were necessarily utilized. Although attempted infections
of invertebrate hosts have been repetitions of, and have
supported much of Webster's (1949) work, some additional
new hosts have been used (Table 7). Only those inverte-
brates which ingested some part of infective, egg-bearing

material have been included in the data. Tribolium,
Tenebrio and Stegobium (beetles) as well as Collembola
and Hyalella were laboratory reared; all other hosts listed
in Table 7 were collected from the wild. The results of
these experiments suggest the desirability of additional
study of these hosts. Of the large number of invertebrates
exposed to eggs of Mesocestoides, only one each of three
species, Necrophorus orbicolis, Tenebrio molitor and
Onthaphagus hecate, was found to be infected with some kind
of helminth or helminth-like larval forms following exposure.
All other invertebrates examined were negative.

The report of Soldatova (1944) of oribatid mites as
first intermediate hosts for Mesocestoides seems premature
in light of evident lack of additional experimental support
and is, hence, rejected on this basis. In this study, large
numbers of oribatid, mesostigmatid and a few other unidenti-
fied mites were fed eggs of Mesocestoides, but none became
infected, regardless of the variety of conditions under
which feeding experiments were conducted. It is also un-
likely that a mite could support the numbers of larval
helminths so often found in some hosts for tetrathyridia,
amphibian or otherwise. The possible occurrence of repeated
infections of tetrathyridial hosts through ingestion of
mites is improbable, since all tetrathyridia in any one
host appear to be of approximately the same age. Similarity
in degree of development of tetrathyridia and of their cyst

walls was used as a criterion for determining comparative age of infections. Evidence of repeated infections of a host, which presumably would result in varying degrees of development of both the parasite and its host-produced cyst wall, was never apparent in exposed organisms. Since no sign of asexual reproduction in natural infections of tetrathyridia, as described by Specht and Voge (1965) and Voge and Coulombe (1966), has been noted, this mechanism, presumably abnormal, does not appear to be a typical, naturally occurring process. Ssolonitzin(1933), in experiments with dogs, reported asexual multiplication of tetrathyridia by budding as an apparently new form of reproductive process for Mesocestoides, but noted that many of the asexually produced larvae were abnormal and were incapable of developing into adult worms. Further discussion of investigations dealing with tetrathyridial reproductive capacity and development has been included in a following portion of this thesis under the heading "Tetrathyridia".

Results of my studies support the investigations of Whittaker[*] who, having fed mesocestoidid eggs to numbers of laboratory reared oribatid mites, suggests that, at best, mites perhaps act as abnormal invertebrate hosts. It is doubtful that the "cysticercoids" described by Soldatova (1944) were viable. She was unable to complete the cycle

---

[*]Whittaker, F. H. University of Louisville, Louisville, Kentucky. Personal communication (letter). 1967.

using her experimental cysticercoids from mites and suggested
that her larvae were not old enough to be infective. No
further work has appeared to establish her contention that
the mite is a normal, first intermediate host of Mesoces-
toides. The validity of Soldatova's proposed mesocestoidid
life cycle must, hence, be considered questionable.

Several hundred adult and larval tenebrionid beetles
(T. molitor) received mesocestoidid eggs fresh or after
having been subjected to conditions of drying, wetting,
aging, cooling, shaking and predigestion in hosts of both
the same and different invertebrate species. One adult
Tenebrio contained at least three oncospheres of Mesoces-
toides. These were observed crawling in or on intestinal
tissue when the intestine was removed and examined under
coverslip pressure. Although active, oncospheres had not
changed form and were identical to those released artificially
from eggs (Figure 32). Measurements of living larvae aver-
aged 48 x 24 u. When fully extended, larvae attained a
length of 68 u. Because no other tenebrionid was ever
found to be infected, it was assumed that the egg shell had
accidentally ruptured and a few oncospheres managed to
escape and remained alive until that particular host was
examined. Numbers of egg shells and isolated hooks ordinarily
seen in the hind gut or faeces of exposed tenebrionids indi-
cate that these invertebrates are not acceptable hosts for
this parasite.

One of several sexton beetles (<u>Necrophorus</u> <u>orbicolis</u>),
caught wild and fed large numbers of fresh proglottids,
contained typical, unarmed cysticercoids (Figures 18 and 19).
These larvae were encysted in host tissue adjacent to the
haemocoel and were especially numerous attached to or in
association with tracheal tubules. Calcareous corpuscles
were numerous, and four distinct suckers were visible. In
general, these larvae measured 0.58 mm. x 0.51 mm.; their
cysts averaged 0.74 mm. x 0.69 mm. Suckers, almost circular,
measured approximately 0.1 mm. in diameter. At the time
this infection was observed, local amphibians were not known
to be hosts of tetrathyridia; consequently, the infected
beetle was fed to a laboratory-reared opposum which ate it
ravenously; upon examination 35 days later, however, this
host was negative. Attempts to repeat the infection of
<u>Necrophorus</u> with mesocestoidid eggs have not been successful,
and no further cestode larvae from this host, similar to
those figured (Figures 18 and 19) have been found. Addi-
tional feedings of <u>Necrophorus</u> beetles are contemplated.

Witenberg (1934) and others have suggested that dung
beetles might be proper first intermediate hosts for <u>Mesoces-</u>
<u>toides</u>. Joyeux and Kobozieff (1928) described "cysticerci"
from <u>Geotrupes</u> <u>sylvaticus</u> which, they indicated, might be
larvae of <u>Mesocestoides</u>; they were, however, unable experi-
mentally to substantiate their opinion. In my studies, dung
beetles, <u>Onthophagus</u> <u>hecate</u>, usually copraphagous but scav-

engers of carrion as well, were collected from beneath cow
dung and, in the laboratory, were fed a constant supply of
proglottids placed on small pieces of decaying meat during
a period of approximately 2 to 4 weeks. Examination of
exposed beetles yielded one which contained, in its haemocoel,
numerous, odd, acephalous, larval organisms shown in Figures
15 and 16. These organisms, containing obvious calcareous
corpuscles, were never experimentally identified, but may
be similar to those acephalous tetrathyridia described by
Neumann (1914). They most certainly bear a surprising like-
ness to those developing stages of Mesocestoides shown by
Voge (1967). Although subsequent feedings did not produce
more of these larva-like forms, it is strongly felt that they
might be early developmental stages of Mesocestoides, but
whether they are normal or abnormal, necessary or unnecessary
to the cycle, is not yet known. Experiments in progress will
attempt to answer some of the questions raised by such un-
usual larvae. It should be noted that at the time of these
findings, the importance of frogs and toads in the cycle of
local mesocestoidid infections was not known. The beetle
carcass containing a number of these organisms was fed to a
mouse which was negative upon examination almost one month
later. The larvae recovered from Onthaphagus, like those
described by Neumann (1914) and Meggitt (1931) were free in
the coelom. They moved with a rapid, gliding effect and
their extremely plastic bodies were capable of twisting,

turning and circumnavigating obstacles. Flexibility of shape exhibited by these individuals is illustrated by Figure 15. In addition to these presumed larvae, spherical structures were also present in the coelom. These did not move or change shape, but like the "larvae", possessed calcareous corpuscles and are possibly different developmental stages of the same organism. The largest of these spherical non-motile organisms was approximately 150-160 u in diameter; the smaller of two motile specimens measured 84 u in length and the larger, 240 u. Calcareous corpuscles appeared typical in both types, and some measured up to 24 u in greatest diameter. Cephaline gregarines, common in these beetles, are easily distinguishable from these unidentified organisms.

The presence of such organisms in Onthophagus and their great resemblance to larvae shown by Voge (1967) suggests that this genus may be a satisfactory host for Mesocestoides. Perhaps, as with adults and tetrathyridia, numerous hosts are susceptible to infection with mesocestoidid eggs, but the role of the coprophagous insect in the life cycle of Mesocestoides deserves renewed experimental attention. It is entirely possible that acephalous larvae observed in vertebrate hosts by Neumann (1914), Meggitt (1931) and those reported from in vitro cultures of Voge (1967) and now, presumably, in the beetle (Onthophagus hecate) are all a functionally parallel part of the cycle, and that inter-

mediate hosts of <u>Mesocestoides</u> are perhaps more diverse than this or any other study has shown.

<u>Vertebrate hosts</u>   Feeding eggs of <u>Mesocestoides</u> to vertebrate hosts has produced almost wholly negative results. In my experiments, eggs were fed to the following vertebrates: 28 <u>Bufo americanus</u> adults and 39 tadpoles, 68 adult <u>Rana pipiens</u>, 1 canary, 6 opossum, 3 mice, 6 rats, and 1 raccoon. All hosts, other than adult frogs and toads, were laboratory-reared. Among vertebrates, frogs and toads appeared to be the most likely hosts, if tetrathyridia were to develop directly under natural conditions. Numbers of these anurans were consequently fed eggs under various conditions and were examined at equally varied time intervals. Observations resulting from these feedings have produced a clearer under-standing of proglottid structure, oncosphere excitory stimuli, and the effect of host digestion on proglottids and their incorporated structures.

Generally, feedings were accomplished by means of pipette, by tubing, or by merely allowing the experimental host to ingest segments placed on or in some acceptable food. Experimental animals were fed from one to a maximum of 20 proglottids. Proglottids were either fresh or whole, fresh and crushed, dried, or whole ones cooled for various periods of time at 5° C. Prior to all feedings, one or more pro-glottids were checked to determine state of oncospheral

activity. Proglottids were generally discarded if oncospheral
activity had ceased. On several occasions, inactive pro-
glottids were purposely fed to hosts under the assumption
that possibly a dormant period for larvae might be required.
Some amphibian hosts were starved and others well fed before
receiving an egg supply. Upon subsequent examination of
experimental animals, no larvae beyond the stage of free
oncospheres were recovered. In some frogs and toads, starved
or well fed, after parenchymatous and muscular tissues of
proglottids had been digested, parauterine organs broke and
released eggs into the small intestine where oncospheres
actively emerged from the enzyme weakened egg shell (Figures
30 thru 33).

Numerous frogs and toads fed in this manner have re-
sulted in the following observations: (1) no additional
larval development of Mesocestoides could be detected; (2)
all proglottid tissue other than that of the parauterine
organ is readily digested, partially in the stomach and
completely in the small intestine; (3) the wall of the
parauterine organ sometimes breaks, but is never digested
by either stomach or intestinal secretions. Known numbers
of proglottids repeatedly fed to these hosts have resulted
in the recovery from the cloaca of the same number of
parauterine organs, often devoid of eggs.

In starved hosts, the proglottid tissue proper is
digested away within approximately one to two hours; and,

within three to five hours, isolated parauterine organs, free from all other proglottid tissue, can be recovered. In well-fed hosts the process is similar, but requires a longer time. Parauterine organs reaching the cloaca may remain there for days or until a subsequent feeding elicits defecation. The large number of parauterine organs devoid of their egg masses cannot be explained, but the presence of residual parauterine organ tissues following otherwise total digestion suggests, perhaps, a collagenous nature of this organ. Most certainly, its resistance to digestion is indicative of a protective function, the exact role of which must await the solution of a life cycle. Isolated hooks, usually an indication that oncospheres were destroyed, have not been recovered, even though egg shells and calcareous corpuscles of all sizes were frequently observed in the cloacal contents. It is possible that hooks, because of their small size, were overlooked, but this is not probable considering the extent and frequency of feedings, and the great care and time taken in making examinations. Hence, the fate of oncospheres released from eggs (as indicated by empty egg shells, the presence of parauterine organs and the absence of hooks) has not been determined.

It has already been stated that oncospheres may be naturally released from proglottids taken into the digestive system of a frog or toad (Figure 33). The mechanism for

release appears to be similar to that indicated for the artificial technique employed for the releasing of oncospheres for injection experiments described in "Materials and Methods" and discussed below. Once muscles and other organs of the proglottid are digested by stomach and intestinal juices, (presumably a pepsin-trypsin enzyme system) the parauterine organ, free from surrounding tissue, is directly subjected to intestinal conditions. Oncospheres are completely inactive in the acid (pepsin-HCl) medium of the stomach as in the similar medium used in artificial hatching procedures. It is not until they reach the digestive juices of the small intestine that they become active. Bile in the amphibian intestine seems to intensify oncospheral movements, but in heavy concentrations it also kills them. In both artificial and natural digestion, acid-pepsin followed by basic-trypsin are required to release and activate the oncospheres (Figures 32 and 33). Released oncospheres quickly die in any acidic medium, but may live for hours (as many as five in my experiments) in a slightly basic normal saline. It must be assumed that whatever the requisite host may be for the natural development of the oncosphere to occur, it must provide, in proper sequence, the conditions observed and described in this study. Presumably, oncospheres are never released in the stomach, and, most probaly, the release site is the small intestine. It is conceivable, however,

that some non-vertebrate host may provide the requisite conditions for release of hexacanth larvae and subsequent successful development.

The negative results obtained from the feedings of eggs to vertebrate hosts in this study strongly suggests that Witenberg (1934), Henry (1927) and others may be correct in assuming a non-vertebrate host for the earliest developmental stage in the mesocestoidid life cycle.

The possibility of having overlooked larval stages in experimentally-exposed invertebrates or vertebrates was considered, and one set of indirect feeding experiments of eggs to vertebrates _via_ previously exposed invertebrates was undertaken. A dung beetle and a silphid beetle which had been fed one and two proglottids each, respectively, were subsequently fed to two laboratory-reared opossum. Examination of these two vertebrate hosts 36 days post feeding, produced no sign of infection. Apparently the mesocestoidid infection does not appear to involve the indirect passage from beetle to opossum for its development.

Additional experiments similar to those described immediately above were designed to determine whether or not the infection could be produced in a seminatural environment. Attempts to stimulate infections were made by placing several opossum, 2 gophers (_Geomys bursarius_) and 13 toads (_B. americanus_) in large cages in order to approximate, as closely as possible, a natural environment for these hosts. The cage

floor was well seeded with proglottids and large mesh (one inch) screening permitted access to most potential invertebrate hosts and even many small vertebrates into the enclosure. Although this experiment was conducted for more than two months, it resulted in no infections. Since mites, mice, small frogs and toads are common inhabitants of the area where these enclosures were set, it would appear that either the proper host or hosts were not present or that some mitigating factor, yet unknown, controls the successful progression of mesocestoidid eggs (oncospheres) to subsequent developmental stages.

A skunk, examined in 1965, was found to be newly infected with Mesocestoides. The worms were so young that they compared well with those recovered from an experimental dog, raccoon and mouse, only several hours after having been fed tetrathyridia. Several of these young worms from the skunk were pipetted into the stomach of two laboratory-reared mice. One month later, the mice proved negative upon examination. Although only two mice were used in this experiment, it can probably be safely concluded that: (1) post tetrathyridial stages of Mesocestoides cannot tolerate the digestive action of a new host and are, therefore, incapable of re-establishing themselves; (2) the mouse, known to be a suitable definitive host for Mesocestoides, may be an incompatible host because of strain differences; (3) a combination of foregoing factors might have prevented development; or

(4) the young worms, although alive when taken from the
skunk, may have been physiologically weak or in the process
of deteriorating. Because of the number of possible detri-
mental factors, these results are inconclusive.

Injection experiments

Oncospheres have repeatedly been observed actively
moving within their restraining egg shells. It was felt
that although some invertebrates might be capable of sup-
porting oncospheral development, they were incapable of
enabling release of oncospheres because of mechanical or
chemical reasons. Apparently, requisite conditions (dis-
cussed above) were not present for weakening the egg shells,
and oncospheres consequently perished within the eggs.
Therefore, living oncospheres, artificially released from
their shells by means of pepsin-trypsin digestion, were
injected into the hemocoel of several laboratory-reared
tenebrionid beetles. Examination of injected beetles after
a few hours or after several days yielded either negative
results or, in a few instances, only dead and usually
deteriorating oncospheres. It is possible that injection
of living oncospheres into a more suitable host would have
permitted them to survive and develop, at least to some
degree. Artificial digestion techniques used in this study
and described under "Materials and Methods" are little dif-
ferent from natural digestive processes of man or other

vertebrates. It is the apparent requirement of a pepsin-trypsin digestive sequence for oncospheral release that has caused some reluctance on my part to accept the need or even the presence of an invertebrate, presumably insect, host in the life cycle of Mesocestoides. However, evidence from feeding experiments outweighs the physiochemical implications and those who propose the presence of an invertebrate host, whether obligatory or facultative, may be correct.

Injections of artificially released oncospheres into two laboratory-reared mice resulted, upon examination 17 days later, in no apparent infection. It is presumed that for vertebrates, as well as for invertebrates into which onco-spheres have been injected, similar problems exist. The fact that apparently normal oncospheres can be successfully released artificially suggests the egg of Mesocestoides to be a valu-able experimental resource, as Voge (1967) has already demon-strated.

Tetrathyridia

Feeding experiments

Tetrathyridia taken from amphibian hosts during this study were shown to be capable of developing into apparently normal adult worms in a variety of definitive hosts (Table 8). Such studies have reaffirmed numerous earlier investigations which established the relationship of tetrathyridia to Mesocestoides. Whenever possible, tetrathyridia of known

lineage (from a single host) were used to infect numbers of
potential host species. All tetrathyridia were obtained
from three amphibian host species, namely: Bufo americanus,
B. cognatus and Rana pipiens. The low percentage of infec-
tion and great similarity between those Mesocestoides re-
covered from various hosts involved in feeding experiments
have strengthened the already suggested concept that this
cestode genus consists of a single but morphologically
variable species. It is improbable that tetrathyridia from
a single, infected, amphibian host are the product of more
than one exposure, nor is it likely that tetrathyridia from
anuran amphibians develop into more than one species of
Mesocestoides.

Freshly removed tetrathyridia used in feeding or injec-
tion experiments were counted and placed into amphibian
Ringer's solution. Those used for wholemounts or sections
were prepared using techniques similar to those used in
handling adult worms. Feedings were accomplished by intro-
ducing larvae into the stomach or mouth by way of pipette or
plastic tubing or, more often, by placing the desired number
of tetrathyridia on a morsel of food. In some instances,
tetrathyridia, in the absence of a particular species of
definitive host, were maintained for as long as several
months in the body cavities of laboratory-reared mice (see
injection experiments below). Hence, laboratory mice so
used in this study may be considered as experimental, paratenic

hosts. Tetrathyridia maintained in mice, when fed to defini-
tive hosts, developed in a normal manner without differing
noticeably from those fed directly from amphibian hosts. It
is assumed that the change from amphibian to mammalian inter-
mediate hosts is not detrimental to development or eventual
viability, since the resulting adult worms have appeared
normal in every respect. Oncospheres produced by worms from
all experimentally-infected hosts were no less active than
those from naturally-infected hosts. Unfortunately, in the
absence of an experimentally-determined life cycle, oncosphere
viability can only be presumed. In this study, normal appear-
ance of eggs and activity of oncospheres have, by necessity,
been used to gauge infectivity although it admittedly is a
weak criterion.

Whenever possible, laboratory-reared hosts were used,
but the difficulty of obtaining laboratory-reared wild
carnivores in the quantities needed was, and continues to be,
a plaguing problem. In the absence of laboratory-reared
definitive hosts, mesocestoidid free animals were used. By
mesocestoidid free is implied those hosts showing no evidence
of mesocestoidid proglottids during an imposed 30-day mini-
mum quarantine period. It was found that 30 days was usually,
although not always, longer than the average prepatent period.
In addition, faeces were checked for eggs of other helminths,
which on most occasions were removed by administering appro-
priate helminthics approximately one week previous to parasite

exposure. Control animals, unexposed to tetrathyridia, were kept and sacrificed at regular intervals throughout the investigation. No control animal became infected with Mesocestoides.

The presence of other helminths in a host does not appear to hinder subsequent infection with Mesocestoides. Hosts known to harbor ascarids, taeniids, Oochoristica or even Mesocestoides and in which these infections were purposely maintained as a means of checking possible immune response, were readily infected by feedings of tetrathyridia. No immunity seems to have resulted from hetero- or homo- parasitic infections or from reinfections. One raccoon, used as a source for needed proglottids, was repeatedly rein- fected. Hyperparasitism occurred commonly; in one raccoon a heavy infection of Mesocestoides alone completely blocked the lumen of the small intestine from which approximately 40 worms were subsequently recovered. Size or state of develop- ment of worms recovered from heavy infections did not appear to have been consistently affected by crowding. In some heavily parasitized hosts, worms were all of a similar size and development, while in others, size and stage of develop- ment showed extreme variability. For example, a few gravid mesocestoidids (30-60 cm.) were present as well as other worms of the same species varying greatly in size and in stages of development; one immature worm was as small as three centimeters. The same variability occurred in some

hosts where crowding was not evident. In one experimental

coyote, two worms, one gravid and one immature, were re-

covered. It would appear that the presence of other

parasites or the occurrence of large numbers of Mesocestoides

is not a major cause for developmental or size variations

shown to be exhibited by this genus. Similar observations

have been made in hosts naturally-infected with Mesocestoides,

but lack of background information of these infections makes

correlation with experimental results difficult. It is

probable that factors other than crowding or hyperparasitism,

namely physiological factors, may be responsible for the

marked extremes of mesocestoidid variability.

The prepatent period in Mesocestoides infected hosts is

as inconsistently varied as are variations in morphology and

development of the cestode. Wetzel and Quittek (1940) have

described a prepatent period of 16 days, and Maskar (1953)

of 80 days in dogs. Additional periods of prepatency in

dogs and other hosts species have been noted by Schwartz

(1927), Srivastava (1939), Carta (1939), Neumann (1896) and

others. The longest prepatent periods (some over 100 days)

have been described by several workers dealing with infec-

tions in domestic cats. Srivastava (1939) has suggested

that an extended prepatent period implies the presence of a

parasite in an insuitable host. The great variations of the

prepatent period among hosts, such as dog and raccoon, known

to be proper definitive hosts, for Mesocestoides, however,

may indicate that the unsuitability, if it exists, is not necessarily due to host species but to some individual incompatibility between worm and host. It is also possible that the state of development of the tetrathyridium at the time of its ingestion might affect the time required by the worm to become gravid and consequently apolytic. Under no circumstances should prepatency be employed as a systematic criterion for Mesocestoides. In this study, prepatent periods of 16 and 52 days have been recorded from the raccoon; about 30 days is average for this and other successfully infected hosts (Table 8).

The long prepatent period repeatedly described for domestic cats is presumably the result of the unsuitability of this particular host species to Mesocestoides, as has been stated by Voge (1965). Schwartz (1927), Henry (1927), Witenberg (1934) and others also found that cats were difficult or impossible to infect and that ordinarily, successfully infected cats yielded gravid specimens of Mesocestoides only after 109 days (Carta, 1939) or more. Histologically, the structure of the cat intestine is substantially different from that of all other host species examined for Mesocestoides during this study. Its muscularis layers are very thick and villi are low, numerous and small, a combination of characteristics which may contribute to the difficulty of establishing mesocestoidid infections in this host. The relatively common reports of tetrathyridia in the cat may also point to its

general unsuitability as a host for adult <u>Mesocestoides</u>. In
this study, tetrathyridia were fed to cats either directly
from amphibian hosts or from a mouse transfer host which had
received, via injection, tetrathyridia from an amphibian.
Of eight cats fed tetrathyridia, none became infected, and
tetrathyridia were not recovered from the body cavity or
from tissues.

Tetrathyridial feedings to hosts other than cats resulted
in a high percentage of uninfected hosts, and it is probable,
aside from unsuitability of host species or physiological
incompatibility, that technical difficulties were responsible.
The host list presented in Table 8 shows many hosts which
could be fed, whether forcibly or naturally, with infective
larvae. However, undomesticated carnivores were often fed
with great difficulty. Narcotizing carnivores proved diffi-
cult and costly in terms of host loss. Some hosts would eat
readily, but others refused to eat; some mouthed the food
and dropped it after several minutes. Hence, it was not
always clear if and when given numbers of tetrathyridia fed
were actually swallowed by a particular host. As a result
of these difficulties, the percentage of infection obtained
experimentally cannot be considered as an indication of
natural infectivity.

Skarbilovich (1945) pointed out that <u>M. lineatus</u>, col-
lected from foxes, sheds segments in autumn, leaving only
the scoleces within the host. She found very few segments in

winter, and strobilization appeared to cease until the following spring, perhaps as a means of reserving generative powers for a greater abundance of suitable vectors. Observations in this study made throughout the year from natural as well as from experimental infections do not support the contentions of Skarbilovich (1945). Adult Mesocestoides, in every conceivable stage of development, from early immature stages of only a few hours infection to fully-developed gravid worms, have been found at all seasons of the year and in a variety of hosts. Apparently, sources of tetrathyridia are available during the winter as well as in other seasons, since young infections in winter were common and infection can and does occur throughout the year. In one skunk and in several raccoons collected during mid-winter in Iowa, the mesocestoidids found were situated in the anterior duodenum; and the probable source of infection, the pocket gopher (Geomys bursarius)*, was the only food organism found in the digestive tract. Gophers were readily identified by tooth and claw structure. Although no tetrathyridia were found in 27 gophers (15 from Iowa and 12 from Vermillion Co., South Dakota), this host remains suspect. Rissky (1962) in a survey of parasites of gophers from southeastern South Dakota reported finding unidentified cestode larvae throughout the body cavity in about 98 percent of hosts examined by him. In this study,

---

*Species determination by the Iowa State University Fish and Wildlife Section.

however, gophers collected from the same general locality were not infected with cestode larvae. Rissky's report contains no drawings or description of these cestode larvae, and specimens are not available for examination, having been either lost or misplaced, according to Rissky.[*] However, experimental feedings have shown that in carnivores there is a marked correlation between the state of digestion of gophers or gopher-like prey and the location in the intestine of early developmental stages of Mesocestoides. It is not likely that the tetrathyridia which infected the previously described skunk and raccoon could have come from any other vertebrate source than Geomys. However, until cestode larvae from this rodent can be examined, the relationship between Geomys and winter infections of definitive hosts of Mesocestoides must remain purely suppositional.

Some attempt was made in this study to determine how diet of the host might affect Mesocestoides. Each of three laboratory-reared racoons was fed five tetrathyridia from a leopard frog. One raccoon was fed on a high carbohydrate diet, another on a high protein diet and the third, starved. Only the starved host animal was positive upon examination 38 days later and harbored two mature, 11 cm. worms. Since development of these two worms was much retarded in comparison with those ordinarily recovered from the raccoon after a

---

[*]Rissky, R. W. Mitchell High School, Mitchell, South Dakota. Personal communication. 1966.

similar period of time following exposure, it is probable that starvation had adversely affected development of the parasite. Three more raccoons were similarly fed and treated except that each received ten instead of five tetrathyridia from a different frog. The "starved" animal received only 150 gms. of Purina Dog Chow every two days. Shedding of proglottids was observed in this animal 23 days after exposure, and only one gravid worm was recovered from the small intestine 11 days later. The host maintained on a heavy protein diet of lean, raw beef also shed proglottids 23 days following infection, and four extremely small worms were recovered. Eggs from these small worms measured approximately 29 x 36 u while those from "starved host" worms averaged 41 x 48 u. The third raccoon, given a high carbohydrate diet, was negative as in the preceding group. All animals were permitted unlimited quantities of water throughout the experimental period. It is difficult to draw any correlation between diet and development of worms on the basis of these few hosts. Paucity of laboratory-reared hosts of the same species and age has curtailed additional experimental feedings.

Ssolonitzin (1933) first described asexual reproduction (by budding) of larval Mesocestoides in the dog, and noted considerable variation of form and viability among these tetrathyridia. He experimentally fed such larvae to other

dog hosts and his reported observations are of interest. He
felt that migration of tetrathyridia in the host followed
circulatory pathways. No indication that this means of dis-
persal within a host has been observed here, nor has it been
observed by other investigators. His suggestion that young
definitive hosts produce larger worms cannot be accepted,
for large worms are known to develop in young or old hosts
and, in Mesocestoides, host age does not appear to affect
infectivity. Feeding of tetrathyridia to dogs produced for
Ssolonitzin(1933) normal adults in the intestine as well as
tetrathyridia which re-established themselves in the body
cavity. More larvae penetrated into the body cavity than
developed normally. He has described four types of mesoces-
toidid larvae from the body cavity: (1) small cystic larvae,
(2) small compact larvae, (3) elongate larvae with parenchymal
spaces, and (4) asexually reproducing types exhibiting endog-
enous budding. Ssolonitzin(1933) stated that sterile forms
were primarily from serosal cavities but could be found co-
existing with normal, infective tetrathyridia. Larvae, inter-
mediate between normal and sterile types, were also described.
Histologically, both sterile and normal larvae were reported
as generally similar. It is important to note that all
tetrathyridia described by Ssolonitzin(1933) were recovered
from animals which had been dead for an unstated period of
time.

Further evidence that asexual multiplication of Mesoces-

toides by budding does occur has been provided by other workers. Specht and Voge (1965) and Voge and Coulombe (1966) were able to obtain true asexual multiplication of Mesocestoides in vivo and in vitro, respectively. However, it is suggested that since neither Ssolonitzin nor Voge and co-workers have been able to obtain normal adult worms from their asexually formed tetrathyridia, perhaps Ssolonitzin's observation that asexually produced tetrathyridia are sterile is correct.

The association of asexually reproducing tetrathyridia with parasitic ascites in dogs has been confirmed both by Ssolonitzin (1933) and by Voge and Berntzen (1963), who have described this pathological finding in dogs containing masses of asexually reproduced tetrathyridia. However, whether tetrathyridia are the etiologic agent of this disease condition or merely accidently present in ascitic animals has never been experimentally determined. Ssolonitzin (1933) also discovered that severing of tetrathyridia did not kill them, for both sections of each severed individual were maintained by him in the living state for two to three days. Hart (1967) has found through recent in vivo and in vitro studies that anterior halves of transected tetrathyridia will proliferate and regenerate or even strobilate. Posterior ends, however, do not regenerate but may survive for as long as 40 days in vitro.

The relationship of the tetrathyridium to other cestode larval stages is still unclear. It has been described variously (as noted above) as solid, cystic, or intermediate between these forms. It has been referred to as cysticercoid, cysticercus, plerocercoid, plerocercus and combinations of these. Whatever its true status, the tetrathyridium is, in fact, a highly variable larval type which apparently may or may not reproduce asexually. The determination of the phylogenetic position of Mesocestoides depends, in part, upon its larval stages as well as upon its development, and hence remains enigmatic. Voge (1967) has recently suggested proteocephalan affinities for the genus, whereas the pseudophyllidean characteristics of Mesocestoides have long been noted by numerous investigators. Tetrathyridia, encapsulated in the tissues of snakes and lizards, were experimentally fed by Joyeux and Baer (1933) to a European green frog (Hyla arborea); they observed that only scoleces of ingested tetrathyridia penetrated the intestinal wall. These investigators subsequently recovered from the frog's body cavity new, normal tetrathyridia which had re-encysted. An attempt by Joyeux and Baer (1933) to repeat this experiment was unsuccessful. They noted that calcareous corpuscles were evenly distributed throughout the parenchyma rather than decreasing toward the posterior end of the larva as they do in plerocercoids. Joyeux and Baer thus implied a cyclophyllidean relationship of the tetrathyridium.

In my opinion, Joyeux and Baer (1933) were probably
dealing with early stages of tetrathyridial metamorphosis
in an abnormal host, a frog. This anterior portion of the
tetrathyridium, instead of developing into a strobilate worm,
invaded frog tissues and became re-established as a typical
larva. It would appear that the only observations on post-
tetrathyridial development of Mesocestoides are those by
Joyeux and Baer (1933) and my own studies recorded below.

The loss of the cystic body and the development of the
scolex into the definitive strobilate cestode is character-
istic of cylophyllidean cestodes. Observations made in this
study on early stages of tetrathyridia within the digestive
tract to early strobilation of young cestodes tend to support
Joyeux and Baer (1933) and have provided additional evidence
in support of the cyclophyllidean nature of Mesocestoides.

An extensive series of feeding experiments of tetrathy-
ridia to laboratory-reared definitive hosts was undertaken
in order to follow the development of Mesocestoides to fully
developed strobilate adults. Tetrathyridia recovered from
the tissues of naturally-infected toads (Bufo americanus)
were fed by pipette to 25 laboratory-reared domestic mice
(Mus musculus). Exposed mice were then sacrificed periodi-
cally from 25 minutes post exposure to a maximum of 9.5 days,
and a series of early developmental stages of Mesocestoides
was obtained from 17 of the mice. Additional experiments
involved the feeding of tetrathyridia from naturally-infected

frogs (R. pipens) to two dogs and to two raccoons, all labora-
tory-reared. Worms from dogs were recovered 8 hours post
feeding. Those from one of the raccoons were recovered after
1.5 to 2 hours. The second raccoon was fed tetrathyridia at
various intervals as follows: an initial exposure of 60
tetrathyridia on June 28, 1966; a second exposure of more
than 100 tetrathyridia on August 27, 1966, and a third ex-
posure of numerous larvae 8 hours before sacrificing the host
on January 12, 1967. These experimental data, coupled with
data from various naturally-infected hosts, have permitted
comparative studies of early development to be made on a
variety of definitive hosts. The following account is based
upon these laboratory infections.

Earliest stages were recovered from the stomach and
duodenum of two mice, 25 and 30 minutes post-feeding and
from the stomach of a raccoon, 9 hours post-feeding (Figure
41). In one mouse, a single tetrathyridium was recovered
from the stomach (Figure 40); in the second mouse, seven
were found in the stomach and seven within the duodenum.
Most of these larvae were unchanged, except that calcareous
corpuscles subjected to acidic digestive juices of the host
had degenerated and darkened (Figures 40 and 41). Within
the duodenum, a few centimeters beyond the stomach, scoleces
had everted but were still attached to the tetrathyridial
body (Figure 45). In another infected mouse examined one
hour post feeding, the scolex and neck had separated from

the remainder of the tetrathyridial body. This separation of scolex may occur whether or not it has evaginated (Figures 43 and 44). It should be emphasized that that portion of the body into which the scolex invaginates is not cystic, for it is devoid of any lumen and is parenchymatous. This is clearly evident in Figure 46, illustrating a 1.5 hour experimental infection from the duodenum of a laboratory-reared raccoon. Scoleces may be withdrawn and evaginated at will, even after separation from the remainder of the tetrathyridial body (Figures 47 and 50).

Following separation of the scolex region, very young worms (Figure 20) are similar to older ones (Figure 21) both in their movement and in their mode of attachment to host villi. Young adults may move actively and may change markedly in their general body configurations. Apparently, however, development is considerably retarded in mice, as may be seen by comparing sizes of worms from mice 37 hours post feeding (Figure 52) with those from raccoons at 8 hours post feeding (Figure 50). This appears to be early evidence that mice are not as suitable as other mammals recorded as definitive hosts for Mesocestoides. Additional evidence is provided by the absence of strobilation, or by only weak strobilation, in worms from mice 6-1/2 days post feeding (Figure 55) and its early presence in adults from raccoons only 8 hours post feeding (Figure 36).

The parenchyma of recently separated scoleces and of

later developing stages contains numerous calcareous corpus-
cles (Figure 38). The body usually also retains a stalk-
like or vesicular remnant of tissue, by means of which the
scolex-neck complex is attached to the remainder of the
tetrathyridium (Figures 47 thru 51). This remnant or "tail"
may persist in young worms and has even been observed still
attached to the posteromost segment of strobilate adults
(Figure 37).

Subsequent development of Mesocestoides involves in-
crease in body length and the attainment of sexual maturity.
Strobilation normally occurs in the lower duodenum, and in
my studies was observed in all experimental feedings involving
raccoons (Figure 36), dogs (Figure 35), and mice (Figure 34).
In the latter, however, it is much delayed, as noted above.
Young adult worms, comparable to those described in these
experimental infections, were also recovered from naturally-
infected fox (Figure 53) and skunk (Figure 56). In additi
an opossum previously maintained in the laboratory for more
than two months was fed tetrathyridia from R. pipiens and a
few hours later, 12 early stages (Figure 51) were recovered.
Young adults (Figure 54) were also recovered from a laboratory-
reared and similarly infected dog. All these hosts contained
representative early stages of worms indistinguishable from
those young Mesocestoides recovered from experimental infec-
tions. The finding of similar stages in both naturally and
experimentally-infected hosts confirms my belief that the

experimental observations herein reported are normal and typical of mesocestoidids in general.

Development of _Mesocestoides_ adults to gravidity has been well documented by numerous investigations and will not be considered here. With the knowledge of post-tetrathyridial development as described above, it becomes clear that the tetrathyridium consists of two distinct regions--an outer, solid protective coat and an inner scolex-neck complex. It is evident that the tetrathyridium is to _Mesocestoides_ what the cysticercus is to most other cyclophyllideans, for only the neck and scolex region of the tetrathyridium are involved in the formation of the adult worm, contrary to the development of pseudophyllidean or proteocephalan cestodes in which the entire larva becomes incorporated into the adult body. However, unlike most cyclophyllideans, the tetrathyridial body is ordinarily solid and apparently is only rarely cystic. Cystic forms, however, are presumably abnormal. The sequence of development from tetrathyridia to young adult worms is typically cyclophyllidean. It offers additional support for the affinities of _Mesocestoides_ to the order Cyclophyllidea.

Injection experiments

This study was in progress when Specht and Voge (1965) reported that, experimentally, tetrathyridia (found naturally in lizards of the genus _Sceloporus_) when injected into laboratory mice were capable of undergoing asexual multiplication.

In my studies, tetrathyridia from naturally-infected amphibian hosts have never shown clear evidence of asexual multiplication. However, because a supply of tetrathyridia from frogs (_Rana pipiens_) and toads (_Bufo americanus_) was available to me, injections similar to those performed by Specht and Voge were carried out in the laboratory. Those tetrathyridia injected into amphibian and mammalian hosts, including laboratory-reared mice (inbred Marker stocks, Iowa State University Genetics Department), have been viably maintained for as long as ten months without any apparent increase in numbers or any substantial increase or decrease in size.

Hosts injected with tetrathyridia from _R. pipiens_ or _Bufo americanus_ included: 3 _R. pipiens_, 5 _B. americanus_, 6 _Ambystoma tigrinum_, 24 _Mus musculus_ (laboratory mice), 1 _Tamias striatus_ and 2 _Rattus_ (laboratory stock). Those hosts in which injected larvae were successfully maintained included: 14 _Mus_, a single _Tamias_, 5 _Bufo_ and 1 _Rana_. The difference in results of these studies from those reported by Specht and Voge (1965) may be due to physiological differences in members of this highly variable genus, but more likely, indicates that the asexual reproduction exhibited by tetrathyridia from the lizard is abnormal in the mice, if not in the reptilian hosts. Those tetrathyridia taken from amphibian hosts have been shown to be viable and develop into normal _Mesocestoides_ when fed to proper definitive hosts either before or after

injection into transfer hosts. It is of interest that the asexually produced tetrathyridia reported by Voge have not developed into normal adults.

PART II. THE MESOCESTOIDID PARAUTERINE ORGAN

MATERIALS AND METHODS

One of several gravid mesocestoidid tapeworms was obtained
from the ileum of a freshly road-killed grey fox (Urocyon
cinereoargenteus (Schreber)) in Ames, Iowa. The worm was re-
moved as rapidly as possible and was fixed by the modified
double aldehyde-osmium technique of Sabatini et al. (1963).
The worm was flooded in a 3 percent phosphate-buffered
glutaraldehyde fixative, then washed in 0.13 m phosphate
buffer containing 0.24 m sucrose and postfixed for two hours
in cold, phosphate-buffered 1 percent osmium tetroxide.
Tissues were gradually dehydrated through a standard ethanol
series and embedded in a mixture of Maraglas (72 parts), Dow
epoxy resin 732 (16 parts), dibutyl pthalate (10 parts), and
benzyl dimethylamine (2 parts). Following the method of
Jenkins[*], the plastic impregnated strobila was cut into lengths
and serially flat-embedded. The boat of unpolymerized epoxy
containing the tissue segments was then evacuated for five
minutes and transferred to an oven for curing at 60° C for
18 hours. Segments of strobila were sawed from the block as
desired. Sections displaying silver to gold interference
colors were cut on a Reichert "Em U 2" microtome using hand-
broken glass knives. Sections were then mounted on copper
grids and doubly stained with a lead citrate (Reynolds, 1963)

---

[*]Jenkins, R. A. Ames, Iowa. Electron microscopy tech-
nique from personal files. Private communication. 1966.

and 2 percent aqueous uranyl acetate (Watson, 1958). Staining
procedures involving 30 minutes in uranyl acetate, followed
by 7 minutes in lead citrate with brief washings in carbonate-
free deionized distilled water, produced good results. Prep-
arations were examined with an RCA EMU 3F electron microscope
operated at 50 kv. throughout. Thick skip sections were cut
serially and examined regularly as a guide to the desired
areas of tissue. Microtome and hand-cut thick sections were
mounted, unstained or stained, in both temporary and/or
permanent preparations; temporary mounts were set in immersion
oil; permanent mounts, in Permount. Plastic embedded thick
tissue sections were stained with 1 percent methylene blue
and 1 percent azure II, each in a 1 percent sodium borate
solution. Stained and unstained material was observed and
photographed under phase using a Zeiss photomicroscope.

Gravid mesocestoidids, used for the histochemical portion
of this study, were removed from their hosts and the following
fixation and staining techniques employed: Fixatives used
were FAAG (Turtox), 10 percent phosphate-buffered formalin
at pH 7.2-7.5, Bouin's, Carnoy's, Zenker's, 70 percent ethanol
and osmium tetroxide. Histochemical and staining techniques
used were Harris's hematoxylin and eosin for general, compara-
tive sections; Van Gieson's stain for collagen fibers; Oil
red O and osmium tetroxide stains for lipids; Best's carmine
stain for glycogen; periodic acid-Schiff reaction (PAS) with

diastase control for carbohydrates; colloidal iron trichrome stain for acid mucopolysaccharides in tissue; alcian blue (short method) without hyaluronidase for acid mucopoly-saccharides; Gomori's iron reaction; and von Kossa's method for demonstrating calcium. All of the above techniques may be found in the Armed Forces Institute of Pat·ology Manual of Histologic and Special Staining Technics, 1960. Additional techniques included: Heidenhain's azan triple stain (Dawson and Friedgood, 1938); malachite green method for glands and egg shell material; brom-phenol blue technique for vitelline globules (Johri and Smyth, 1956); Bonhag's modification of the mercury-brom-phenol blue method for protein; Mazia's total protein (Pearse, 1960); azure B bromide for RNA and DNA (Flax and Himes, 1952); hot and cold perchloric acid with RNAse (crystalline, salt-free) controls, and the Feulgen reaction for DNA (Lillie, 1965).

GENERAL CONSIDERATIONS

The presence of a parauterine organ is not restricted to
the mesocestoidids, for similar organs are present among the
avitellinid Anoplocephaloidea Spasskii, 1951, and other
cestodes. The biological significance of the parauterine
organ, as well as variations in its development and morphology,
have been briefly but well reviewed by Spasskii (1951).
Spasskii and earlier workers have ascribed to this usually
thick-walled and fibrous structure (Figures 14 and 72) a
protective role in keeping eggs, contained within the shed,
gravid segments, from hazards of the external environment.
Experiments by Dubnitskii (1949) have shown that mesocestoidid
oncospheres, in eggs removed from the parauterine organ, be-
came dessicated and died in 0.5 minutes; but when left in the
parauterine organ, the oncospheres remained active for 25 to
40 days, clearly demonstrating one protective role of this
organ. Observations on oncospheres, both in and out of the
proglottid, subjected to various environmental conditions,
have supported Dubnitskii's findings and have provided addi-
tional information concerning the functional aspects of the
mesocestoidid parauterine organ (see Tables 5 and 6). The
oncospheres subjected to controlled conditions of temperature,
humidity, salt, and pH concentrations have been shown in this
study to survive best within the parauterine organ and even
better when the parauterine organ itself was retained within
the protective envelope of the proglottid. It was found that

distilled water killed oncospheres as rapidly as desiccation and that a cool and humid environment increased this longevity. I have kept oncospheres alive, when contained within the intact proglottid, for a maximum of 51 days. Hence, a multiple protective role of the parauterine organ is indicated.

Spasskii (1951) has suggested that the parauterine organ, in addition to its protective role, plays a part in attracting the attention of the potential host organism which, possibly assuming it to be food, ingests masses of infective oncospheres rather than a scattered one or few. But in Mesocestoides it is the entire proglottid and not the parauterine organ which probably attracts the host. In all freshly-dropped faecal masses from hosts infected with Mesocestoides, I have observed that proglottids actively migrate to the exterior of the faecal mass (Figure 17) and, standing on end, undulate like some white grub, such as fly larvae. Spasskii (1951) has pointed out, at least for anoplocephalans, that in proglottids with large numbers of eggs, parauterine organs are numerous; conversely, in proglottids with low numbers of eggs, parauterine organs are few. In Mesocestoides, neither of these observations holds. Usually, large numbers of eggs, about 100 to several hundred, are contained within a single parauterine organ, a most significant factor among several contributing to the possible life cycle proposed for this genus.

Indeed, if the eggs are transmitted en masse within the parauterine organ as is suspected, no small intermediate host

such as a mite could support the obviously large numbers of oncospheres which would be released--even if the entire parauterine organ were ingested by a mite. On the other hand, it is well known that hosts for tetrathyridia often contain several hundred to thousands of these larvae (James and Ulmer, 1967) and that most of these hosts are somewhat insectivorous and would probably be naturally attracted to a grub-like proglottid on a faecal mass. Also, since dried proglottids containing the protective egg capsule look much like plant seeds, it seems reasonable that even the seed-eating hosts for tetrathyridia might be attracted to the dry, but infective, mesocestoidid proglottids. It is, therefore, here suggested that both fresh and dried proglottids might be equally attractive to potential and available host organisms, but that only larger invertebrates would be able to support the large numbers of oncospheres which would be freed from one or more ingested parauterine organs. Other evidence supporting the suggested one intermediate host cycle for Mesocestoides has already been introduced in Part I.

Spasskii (1951) has postulated that a large, single infection might be advantageous to the parasite, considering that acquired immunity of hosts to helminths is known to occur. But, whatever role the parauterine organ takes in the elusive life cycle of Mesocestoides, it is morphologically and functionally a most intriguing structure. In the words of Spasskii (1951):

....the formation of capsules with eggs in parasitic worms is an adaptation which has arisen in the course of phylogenesis, an adaptation to the parasitical mode of existence, one which ensures better infective conditions (intermediate and definitive) for entering the host, and better conditions of existence by the parasites in the organism of the definite host.

Clearly, the mode of group infection endows many parasites with decided advantages, since the possibility of forming capsules makes its appearance independently in highly different groups of helminths (convergence).

MORPHOLOGY AND HISTOCHEMISTRY

In perhaps no other organism can morphogenesis be so readily studied as in the cestodes. Because of the chronologically graded, serial arrangement of proglottids, organs can be found in all stages of development, from primordial stages to old age, within a single worm. Although morphological descriptions of isolated segments or structures are common, few recent extensive investigations into cestode organogeny have been made. Byrd and Ward (1943) and Chandler (1946, 1947) have described the segmental anatomy and morphogenesis of the parauterine organ in Mesocestoides. Several errors, made by Chandler (1946), although partially corrected by him (1947), make his work useful only in connection with that of Byrd and Ward (1943), whose findings this study substantiates.

Byrd and Ward (1943), in agreement with Joyeux and Baer (1936), have stated that "The egg-capsule is formed as an evagination from that part of the uterus which was embedded in the parauterine cells of the mature segment. Its lumen is derived directly from the lumen of the uterus and the major portion of the wall comes from the parauterine cells." Chandler (1946), on the other hand, accepts Fuhrmann's (1928-1933: in Chandler, (1946)) description of the development of the parauterine organ in other genera, stating that it "forms independently of the uterus, between it and the ovary in the median line of the proglottid, as a solid mass

of deeply-staining cells. It soon acquires a cavity and then establishes a broad connection with the uterus at a point posterior to the entrance of the uterine duct .... [and] the walls condense and become fibrous, leaving a twisted passageway to the parauterine duct...."

Of these two diametrically opposed conclusions relative to the morphogenesis of the mesocestoidid parauterine organ only that of Byrd and Ward (1943) appears to be valid. This study, employing techniques in light and electron microscopy, has involved the examination of the origin, organization and fate of the parauterine cells, in the development of the parauterine organ, from its inception in the early mature proglottid (Figure 7) to its definitive structure in the gravid proglottid (Figure 14).

The parauterine cells, in very early mature proglottids, appear to arise in the medullary tissue approximately on the midline of the segment, between the anterior margins of the ovarian lobes and directly anterior to the oöcapt (Figures 5 and 9). Thick skip-sections of this region illustrate the relationship of the developing parauterine organ to the remaining structures in a mature proglottid (Figures 87 thru 92). It is possible that some fibrous extensions of these cells may extend posterior to the oöcapt and become intimately associated with parenchymal fibers, thus anchoring the posterior end of the organ. Further study is needed to support this observation. It is suspected that the "caudal

appendage" (Figures 64, 65, 134) of Byrd and Ward is a combination of these binding fibers plus degenerated ducts. Initially, the parauterine cells show no apparent organization (Figure 89). In later stages, and in a manner not yet apparent, these cells form a syncytial, circumferentially arranged, network of cells and fibers (Figures 92 and 93). The entire reticular syncytium of cells seems to flatten out and to send out cytoplasmic processes (Figures 93, 98, 99) until the final structure of the organ, resembling wood in tangential section--rather than any familiar cellular pattern-- has been completed (Figures 85, 86, 94 thru 97).

Observations indicate that parauterine cells, even in their lamellar state, remain active and are often associated with granules of varying sizes, texture, and chemical constituency as well as with rough endoplasmic reticulum, particularly in the region of the egg mass (Figures 104 and 106). It is possible that numerous cellular structures (ordinarily associated with high cellular activity) found within the viscous medium surrounding the eggs and contained within the parauterine organ wall are a product of this remarkable organ. Observations made thus far on the parauterine organ suggest, not only the previously described passive, protective function, but a highly active one as well. It is highly probable that the mitochondria, myelin figures, endoplasmic reticulum, and various granules found within the confines of the parauterine wall may be involved with the development of the

embryos and/or egg shells. Histochemical studies appearing in the following section were undertaken in order to understand better the possible function of the parauterine organ in relation to its origin and structure.

Although anatomical observations have been useful in clarifying some of the problems concerned with the mesocestoidid parauterine organ, the static nature of an anatomical study alone, at any level, is not conducive to understanding the dynamics of development and/or function. In this study, combined histochemical and anatomical findings have hopefully resulted in a clearer understanding of parauterine organ structure, its mode of development, and suggested function. Evaluation of histochemical observations has been based primarily on Pearse (1960), von Brand (1966) and the cited references for each technique used. Results of histochemical staining appear in Tables 9 thru 13.

Origin of the Parauterine Organ

Chandler (1946) stated that the parauterine organ forms independently of the uterus, later unites with it, and implied that each retains its identity. Byrd and Ward (1943) have indicated that the uterus itself becomes an integral part of the parauterine organ. My own observations of whole-mounts and sections support the contentions of Byrd and Ward (1943). Progressive development of organs is illustrated by Figures 6 thru 14. In early development, the uterus (Figures 5, 87 thru 92, 109), becomes surrounded posteriorly by

parauterine cells whose nuclei are obvious. Because the inner lining of the uterus is morphologically and chemically different from its outer glandular cells, this lining may be readily observed within the parauterine organ (Figure 72), Azan triple (Figures 112 and 113), Mazia's total protein (Figures 134 and 135) and van Gieson's stains differentiate outer and inner uterine cell layers which, although continuous, appear to be morphologically and functionally modified when enclosed by parauterine tissue. Posterior to the parauterine organ, the remnants of the uterus and other genital ducts are clearly visible (Figures 72 and 134) and form, in part, the caudal appendage of Byrd and Ward (1946). van Gieson's collagen stain, Mazia's total protein or azure B bromide stain illustrate exceptionally well the uterine origin of this appendage. The differentially staining outer uterine gland cells can be seen within the anterior part of the parauterine organ (Figures 134 and 135). These cells maintain their histochemical identity even when enveloped by parauterine organ tissue; they appear never to merge with it as do cells of the inner uterine lining. Neither parauterine organ nor uterus show positive PAS or glycogen reactions with Carnoy-fixed tissue, but strong PAS and weak glycogen reactions have been demonstrated with Zenker-fixed material; perhaps this is another indication of the close structural and functional relationship of these two organs.

Histochemical evidence clearly shows that the parauterine organ is formed from both uterus and differentiated parauterine cells; the inner lining is continuous with that of the uterus, and the outer, fibrous-appearing layers are formed from the parauterine cells.

Structure

Although several aspects of the developing and fully developed structure of the parauterine organ have been discussed, details of combined histochemical and anatomical findings may be of value. The overall appearance of the parauterine organ when observed by means of standard light microscope techniques is that of a homogeneous, fibrous capsule (Figures 68 and 72). Not until the capsule is examined under phase (Figures 83 and 84) or by means of differential staining can its true laminated nature be observed. Full appreciation for its intricate and morphologically varied stratification is gained only through ultrastructural and histochemical studies.

The innermost layer of the parauterine organ is a continuation of the thin epithelial-like lining of the uterus (Figures 72 and 135) which extends throughout the parauterine organ, enfolding the egg mass. This lining emerges at the posterior end of the organ as part of the caudal appendage (Figure 134). Immediately peripheral to the lining membrane is a loose but apparently metabolically active lamina con-

taining the previously noted rough endoplasmic reticulum,
mitochondria and various granules (Figure 106), some of
which (mitochondria and granules) also appear internal to
the inner membrane and constitute a part of the viscous media
of the egg mass (Figure 104). The viscous matrix is so dense
and sticky that it does not flow when the parauterine organ
is broken, but encases and holds the eggs as though they
were embedded in soft gelatine. However, from the inner
laminated region to the outermost region of the parauterine
organ, no sharp boundaries exist between laminae (Figures
100 thru 104). As a result of fixation, membranes frequently
separate but remain connected by reticular-like fibers (Fig-
ures 68, 100, 104). Scattered throughout the membranes of
the layers are found discrete bundles of granules of two
types which, because of their abundance and location, when
compared with similar granules elsewhere throughout the
tissue, are assumed to be glycogenic (Figures 85, 86, 102,
103) and proteinaceous (Figures 97 and 102). The glycogen
granules are minute, of relatively consistent shape, widely
scattered, and strongly osmiophilic (Figures 97 and 108).
The protein granules are generally much larger, variable in
shape, appear to be amorphous, and are weakly osmiophilic.
Granules of glycogen far surpass the proteinaceous ones in
number, volume, and distribution in the parauterine organ.
Whether these granules are remnants of storage products un-
used in the formative processes of the organ, or are formed

within the developed parauterine tissue is not known. It is my contention that the former is true, since the areas of the fully developed parauterine organ which contain most of these granules do not show typical cellular structure denoting active metabolic synthesis. It might be possible for the granules to move inward from rich storage sources in the parenchymal cells (Figure 108) and to pass between laminae to reach the position from which they have been described. However, the tightly laminated structure of the parauterine organ and the apparent intramembranous position of the granules (Figures 94 and 96, 101 thru 103) tends to negate the latter point of view. The true source and destiny of these granules remains unanswered.

The laminae between the outermost and the innermost layers are substantially homogeneous in appearance. They look not unlike tangentially cut wood (Figures 85 and 86, 94 thru 97) and little of their cellular structure can be recognized at this final stage of development (Figure 96). Active cell structures are wholly or almost absent throughout this central region, and the membranous character of the laminae is vague. Although the typical unit membrane could not be demonstrated, the histochemically positive lipid and protein reactions substantiate the proposed cellular nature of the laminae. Hence, the structure of the greater part of the mesocestoidid parauterine organ is not fibrous as previously suggested (Chandler, 1946), but membranous,

formed from a flattening and layering of the parauterine cells (Figures 91, 92, 93). The outermost region of the parauterine organ closely interconnects with invading fibers of muscle and parenchyma. Muscle fibers may act to move the eggs into the organ from the uterus and perhaps to move them about in situ, but this is not known. Muscle fibers attached to a parauterine organ, teased from its muscular and parenchymal moorings into normal saline, contract spasmodically but rhythmically in ever increasing time intervals. Regrettably, no records were kept of the contractile time intervals, but early contractions occurred every few seconds.

Closely associated with the muscle fibers are parenchymal fibers which interlace with muscle fibers and peripheral fibers of the parauterine organ. These parenchymal fibers are syncytial and most probably strengthen and support the organ. Like muscle fibers, parenchymal fibers can be demonstrated histochemically and are especially clear in Zenker-fixed, Azan stained sections.

The earliest stages of the developing parauterine organ studies indicate it to be histologically cellular. The cells closely approximate typical stellate cells of the parenchyma and are apparently syncytial. Nuclei, although distinct in early parauterine cells (Figure 93) are sparse in the late gravid stage of the parauterine organ. Nuclei have not been observed in studies on the fine structure of the late-stage

parauterine organ, but they may be demonstrated with Feulgen's or other nuclear stains. The early developing parauterine organ (Figure 93) has fewer organelles of the kind usually associated with metabolic activity than do early gravid stages which contain developing eggs (Figures 100 and 106). It is possible that energy for the laminating activity of these cells is provided from some extraneous storage source, since large quantities of lipids, proteins, and carbohydrates are found in the parenchyma (Figure 108). The early parauterine cells are filled with a poorly osmiophilic, homogeneous material (Figure 93) which seems to be similar in appearance to the individual laminae of the fully developed parauterine organ (Figure 94).

All parauterine organs are attached, by means of the muscle and parenchyma associated with the caudal appendage, to the posteromost aspect of the proglottid which is in turn connected with the parenchyma of the anterior part of the next posterior proglottid. In effect, parauterine organs are linked to one another throughout a strobila. Presumably, these interparauterine connections provide further structural support for the organ.

Incidental Observations
on Eggs and Developing Oncospheres

Incidental observations were made on eggs and developing embryos. The malachite green method (Johri and Smyth, 1956) was employed to demonstrate origin and/or distribution of

egg shell material. The shell of trematodes and pseudophylli-
dean cestodes is known to be predominantly sclerotin, a
quinonetanned protein (Smyth, 1957 and Smyth and Clegg, 1959).
Malachite green is known to have an affinity for vitelline
globules, the precursors of sclerotin (Johri and Smyth, 1956
and Smyth and Clegg, 1959). In Mesocestoides, only the
uterine gland cells and oncospheral hooks gave positive re-
actions as indicated by their light green color (Figures 121
and 138). No hooks were found in the developing embryos far
removed from gland cells. Hooks, egg shell, and gland cells
were protein positive with mercuric bromphenol blue. The
"shell" showed a positive reaction for glycogen (Best's
carmine), but the hooks did not. Gland cells of the uterus
gave a high positive test for carbohydrate (PAS), a low test
for glycogen (Best's carmine), and demonstrated an extremely
rich RNA content (Azure B bromide). The malachite green
positive gland cells and hooks, in conjunction with the
absence of carbohydrate, suggest that hooks of mesocestoidid
oncospheres are formed, at least in part, by some substance,
probably proteinaceous, produced in the uterine gland cells.
The problem of the composition and development of oncospheral
hooks requires further investigation.

It would seem that the mesocestoidid egg shell is chemi-
cally different from egg shells of trematodes and pseudo-
phyllidean cestodes (Smyth and Clegg, 1959). It is most
probably similar to the shell (embryophore) of Multiceps,

shown by Smyth (1957) to be a combination of a protein and of a polysaccharide of the disulfide type. The PAS test for carbohydrate was far stronger than glycogen in the uterine gland cells indicating the presence of a non-glycogenic carbohydrate. A performic acid Schiff test for disulfides was not made, and the composition of the mesocestoidid egg shell can only be inferred. It is presumably a disaccharide and protein complex, similar to that described for the Multiceps embryophore by Smyth (1957). The apparently similar composition of the egg shell of Mesocestoides and Multiceps, coupled with additional factors noted above, adds further evidence to the cyclophyllidean nature of mesocestoidid tapeworms.

Metabolic Activities

Histochemical evidence adds support to the supposition that the parauterine organ has an active role in the development of eggs and developing oncospheres. High concentrations of carbohydrates (the greater proportion of which has been identified as glycogen) and moderate amounts of protein as well as RNA are present in both the uterine and parauterine contents in which developing and fully formed eggs are bathed. Read (1959) has discussed in detail the metabolic need of carbohydrates by cestodes. Read and Rothman (1958) found high concentrations of glucose and moderate amounts of galactose in Mesocestoides. Read (1959) has pointed out that

carbohydrate requirements are far greater for young and growing worms than for older or senescent ones, and that the deleterious effects caused by the absence of carbohydrates is most pronounced among young worms. A search of the literature indicates little information concerning carbohydrate requirements for developing embryos, but since they represent an active state of cestode development, it is assumed that their need for carbohydrates parallels or exceeds that of young worms. Both uterine and parauterine contents of Mesocestoides show quantities of carbohydrate. The highest concentration of PAS positive material was found in parauterine organs of younger proglottids; the converse is true of older proglottids.

Von Brand (1966) has pointed out that little is known of the biochemistry of nucleic acids in helminths. However, the well established function of RNA in protein metabolism would strongly support a similar need and function in them. The high protein content of developing embryos and organs of developing and fully developed worms suggests that the RNA existing in the viscous matrix of the parauterine organ and uterus is active in protein production. The glandular cells surrounding the posterior two thirds of the uterus are rich in RNA and probably secrete their products into the uterus for use by developing eggs and embryos. Histochemical observations in this study (Azure B bromide and Feulgen's stains) on developing embryos of Mesocestoides

have supported the work of Ogren (1952), who obtained similar results with the methyl green-pyronin technique for RNA-DNA.

The high concentrations of proteins and carbohydrates in eggs of Mesocestoides, coupled with the presence of like substances in the uterine and parauterine matrix surrounding the eggs, lend strong support to the previously noted observations on ultrastructure. It would be difficult to doubt the active role of the mitochondria, rough endoplasmic reticulum, myelin configurations and granules (presumably carbohydrate and protein) observed in the inner wall and in the contents of the parauterine organ. The higher concentration of organelles and cell products and their close approximation to the egg mass in younger parauterine organs strongly support the active nature of the mesocestoidid parauterine organ.

Calcareous Corpuscles

Calcareous corpuscles, as in most cestodes, are a major and characteristic constituent of larval and adult forms of Mesocestoides (Figures 39, 63, 130, 131). Von Brand (1966) has written an extensive review on these structures in which he suggests that the deposition of corpuscles parallels the formation of new living tissue, thus implying localized sites of metabolic activity. According to von Brand (1966), corpuscles have been shown to contain a protein base into which may be incorporated

glycogen, mucopolysaccharides, lipids, and alkaline phos-
phatases as well as calcium, magnesium, carbon dioxide, and
phosphorous. Some minor constituents are aluminum, boron,
copper, iron, lead, manganese, silicon, sodium, and tin.
The calcareous corpuscles of Mesocestoides observed in this
study have shown positive staining reactions for proteins,
including collagen, glycogen, acid mucopolysaccharides,
lipids, calcium, and iron (Fe^{+++}) (Tables 9 and 12). Little
is known of the formative mechanism of these corpuscles, but
several ideas have been proposed concerning their biological
significance. The view that they may act as neutralizing
buffers has been expressed by several authors (as noted by
von Brand) and has recently received some experimental sup-
port; it is also conceivable that they may serve as a phos-
phorous reserve (von Brand, 1966), but Timofeev (1964) has
suggested that they are waste products and may possibly have
an additional buffering capacity.

Observations on these corpuscles have been made on
Mesocestoides of all ages throughout this study. Their num-
ber increases in older proglottids. They appear within the
parauterine organ during later stages of oncospheral develop-
ment and are seldom present during the most active develop-
ment of eggs and embryos. The uterus of mature proglottids
contains high concentrations of acid mucopolysaccharides,
but in corpuscles found within such proglottids, no evidence
of acid mucopolysaccharides can be demonstrated. In late

gravid proglottids, however, acid mucopolysaccharides have almost disappeared from the uterus, but heavy concentrations appear in the calcareous corpuscles. Corpuscles may be found scattered throughout the parenchyma and within the confines of the parauterine organ, but they are especially numerous immediately surrounding the parauterine organ. Within it, corpuscles are almost always at the periphery of the egg mass and seldom embedded in it. If these structures were buffering mechanisms, their buffering capacity would probably be much reduced considering the extremely viscous nature of the parauterine matrix surrounding the eggs. It appears, at least for Mesocestoides, that calcareous corpuscles may be associated with regions of high metabolic activity, and that they appear late in the tissues as a by-product and marker of that activity. According to observations in this study, Timofeev (1964) is probably correct in assuming that calcareous corpuscles are metabolic by-products. Regardless of their true function, such corpuscles are most likely associated with metabolic activity. Their presence in older active regions of the worm implies previous or ongoing metabolic activity in that region, adding further support to the suggested active role of the parauterine organ.

Histochemical results noted above have been used to corroborate and/or explain anatomical, including ultrastructural, observations. In the following sections, a more detailed analysis of histochemical findings is presented to-

gether with a discussion of their histochemical implications as related to cestodes.

Proteins

Proteins have been shown to make up a large percentage of body weight of parasites, particularly among cestodes. For various species of cestodes, von Brand (1966) lists protein compositions ranging from 21 to 67 percent of dry body weight. Complex heteroproteins conjugated with lipids or carbohydrates are common. Keratin-like proteins have been shown to be present, as noted by von Brand (1966), but differ from known vertebrate keratins. Pence and Miller (unpublished abstract) have reported the presence of keratin in the embryophore and oncospheral hooks of Dipylidium caninum. Von Brand (1966), citing Gallagher (1964), states that the hooks of Echinococcus granulosus are keratin-like. Collagenous proteins have been reported, but like keratin, are different from collagen of vertebrates according to von Brand (1966). Sclerotin or quinone-tanned protein, the major component of egg shells of trematodes and pseudophyllidean cestodes, is almost entirely a product of vitellaria. Numerous other proteins and/or protein products have been described, and the higher concentrations of proteins have usually been associated with the more highly active or growing organs or organisms.

In Mesocestoides, proteins appear to be second only to

carbohydrates in quantity. In this study, dye tests for proteins included Mazia's mercuric brom phenol blue (HgBPB) for total protein content (Pearse, 1960) (Figures 134 and 135); van Gieson's stain for collagen (Armed Forces Institute of Pathology, 1957) (Figures 122 and 123); brom phenol blue for vitelline contained proteins (Johri and Smyth, 1956); and malachite green for sclerotins (Johri and Smyth, 1956) (Figures 121 and 138). Pepsin--HCl controls were used in conjunction with Mazia's total protein stain, and a five-hour incubation period at 56° C was required to remove all proteins from all tissues. A three-hour digestive period at the same temperature resulted in heavy protein deposits remaining in tegument, inner lining of uterus, caudal appendage, and outer lamina of the parauterine organ. In non-control sections, glandular cells of the uterus, egg shells, debris of egg mass matrix, as well as deposits in parenchymal and parauterine calcareous corpuscles showed a light staining reaction.

With protein staining, a definite relationship of the caudal appendage and uterus may be demonstrated. The proteinaceous nature of egg shell and hooks is evident, and in conjunction with the malachite green technique, one may infer that the protein composition of the hooks in mesocestoidid oncospheres is perhaps similar to the quinone-tanned proteins of trematodes and pseudophyllidean cestodes. In Mesocestoides, however, the source of malachite green posi-

tive material is not vitelline glands but rather, uterine glands which exhibit the same staining reaction as do the hooks. No other malachite green positive structures were observed in sections of Mesocestoides.

Other highly proteinaceous organs of the mature proglottids are ovaries, vitellaria, linings of male and female ducts, although neither the vas deferens nor the sperm in them stain significantly. Testes, however, exhibit protein positive follicles and the outer fibers of the cirrus react strongly.

Presence of collagen was observed with van Gieson's stain in sections of gravid and mature worms, but no collagenase controls were used. The inner lining of the uterus, as well as inner and outer laminae of the parauterine organ, were positive. Hooks of oncospheres were entirely negative for collagen, but shells and internal structure were positive and aggregates of collagen rich substance were apparent throughout the egg mass.

In addition, the tegument of Mesocestoides shows a division into three distinct, histologically-defined layers similar to those described by Monne (1959) and not into two layers as suggested by Rundell (1957). Of those mesocestoidid sections examined in this study, a thin collagenous inner layer (Figure 115) and a thicker positive outermost layer could be distinguished; the region, or layer, between these two layers was entirely devoid of van Gieson positive material

and is here considered a third layer. These three distinctly different and chemically discrete layers of the tegument described for <u>Mesocestoides</u> substantially supports the work of Monne (1959). Perhaps Rundell's (1957) interpretation, based solely upon his observations on anatomy, emphasizes the need for microscopists to employ correlative techniques whenever possible.

Carbohydrates

Carbohydrates are known to be an excellent source of anaerobic energy. Their oxidized carbon atoms of intermediate metabolism ideally supply the requirements of most intestinal parasites inhabiting oxygen low or oxygen free environments. Therefore, it is not unexpected that carbohydrates, as storage products and/or structural components, constitute the largest chemical fraction of cestode tissues.

Free glucose, trehalose, glycogen, and various polysaccharides are known to occur in tapeworms (von Brand, 1966). Simple sugars are probably polymerized into polysaccharides or utilized as constituents of conjugated proteins and lipids; carbohydrate studies, by several investigators according to von Brand (1966), have demonstrated little free sugar in the bodies of tapeworms. Von Brand (1966) has pointed out that reserve polysaccharides described from parasites have all been polymers of either amylopectin or glycogen, and it would appear that glycogen is by far the

most abundant and widely distributed form of energy reserve
in helminths. Analysis of physical and chemical character-
istics of glycogens from parasites has shown that they differ
slightly from typical glycogens of vertebrates, and that poly-
saccharide-protein complexes occurring in tapeworms are not
necessarily restricted to conjugates of glycogen (von Brand,
1966). Perhaps, as von Brand (1966) has noted, the stage
of development and eventual site of the parasite within its
host, as well as species and physical condition of the host,
are important factors governing the amount and distribution
of reserve carbohydrates.

Of the polysaccharides, the mucopolysaccharides are
probably the most abundant type in cestodes, having been
reported, according to von Brand (1966) from scoleces, lami-
nated membranes, cystic fluids and larvae of various
cyclophyllidean species. Although histochemically identified,
analytical evidence for the true nature of mucopolysaccharides
has been sparse, Monne' (1959) has identified acid mucopoly-
saccharides in the "main cuticular layer" (middle tegumental
layer, in this study) of several cestodes, including Mesoces-
toides. However, von Brand (1966) has clearly shown that
without chemical analysis great difficulty exists distinguishing
histochemically between types of mucopolysaccharides without
chemical analysis and that histochemical evidence alone may
prove misleading. He also contends that helminths probably con-
tain a far greater variety of polysaccharides than have thus far

been recognized, but this supposition, he cautions, has had only little analytical support.

Pearse (1960) has stated that glycogen is the only naturally-occurring polysaccharide to remain in animal tissue following aqueous fixation and standard paraffin embedment. Of the five major carbohydrate groups treated by Pearse (1960), glycogen alone was labile following treatment with diastase and is, therefore, readily identified from other polysaccharides in tissue sections. Glycogen does, however, form diastase-fast complexes such as glycolipids and glycoproteins, and it is not unreasonable, as suggested by Pearse (1960), that other carbohydrates, ordinarily soluble or enzyme digestible, also form insoluble or indigestible conjugate complexes.

For this study, the PAS reaction (Figures 116 and 117) was used to determine the general concentration and distribution of carbohydrates in mesocestoidid tissues (Tables 9 thru 13). Known PAS-positive tissues, reported by various investigators have been listed by Pearse (1960). Although Tables 9 thru 13 summarize results of staining, the extent of carbohydrate concentration and distribution in Mesocestoides prompts a more detailed discussion of this biochemical group. In sections of mature and gravid proglottids examined, carbohydrate content was sufficiently extensive to obscure much of the counterstained, supposedly carbohydrate-

negative, tissues. PAS positive regions in gravid proglottids included tegument (exclusive of its innermost layer), subtegumental parenchyma, scattered granules and globules among the medullary muscles and cortical parenchyma, as well as extremely heavy deposits associated with the osmoregulatory canals and medullary parenchyma. Throughout most of the parauterine organ, fine carbohydrate globules and granules of carbohydrates of varying sizes and concentrations were apparent; but its inner lining, continuous with inner uterine lining, was generally negative. Scattered carbohydrate deposits were also observed in the egg mass matrix. Egg shells and oncospheres were strongly reactive, and carbohydrate granules within the oncospheres were approximately the same size as those in the vitellaria. The uterus, both outer and inner layers, appeared to be negative in some gravid proglottids but positive in others. Since carbohydrate deposits in uteri of mature proglottids vary, it is probable, if the energy requirement of developing segments is similar to that of mature segments, that depleted reserves of gravid segments would be comparable to the initial storage deposits of their earlier stages. Thus, in view of the suspected active nature of uterus and parauterine organ, the variable results reported (Tables 9 thru 13) are not surprising. Generally, PAS stained tissues of mature proglottids reacted similarly to those of gravid proglottids. Ovary and testes, however, which degenerate in late gravid proglottids of

<u>Mesocestoides</u>, were negative.

Diastase controls, subjected to enzyme action prior to oxidation with 0.5 percent periodic acid, resulted in an almost unstained tissue. However, if digestion followed oxidation, action of diastase was inhibited, and PAS staining was similar to, but for some unexplained reason, slightly more dense than non-control tissues. It is clear that the carbohydrate make-up of <u>Mesocestoides</u> like part of other species of cestodes is predominately glycogenic.

Best's carmine stain for glycogen (Figures 118 and 119) indicated a close similarity of glycogen deposition to the PAS-stained total carbohydrate content. However, calcareous corpuscles in the parenchyma and parauterine organs of gravid proglottids were weakly to moderately positive for glycogen; thus, all calcareous corpuscles in examined tissues of mature proglottids appeared negative. Only the outermost of the two outer tegumental layers was positive; the middle layer, which stained heavily for carbohydrate with PAS, did not stain with Best's carmine and is presumably other than glycogen. It is at least in part an acid mucopolysaccharide for which a slightly positive reaction was obtained. In view of the strong staining reaction of the middle layer, it is more probable that its major carbohydrate component is other than those tested in this study. Monne' (1959) also found difficulty in staining the PAS positive middle layer of the tegument and concluded that it was an acid mucopolysaccharide

of much reduced acidity. The difference in staining density between the PAS positive reaction of the middle layer and other staining reactions employed for acid mucopolysaccharides in this study and by Monné (1959) suggests that this tegumental layer is, in part, a non- or weakly acidic mucopolysaccharide consisting of some, as yet unidentified, non-glycogenic carbohydrate. Throughout the parauterine organ, a weak Best's carmine positive reaction occurs. The outer wall of the parauterine organ in mature proglottids shows moderately strong staining as do shells of intrauterine and parauterine eggs. Glandular cells of the uterus were negative, but the inner wall exhibited a variable, weakly positive reaction. Ovary and testes proper were negative, but vitellaria contained scattered positive staining granules; parenchymal structures of all reproductive organs were medium to strongly glycogen-positive in both mature and gravid proglottids of Mesocestoides.

Results of alcian blue and colloidal iron staining for acid mucopolysaccharides (Figures 132, 133, 136, 137) correspond well (Tables 9 thru 13). The greatest concentration of acid mucopolysaccharide material was observed in vaginal and uterine material (Figures 133 and 137) from mature proglottids, the density of which decreased as development of the proglottid progressed. However, as previously indicated, the converse is true of calcareous corpuscles which, in late gravid proglottids, showed the greatest

affinity for acid mucopolysaccharides. Weakly stained acid mucopolysaccharide deposits were observed scattered inconsistently throughout the parenchyma of both mature and gravid proglottids (Tables 9 thru 13). The inconsistent appearance of these deposits, pending further investigation, remains unexplained. The tegument, discussed above, gave in part a weak to strong positive reaction. Monne' (1959), having obtained similar results, suggested that this "staining is probably not due to the rods of the brush border, but to an acid mucopolysaccharide which is present in the interstices between the rods." He further states, "It is probable that the mucopolysaccharides of the main [= middle layer of this study] cuticular layer extend down into the interstices between the rods where the degree of its acidity is changed." This is an interesting point of view and would partly explain the more weakly staining activity of the middle tegumental layer. Worthy of note, but incidental to this study, is the fact that Monne' (1959) found the tegument of Mesoces-toides from the scolex to the late-gravid proglottids of the strobila, to have identical histochemical properties.

Lipids

In accordance with the definition of Pearse (1960), and as used in this study, lipids include all naturally occurring fat-like substances, insoluble in water, but soluble in organic solvents. For mammalian tissues at least, most

lipids are associated with proteins as complex conjugates (Pearse, 1960), and there is no reason to expect that lipids of cestodes should be significantly different. Substantiating evidence for their complex nature has been presented by von Brand (1966).

Like carbohydrates, lipids presumably fulfill a number of physiological functions including, according to von Brand (1966), storage of energy reserves and of waste products from anaerobic metabolism. Von Brand (1966) has pointed out that cestodes are the only parasitic invertebrates from which have been reported galactosides, glycolipids similar to the cerebrosides, and gangliosides of vertebrates. In addition, he lists the following lipid substances reported from cestodes: unidentified sterols, cholesterols, several phospholipids, and sterol or phospholipid-bound fatty acids. He further cautions that lipid distribution has been primarily studied by means of standard histochemical staining techniques which show only a part "often only a small fraction" of these chemically extractable substances. Therefore, more often than not, negative results are misleading, and tissues reported in this study and elsewhere as negative are, more likely, positive. Von Brand (1966), citing several investigators, indicates that parenchyma ordinarily is the most important site for lipid storage in cestodes. Other organs, reportedly, contain little or no lipids. Lipids have, however, been observed in cuticle, eggs, lumina of

uteri and of osmoregulatory canals. He further reports that investigations have shown a close connection between lipids and "excretory systems" of some tapeworm species, and that calcareous corpuscles also contain lipid deposits. It is the point of view of von Brand (1966) and of others reported in von Brand (1966) that lipid deposits, perhaps less toxic than other metabolic residues, probably are stored waste products of cestodes as well as of other anaerobes. If lipids are metabolic waste products, their presence in calcareous corpuscles lends support to proposals that these structures function as repositories for metabolic wastes.

Because of the technical difficulty in accurately separating lipid groups, no attempt has been made in this study to identify them. Total lipid content and its localization in tissues of Mesocestoides is presented in Tables 9 thru 13. Oil red O (Figures 124, 126, 127) and osmium tetroxide (Figures 77 and 82) both of which are general, broad spectrum stains for lipids, were used to locate lipid deposits in mature and gravid segments; both stains produced similar results in comparable sections.

In mature mesocestoidid proglottids, concentrations of lipid material consistently vary relative to internal localization and age of segments. Early-mature to early-gravid developmental stages contain the greatest density of lipid-staining substance (Figures 77 thru 82, 124). Distribution of lipids within mature proglottids appears to be

correlated with metabolically active organs or areas. The
greatest concentration accumulates around the early uterus
(Figure 70). Heavy lipid positive areas also surround active
testes, ovaries, vitellaria, and other related genitalia.
When compared with deposits in the reproductive region of
the proglottid, concentration of lipids in peripheral and
medullary parenchyma, as well as that associated with the
osmoregulatory canals, must be considered moderate to light
(Figures 79, 80, 82).

In gravid proglottids, parenchymal deposits are generally
similar to those described for mature proglottids, but major
concentrations noted in association with reproductive organs
of mature proglottids are reduced or absent. The parauterine
organ shows an overall, low-density staining reaction (Fig-
ures 126 and 127), but heavy conglomerate deposits occur
between the egg mass and the inner parauterine wall at both
posterior and anterior ends of the organ (Figure 127). Egg
shells, like much of the proteinaceous tissue, are weakly
positive. The sharp decrease of lipids found in older
proglottids possibly indicates that developing embryos, egg
shells, and other tissues have incorporated some of the
supposed reserves into structurally bound lipids.

Histochemical observations of Mesocestoides strongly
infer that lipids in this cestode fulfill two functions,
namely: (1) they serve as energy sources or storage reserves;
and (2) they may represent waste products, and like acid

mucopolysaccharides, residual lipids or their metabolic
by-products are stored to some degree in the calcareous
corpuscles. There is good reason to assume a polyfunctional
role for other histochemically identifiable metabolites as
well.

Nucleic Acids

The role of RNA and DNA in biological systems is well
known, but as von Brand (1966) has noted, chemical observa-
tions on nucleic acids of helminths are scant. Chemically,
according to von Brand (1966), "nucleic acids of parasites
seem to correspond more or less to those of free living
organisms." Lillie (1965), on the other hand, has noted
that in Feulgen-stained tissues of intracellular parasites
a positive but paler reaction than that observed in their
host cells usually occurs.

In this study, RNA was identified by the azure B
bromide method of Flax and Himes (1952). This technique
has been, for both qualitative and quantitative determination
of both RNA and DNA, reviewed in and highly recommended by
Wied (1966). In my studies, appropriate control tissues
were subjected to 10 percent perchloric acid extraction
and RNAse digestion, although Pearse (1960) has suggested
that results obtained by PCA extraction do not parallel
those of RNAse digestion and are, therefore, open to question.
While RNAse is highly selective, PCA may remove, depending

upon time and temperature, any or all nucleic acids as well as some glyco- and lipoproteins, and it is, therefore, suggested that RNAse is the better control measure for determining the presence of RNA (Pearse, 1960). DNA, which in azure B stained mesocestoidid tissues could not be satisfactorily differentiated from RNA, was positively identified using the standard Feulgen technique (Lillie, 1965). Contrary to Pearse (1960), PCA-treated control tissues (37° C for 15 minutes) were similar to RNAse-treated material (tris buffered, 37° C for two hours). However, neither method resulted in complete extraction or digestion, but the staining reaction was sufficiently reduced to permit positive identification of RNA sites. Pearse (1960) is probably correct, however, in suggesting that these two control methods for RNA are not comparable since PCA treatment at increased temperature (90° C for 15 minutes) resulted in azure B negative tissues, and it must be assumed that use of intermediate times or temperatures would have produced results between the extremes indicated.

Azure B bromide staining of mesocestoidid tissue (Figures 114, 115, 125, 139) did not produce the expected metachromasia described by previous users of the technique (Wied, 1966) or that observed, in this study, in control slides of simultaneously processed vertebrate tissue. The blue-green nuclear DNA, clearly visible in vertebrate tissue, was never apparent in the cestode material. However, the blue to purple hues, representing presence of RNA (Tables 9 thru 13), were strong.

Identification of DNA by means of Feulgen's stain, in conjunction with the noted PCA and RNAse controls for RNA, substantiated the azure B positive sites as being predominantly RNA. In mesocestoidid tissues, DNA was demonstrated only with difficulty. Extended staining or periods of oxidation, as well as increased concentrations of stain, did not improve nuclear affinity for DNA in tissues of Mesocestoides. A comparison of Feulgen stained mesocestoidid and vertebrate tissues suggests a difference in the chemical nature of the DNA found in Mesocestoides (Figures 128 and 129) from that in its vertebrate host. This difference with reference to other parasites has already been inferred by Lillie (1965). That RNA and DNA in cestode tissue should apparently differ from that in tissues of free living organisms, particularly vertebrates, is not surprising in view of the already noted biochemical differences between some carbohydrates and proteins of parasites and their counterparts in free living organisms.

Inorganic Constituents

The only inorganic substances histochemically tested in this study were iron and calcium. Both of these elements, together with organic complexes, were found to be components of the calcareous corpuscles. Other than the calcareous corpuscles, the nature and evaluation of which have been presented above, no tissues in Mesocestoides were found to

be calcium or iron positive. Gomori's iron reaction (Armed Forces Institute of Pathology, 1957) produced in the calcareous corpuscles a prussian blue, denoting presence of ferric ion rather than the lighter Turnbull's blue, indicative of ferrous ion (Figure 120). Solutions of Fe^{+++} and Fe^{++} were used, as a control, for comparison of color reactions obtained in tissues. Identification of individual calcium or iron compounds was not attempted, but it is suspected from results obtained from this and other studies that calcareous corpuscles of _Mesocestoides_ differ little from those described from other helminths and that they are proven sites for inorganic elements, particularly calcium.

DISCUSSION

The absence of a substantial mesocestoidid life cycle has been and continues to be a barrier to any definitive study of these cyclophyllidean tapeworms. However, experimental and descriptive data from this investigation, coupled with parallel data from other studies, have extended the systematic, phylogenetic, developmental, and morphological knowledge of this enigmatic cestode group.

Few, if any, categorical statements may be made concerning the systematic state of Mesocestoides. When the parameters used in identifying species of this genus are compared (Tables 2, 14, 15; Figures 2 and 3), it is apparent that the numerous so-called species described in the literature (Table 1) are merely morphological variants of one highly adaptable species. The similarity of form and mode of development of tetrathyridia from various hosts, as well as the number of species of definitive hosts susceptible to infection with larvae of known lineage, implies a high degree of adaptability for both larval and adult mesocestoidids. Unlike the trematode Telorchis bonnerensis shown by Watertor (1967) to exhibit host-induced variability, mesocestoidid variation may be, but is not necessarily, host-induced. Data from this study show that different species of hosts may produce similar adult worms from larval forms of known lineage. Furthermore, a single species of host may provide

specimens which differ considerably from one another. Because variation and variability of mesocestoidids at all known levels of development do occur, the currently accepted criteria for species determination are totally inadequate. Hence it seems valueless to continue to propose new species or to even accept as valid more than one, namely Mesocestoides lineatus (Goeze, 1782) which, by priority, is the earliest validly named species. It is here proposed that all currently known species of Mesocestoides be considered as M. lineatus.

If Mesocestoides is a monotypic genus, its extreme lack of host specificity implies a recent phylogenetic origin of the group, as Freeman (1957) has suggested. Whether Mesocestoides first infected carnivorous birds or mammals is uncertain. The apparent absence of the genus from Australia and nearby islands lends further support to the idea of a recent origin. Because the distribution of rapacious bird hosts extends from the mainland of southeastern Asia to Australia, one might expect these avian hosts in Australia to carry the infection. However, since they do not, it would seem that carnivorous mammals of other continents rather than birds were and continue to be principal hosts for this tapeworm genus. The few examples of non-carnivorous birds and non-carnivorous mammals reported as harboring adult Mesocestoides must be considered as being of minor importance. Apparently, they serve as hosts because of the extreme physiological

adaptability of this cestode group.

The concept of Mesocestoides as a more recently evolved cestode group is in full accord with its generally accepted position as a cyclophyllidean rather than as a proteocephalan (Wardle and McLeod, 1952; Yamaguti, 1958). Cyclophyllidean characteristics of Mesocestoides outweigh any pseudophyllidean aspects exhibited by them. The mesocestoidid hexacanth is adapted to a terrestrial mode of infection and resembles in most respects its typical taeniid counterpart, and the egg itself is substantially taenia-like. Histochemical observations of Johri, Smyth and Clegg (1959) have shown that both trematode and pseudophyllidean egg shells are composed of tanned quinones, whereas those of cyclophyllideans tested do not show this chemical composition. Histochemical tests performed on the egg shell of Mesocestoides in this study have shown it to be apparently without tanned quinones and, hence, more like those of the cyclophyllideans. It is probable that further histochemical observations on the eggs of parasitic and non-parasitic platyhelminths may prove to be of value in determining intraphyletic phylogenetic relationships.

In addition, the larval development of Mesocestoides, traced during the course of this investigation from tetrathyridium to adult, has provided further support for the cyclophyllidean nature of the group. Freeman (1957) has discussed the possible plerocercoid nature of the tetrathyridium, suggesting, in support of Joyeux and Baer (1936) and Wardle and

McLeod (1952), that a plerccercoid (similar to that of Par-
uterina) and not a cysticercoid or cysticercus best character-
izes the tetrathyridium. More recently, Voge (1967a, 1967b),
Voge and Coulombe (1966), Voge and Seidel (1968) have shown
that pretetrathyridial stages of Mesocestoides oncospheres,
hatched and developed in vitro, are procercoid-like. However,
it has been shown in this study that the development of
tetrathyridia to adult worms is remarkably similar to develop-
ment of cysticercoids to adults. Tetrathyridia observed
have shown a developmental pattern identical to that described
by Sawada (1959) for Raillietina kashiwarensis which, states
Voge (1967b) in her excellent review on "Post-embryonic
Developmental Stages of Cestodes," is typical of cysticercoid
development. As with Raillietina, only the scoleces of
larval mesocestoidids, after detaching from the tetrathyridial
body, become adult tapeworms. That tetrathyridia may repro-
duce asexually is of great interest, but asexual development
of other cestode larvae, both pseudophyllidean and cyclo-
phyllidean, is well documented. Thus it would seem that the
tetrathyridium larva of Mesocestoides, which possesses
characteristics common to procercoid, plerocercoid and
cysticercoid larval, is as unique in development, structure,
and host association as the adult into which it develops.

Whether or not two or three intermediate hosts are re-
quired in the life cycle of Mesocestoides remains questionable.
Attempts in this study to determine the pre-tetrathyridial

portion of the life cycle have been unsuccessful. Webster (1949), Witenberg (1934), Henry (1927) and others have suggested the necessity of two intermediate hosts. Although the present study provides some evidence for the likelihood of a second intermediate host, more compelling reasons exist for considering the cycle to involve only one such host. That a direct 1-host cycle exists is improbable because numerous feedings of eggs to known definitive hosts in this study and others has never resulted in an infection. Two reasons for the likelihood of a 3-host cycle may be cited. First, the odd larval forms found in the coprophagous beetle Onthaphagus resemble so much the developmental stages shown by Voge (1967) in her in vitro experiments with mesocestoidid oncospheres, that this insect is highly suspect as a potential intermediate host. Secondly, most hosts that harbor tetrathyridia are insectivorous to some degree, yet feedings of eggs to these hosts produced no infection. Data in support of the likelihood of a 2-host cycle include the cysticercoidlike form of early (small) tetrathyridia, as well as the large numbers of larvae of apparently the same developmental stage. Most recent in vitro studies by Voge and Seidel (1968) in which development of tetrathyridia from oncospheres was attained has added strong support to the latter contention.

Anatomically as well as developmentally, Mesocestoides is a unique cyclophyllidean. Although its central dorsal reproductive pore (Byrd and Ward, 1943) is analogous to the central,

<u>ventral</u> reproductive opening of the pseudophyllideans, it is more likely, in view of the absence of any uterine opening in mesocestoidids, that these cestodes are probably not related to the more primitive Pseudophyllidea. On the other hand, the presence of a parauterine organ is so typical of the anoplocephalan cyclophyllideans that Spasskii (1951) has included <u>Mesocestoides</u> in his monograph on the Anoplocephala. His opinion that <u>Mesocestoides</u> is probably an early offshoot of the cyclophyllidea seems likely in view of evidence presented. That the parauterine organ itself is of considerable consequence from the systematic and phylogenetic point of view prompted the detailed study made of this organ during this investigation. The segmental anatomy proposed by Byrd and Ward (1943) for <u>Mesocestoides</u> is fully accepted in this thesis, and the proposal by these authors that the outer parauterine wall is formed by discrete special parauterine cells which envelop the uterine wall and which together with the uterus form the parauterine organ appears to be a correct interpretation. The ideas of Chandler (1946) relative to the formation of this organ have been rejected.

Ultrastructural and histochemical studies made during this investigation have provided a clearer understanding of the mode of formation of the parauterine organ. This structure, in addition to its accepted passive role of protection for eggs, is presumably active in supplying energy to the embryos within it. It is possible that the laminated struc-

ture of the parauterine organ wall and the large number of
cell products found within the organ occur as a result of
cellular breakdown. It is well known that cellular disin-
tegration and later reorganization occurs in metamorphosing
insects (Lockshin and Williams, 1965). The apparent simi-
larity of the parauterine cells of Mesocestoides and those
cells undergoing reorganization in some metamorphosing
arthropods is striking. As in the development of metamor-
phosing insects, the developing and definitive parauterine
organ shows evidence of such cellular breakdown. Structurally,
cellular organelles unbounded by cell membranes are common to
both. It has been shown in this study that mitochondria,
myelin configurations, endoplasmic reticulum, and other cell
substances are common in the parauterine region. The appar-
ent absence of any secretory cells associated with this organ
is suggestive of cellular breakdown and, correspondingly, of
lysosomal activity even though, structurally, no lysosomes
have been demonstrated. Chemically, however, a high acid
phosphatase reaction was obtained in the parauterine tissue
and its viscous contents. In insects, such activity in
association with free cellular organelles has been shown to
indicate the presence of active lysosomes. It is possible
that as in insects, the products of cellular breakdown in
Mesocestoides may be utilized as an energy source.

Further support for the purported active nature of the
parauterine organ is provided by the increased number of

calcareous corpuscles suggested by von Brand (1966) as possible reservoirs of metabolic waste. In addition, high concentrations of glycogen within the parauterine organ and its depleted state in old proglottids seems indicative of the active nature of this organ. The fact that the parauterine organ itself is non-digestible in proteolytic enzymes provides additional evidence for the passive, protective function of this organ. As with many other aspects of Mesocestoides, the exact role of the parauterine organ in the life cycle and development of this cestode must await knowledge of a completed cycle.

SUMMARY AND CONCLUSIONS

1. Species of adult <u>Mesocestoides</u> from both naturally and experimentally infected hosts, principally from Iowa, were critically examined. Criteria employed for differentiation of species were found to be inadequate and it is hence proposed that all species of the genus be relegated to <u>M</u>. <u>lineatus</u> (Goeze, 1782).

2. Studies on world geographic distribution, host specificity, tetrathyridial development, and histochemistry of the adult indicate that mesocestoidids are primitive, highly adaptable, and recently evolved cyclophyllidean cestodes.

3. The well known tetrathyridium of mesocestoidid tapeworms has been shown to have a distinct cysticercoid-like developmental pattern leading to the young strobilate adult.

4. Mesocestoidids are geographically ubiquitous although apparently absent from Australia and nearby islands. <u>Mesocestoides</u> has been most commonly reported from Europe and northern Asia, but collections made in this study show it to be a common cestode of numerous North American hosts as well.

5. A survey of reports of mesocestoidid infections made in this study has shown that no less than 203 species of mammals, birds, reptiles and amphibians have been reported as hosts for either adult or larval <u>Mesocestoides</u>. Larval

infections are restricted to poikilotherms, whereas homoiotherms may harbor larvae and/or adults. Both tetrathyridia and adult worms are known to infect the same host species, sometimes simultaneously.

6. Although asexual development of tetrathyridia was not demonstrated or evident, large numbers of these larvae, up to 4,000, were found to be present in a single toad (Bufo americanus).

7. As a result of this study, amphibians (Bufo americanus, B. cognatus and Rana pipiens) have been shown to be normal and common hosts of tetrathyridia.

8. A detailed morphological (including electron and light microscopy) and histochemical study of the parauterine organ confirms that it is formed from cells lining the uterus as well as from differentiated parenchymal cells; hence, it is a true parauterine structure.

9. Functionally, the parauterine organ seems to have an active role in providing energy, and a passive role in providing protection for developing eggs and embryos.

10. Increasing numbers of calcareous corpuscles within the maturing parauterine organ, large quantities of carbohydrate, particularly glycogen, associated with this structure, as well as the presence of cellular organelles presumed to be a product of cellular breakdown, all contribute to the idea of an active role for the parauterine organ, whereas, a passive, protective function for it is supported by the

undigestible, apparently collagenous, nature of its wall.

11. Chemically, the mesocestoidid egg shell does not appear to be a quinone-tanned scleroprotein, but oncospheral hooks and uterine gland cells, when stained with malachite green, gave a positive reaction for scleroproteins. It is probable that the uterine gland cells may contribute to the composition of hooks in the mesocestoidid hexacanth.

12. The histochemical composition of maturing and gravid proglottids of Mesocestoides has been discussed in light of currently known information from related studies.

LITERATURE CITED

Alessandrini, G.
    1907.   Su un Dithyridium Rud. del polmone di gallina.
        Bollettino Soc. Zool. Ital. Roma. Ser. 2, 8:49-52.

Anantaraman, M.
    1954.   On the scheme of development in the genus Mesoces-
        toides of tapeworms. Indian Vet. J. 31:98-101.

Armed Forces Institute of Pathology.
    1949.   Manual of Histologic and Special Staining Technics.
        2nd Ed. New York, N.Y., McGraw-Hill (Blakiston)
        Book Co., Inc.

Baer, J. G.
    1933.   Contribution a l'étude de la faune helminthologique
        africaine. Rev. Suisse Zool. 39:31-84.

    1951.   Ecology of Animal Parasites. Urbana, Ill.,
        University of Illinois Press.

Batsch, A. J. G. C.
    1786.   Naturgeschichte der Bandwurmgattung überhaupt und
        ihrer Arten insbesondere, nach den neuern
        Beobachtungen in einem systematischen Auszuge.
        Halle.

Baumann, R. and L. K. Böhm.
    1941.   Cysticercose des Lendenmarkes beim Hunde. Wien.
        Tierärztl. Monatschr. 28:7-14.

Blumberg, C.
    1882.   Über einen neuen Parasiten beim Hunde und der
        Katze (Cysticercus elongatus). Deutsche Ztschr.
        Thiermed. 8:140-147.

Buck, S. C., F. R. N. Pester and J. J. C. Buckley.
    1959.   1. "Sparganum" from man, Uganda. 2. "Tetrathy-
        ridum" from genet cat, Kenya. Abstract of demon-
        stration. Roy. Soc. Trop. Med. and Hyg. Tr. 53:
        303-304.

Byrd, E. E. and J. W. Ward.
    1943.   Observations on the segmental anatomy of the tape-
        worm, Mesocestoides variabilis Mueller, 1928, from
        the opossum. J. Parasitol. 29:217-226.

Cadeac, C.
1909.   Dithyridiose peritoneale et pleurale du chat.   J.
Med. Vet. et Zootech. Lyon 60:477-478.

Cameron, T. W. M.
1925.   The cestode genus Mesocestoides Vaillant.   J.
Helminth. 3:33-44.

Carta, A.
1939.   L'identificazione del ciclo evolutivo del Mesoces-
toides lineatus provata sperimentalmente (With
summaries in Fr., Eng., and Germ.).   Riv. Para-
ssitol., Roma 3:65-81.

Chandler, A. C.
1942.   Mesocestoides manteri n. sp. from a lynx, with
notes on other North American species of Mesoces-
toides.   J. Parasitol. 28:227-231.

_____ 1944.   A new species of Mesocestoides, M. kirbyi, from
Canis latrans.   J. Parasitol. 30:273.

_____ 1946.   Observations on the anatomy of Mesocestoides.   J.
Parasitol. 32:242-246.

_____ 1947.   The anatomy of Mesocestoides--corrections.   J.
Parasitol. 33:444.

Ciordia, H.
1955.   Mesocestoides jonesi, n. sp. from the gray fox,
with descriptions of the chromosome complement and
a dicephalic specimen.   J. Tenn. Acad. Sci. 30:57-
63.

Creplin, F. C. H.
1825.   Observations de Entozois.   Greifswald.

_____ 1829.   Novae observationes de Entozois.   Berolini.

Dawson, A. B. and H. B. Friedgood.
1938.   Differentiation of two classes of acidophiles in
the anterior pituitary of the female rabbit and
cat.   Stain Tech. 13:17-21.

Diesing, K. M.
1850.   Systema helminthum.   Vol. 1, Vindobonae.

Dollfus, R. P.
    1965.   Cestodes de Carnivors, Rongcurs, Insectivors,
             Reptiles et Batraciens. Ann. Parasitol. Hum.
             Comp. 48:61-86.

Dubinina, M. N.
    1950.   Ecological investigations of the parasite fauna of
             Rana ridibunda Pall. in the Volga delta (Russian
             text). Parazitol. Sborn., Zoo. Inst., Akad. Nauk
             SSSR 12:300-350.

Dubnitskii, A. A.
    1949.   The principal cestode diseases of foxes and polar
             foxes. Abstract of dissertation (Russian text).
             Trudy Lab. Gel'mint., Akad. Nauk SSSR 2:222-225.

Ejsmont, L.
    1928.   Sur la question du cycle evolutif de Mesocestoides
             lineatus (Goeze) (French summary). Wiadom. Wet.
             7:1-8.

Fagasinski, A. and W. Piusinski.
    1960.   Infestation of Tetrathyridium elongatum (Blumberg,
             1882) in a dog (English summary). Wiadom.
             Parazytol. 6:533-536.

Flax, M. H. and M. Himes.
    1952.   Microspectrophotometric analysis of metachromatic
             staining of nucleic acids. Physiol. Zool. 25:297-
             311.

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

Fuhrmann, O.
    1909.   Die Cestoden der Vögel des weissen Nils. Results
             of Swedish Zool. Exped. Egypt and White Nile,
             1901. Pt. 3, No. 27. Jagerskiold.

    1932.   Les ténias des oiseux. Neuchâtel. Memoires de
             l'Universite de Neuchâtel, Vol. 8.

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

Gmelin, J. F.
    1790.   Caroli a Linné.   Systema Naturae; Part 6 (Vermes),
        pp. 3021-3910.

Goeze, J. A. E.
    1782.   Versuch einer Naturgeschichte der Eigeweidewurmer
        thierischer Korper. Blankenbburg.

Grundmann, A. W.
    1956.   A new tapeworm, Mesocestoides carnivoricolus,
        from carnivores of the Great Salt Lake desert
        region of Utah. Proc. Helminth. Soc. Wash. 23:
        26-28.

Hart, J. L.
    1967.   In vivo and in vitro regeneration of Mesocestoides
        tetrathyridia. An abstract of a paper read before
        the 42nd A.S.P. Meeting at Tuscon, Arizona (Ab-
        stract No. 87). Author's library.

Henry, A. C. L.
    1927.   Tetrathyridium et Mesocestoides. Bull. Soc.
        Centr. Med. Vet. 80:147-152.

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

Imperial Bureau of Agricultural Parasitology--Notes and
Memoranda, No. 10.
    1933.   The Helminth Parasites of Marsupials (Reprinted
        from the J. of Helminthology). Imp. Bureau of
        Agric., St. Albans, England.

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

Johri, L. N. and J. D. Smyth.
    1956.   A histochemical approach to the study of helminth
        morphology. Parasitol. 46:107-116.

Joyeux, C. E.
    1933.   Le réencapsulement de quelques larves de cestodes.
        Compt. Rend. Acad. Sci. Paris 197:493-495.

               and J. G. Baer.
    1932.   Récherches sur les cestodes appartenant au genre
        Mesocestoides Vaillant. Bull. Soc. Path. Exot.
        25:993-1010.

Joyeux, C. E. and J. G. Baer
    1936.   Cestodes. Faune de France. Paris.

_____ and R. Martin.
    1933.   Sur le cycle évolutif des Mesocestoides. Compt.
    Rend. Soc. Biol., Paris 114:1179-1180.

_____ and N. J. Kobozieff.
    1928.   Recherches sur l'Hymenolepis microstoma (Dujardin,
    1845). Ann. Parasitol. 6:59-79.

Krabbe, H.
    1865.   Helminthologiske Undersøgelser i Danmark og paa
    Island med saerligt Hensyn til Blaereormildelserne
    paa Island. Kjøbenhavn.

Leuckart, K. G. F. R.
    1874.   Bericht über die wissenschaftlichen Leistungen in
    der Naturgeschichte der niederen Thiere während
    der Jahre 1872-1875 (A continuation of same title,
    1873). Arch. Naturg., Berlin, 40 J. (6), Vol. 2:
    401-505.

Lillie, R. D.
    1965.   Histopathologic technic and practical histochem-
    istry. 3rd ed. New York, N.Y., McGraw-Hill Book
    Co.

Lockshin, Richard A. and C. M. Williams.
    1965.   Programmed Cell Death--I. Cytology of Degeneration
    in the Integumental Muscles of the Pernyi Silkmoth.
    J. Ins. Physiol. 11:123-133.

Lopez-Neyra, C. R.
    1947.   Helmintos de los Vertebrados Ibericos. In three
    parts. Granada.

Lühe, M. F. L.
    1910.   Parasitische Plattwürmer. Vol. 2, Cestodes. In
    Süsswasserfauna Deutschlands (Brauer), Heft 18,
    pp. 1-153.

MacCallum, G. A.
    1921.   Studies in Helminthology. Part 1, Trematodes,
    Part 2, Cestodes. Part 3, Nematodes. Zoopatho-
    logica, 1:135-284.

Markowski, S.
    1933.   Beitrag zur Kenntnis der Entwicklung der Larve
    Tetrathyridium variabile (Diesing, 1850). German
    text. Mem. Acad. Polon. Sci. et Lett., Cracovie,
    Cl. Sci. Math. et Nat., s. B:Sci. Nat. 6:43-51.

Maskar, U.
1953.   Über die experimentell erzeugte Entwicklung des Hundebandwurmes (Mesocestoides lineatus) aus den Dithyridien von Huhn und Igel (English summary) Schweizer Arch. fur Tierheilkunde 95:188-193.

Meggitt, F. J.
1924.   The cestodes of mammals. London.

_____ 1928.   Report on a collection of Cestoda, mainly from Egypt. Part III. Cyclophyllidea (conclusion): Tetraphyllidea. Parasitol. 20:315-328.

_____ 1931.   On cestodes collected in Burma. Part II. Parasitol. 23:250-263.

Moniez, R. L.
1880.   Essai monographique sur les cysticerques. Thesis. Lille. 1880. Same title published in: Trav. Inst. Zool. Lille 3:1-190.

Monné, L.
1959.   On the external cuticles of various helminths and their role in the host-parasite relationship: A histochemical study. Ark. Zool. Stockholm, 2nd series, Vol. 12, Häfte 4 No. 23:343-358.

Monticelli, F. S.
1889.   Notes on some Entozoa in the collection of the British Museum. Proc. Zool. Soc. London (21-22): 321-325.

Muehling, P.
1898.   Die Helminthen-Fauna der Wirbeltiere Ostpreussens. Arch. Naturg., Berlin 1:1-118.

Mueller, J. F.
1927.   Two new species of the cestode genus Mesocestoides. Tr. Am. Micr. Soc. 46:294.

_____ 1928.   The genus Mesocestoides in mammals. Zool. Jahrb., Jena, Abt. Syst. 55:403-418.

Neumann, L. G.
1896.   Notes sur les téniades du chien et du chat. Sur le genre Mesocestoides. Mémoires de la Soc. Zool. de France 9:171-184.

Neumann, L. G.
    1905.   A treatise on the parasites and parasitic diseases
             of the domesticated animals. A translation of
             (1892) by G. Fleming, 2nd ed. Revised and edited
             by J. MacQueen. London.

    1914.   Traité des parasites et des maladies parasitaires
             du chien et du chat. Paris.

Neveu-Lemaire, M.
    1936.   Traité d'helminthologie medicale et veterinaire.
             Paris.

Ogren, R. E.
    1952.   Demonstration of ribonucleic acid in the onchosphere
             and adult of Mesocestoides by means of ribonuclease.
             J. Parasitol. 38:498.

Pearse, A. G. E.
    1960.   Histochemistry (Theoretical and Applied). 2nd ed.
             London, England, J. A. Churchill Ltd.

Pence, D. B. and J. H. Miller.
    1967.   Fine structure and histochemistry of Dipylidium
             caninum eggs. Unpublished abstract of a paper
             presented at the 42nd A.S.P. Meeting in Tuscon,
             Arizona. Aug., 1967. Author's library.

Penrose, F. G.
    1882.   On a Cysticercus from the peritoneal cavity of a
             raccoon-like dog (Nyctereutes procyonides). Ann.
             and Mag. Nat. Hist. Series 5, 10:1-7.

Perrier, E.
    1897.   Traité de Zoologie. Part 2, Fasc. 4. Vers (suite)
             mollusquestuniciers. Paris.

Petrov, A. M.
    1940.   Parasitic worms of Mustelidae of the Moscow
             Zoological Gardens. Russian text with English
             summary. Trudy Moskovsk. Zooparka, 1:202-231.

             and A. A. Spasskii
    1954.   Cestodes--Mesocestoidata of domestic and wild
             animals. Russ. Trudy Gel'mint. Lab., Akad. Nauk
             SSSR 7:320-330.

Polonio, A. F.
    1860.   Catalogo dei cefalocotilei italiani e alcune
             osservazioni sul loro sviluppe. Atti. Soc. Ital.
             Sci. Nat., Milano (1859-60) 2:217-229.

Railliet, A.
 1893.   Traité de Zoologie médicale et agricole.   2nd ed.
         Fasc. 1.   Paris.

Ranson, B. H.
 1907.   Tapeworm cysts (Dithyridium cynocephali n. sp.)
         in muscles of a marsupial wolf (Thylacinus
         cynocephalus).   Tr. Am. Micro. Soc. 27:31-32.

Read, C. P.
 1959.   The role of carbohydrates in the biology of
         cestodes.   VIII.   Some conclusions and hypotheses.
         Exper. Parasitol. 8:365-382.

         and A. H. Rothman.
 1958.   The role of carbohydrates in the biology of
         cestodes.   VI.   The carbohydrates metabolized
         in vitro by some cyclophyllidean species.   Exper.
         Parasitol. 7:217-223.

Reynolds, E. S.
 1963.   The use of lead citrate at high pH as an electron
         opaque stain in electron microscopy.   J. Cell.
         Biol. 17:208-212.

Riggenbach, E.
 1896.   Das Genus Ichthyotaenia.   Rev. Suisse Zool. 4:
         165-275.

Riley, W. A.
 1921.   An annotated list of the animal parasites of foxes.
         Parasitol. 13:86-96.

Rissky, R. W.
 1962.   Parasites of the Plains Pocket Gopher, Geomys
         bursarius (Shaw) in Clay County, South Dakota.
         So. Dakota Acad. Sci. 41:83-90.

Rudolphi, C. A.
 1810.   Entozoorum sive vermium intestinalium historia
         naturalis.   Vol. 2.   No. 2.   Amstelaedami.

 1819.   Entozoorum synopsis cui accedunt mantissa duplex
         et indices locupletissimi.   Berolini.

Rundell, H. L.
 1957.   Electron microscope studies of the body covering
         of cestodes.   Unpublished Ph.D. thesis, State
         University, Iowa.   Library, Iowa City, Iowa.
         Title and abstract published in: Diss. Abstr.,
         17:3138.

Sabatini, D., K. Bensch, and R. Barrnett.
    1963.   Cytochemistry and electron microscopy. The
             preservation of cellular ultrastructure and
             enzymatic activity by aldehyde fixation. J. Cell.
             Biol. 17:19-58.

Sawada, I.
    1959.   Experimental studies on the evagination of the
             cysticercoids of Raillietina kashiwarensis. Exp.
             Parasitol. 8:325-335.

Schwartz, B.
    1927.   The life history of tapeworms of the genus Mesoces-
             toides. Science, n.s. (1696) 66:17-18.

Setti, E.
    1897.   Nuovi elminti dell' Eritrea. Boll. Mus. Zool.,
             Genova, No. 57. Genova. 1897. Idem.: in Atti
             Soc. Ligust. Sci. Nat. e Geogr. 8:198-247.

Skarbilovich, T. S.
    1945.   Seasonal destrobilization in the cestode Mesoces-
             toides lineatus parasitic of silver-black foxes.
             Dokl. Akad. Nauk SSSR n.s., an. 13, 46:171-172.

Skrjabin, K. I. and R. E. S. Shul'ts.
    1926.   Affinités entre le Dithyridium des souris et le
             Mesocestoides lineatus (Goeze, 1782) des
             carnivores. Ann. Parasitol. 4:68-73.

Smyth, J. D.
    1957.   Studies on tapeworm physiology, IX. A histo-
             chemical study of egg shell formation in
             Schistocephalus solidus (Pseudophyllidea). Exp.
             Parasitol. 5:519-540.

_____ and J. A. Clegg.
    1959.   Egg shell formation in Trematodes and Cestodes.
             Exp. Parasitol. 8:286-323.

Soldatova, A. P.
    1944.   A contribution to the study of the development
             cycle in the cestode Mesocestoides lineatus
             (Goeze, 1782) parasitic of carnivorous mammals.
             Dokl. Akad. Nauk SSSR, an 12, n.s. 45:310-312.

Ssolonitzin, I. A.
    1933.   Mehrfacher Tetrathyridios der serösen Höhlen des
             Hundes. Ztschr. Infektionskr. Haustiere 45:144-156.

Spasskii, A. A.
    1951.   Essentials of Cestology. Under direction of K. I.
             Skrjabin, Acad. of Sci. of the USSR. Vol. 1.
             [Translation: USDA; P.S.T. Cat. No. 108; (1961)]

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

Sprehn, C. E. W.
    1932.   Lehrbuch der Helminthologie. Eine Naturgeschichte
             der in deutschen Saugetieren und Vögeln schmarot-
             zenden Würmer, unter besonderer Berücksichtingung
             der Helminthen des Menschen, der Haustiere und
             wichtigsten Nutztiere. Berlin.

Srivastava, H. D.
    1939.   A study of the life-history of a common tapeworm,
             Mesocestoides lineatus, of Indian dogs and cats.
             Indian J. Vet. Sci. and An. Husb. 9:187-190.

Stossich, M.
    1896.   Ricerche elmintologiche.   Boll. Soc. Adriat. Sci.
             Nat. Trieste 17:121-136.

Swift, H.
    1966.   The quantitative cytochemistry of RNA. In Wied,
             George L., ed. Introduction to quantitative
             cytochemistry. Pp 355-386. New York, N.Y.,
             Academic Press.

Timofeev, V. A.
    1964.   An electron microscope study of calcareous bodies
             in the plerocercoid and sexually mature stages of
             Schistocephalus pungitti. Dokl. Akad. Nauk SSSR
             156:1244-1247 (422-426 in English translation).

Vaillant, L.
    1863.   Sur deux helminthes cestoides de la genette.
             Institut, Paris, 1. Sect. 1524 31:87-88.

Viborg, E. N.
    1795.   Sammlung von Abhandlungen für Thierärzte und
             Oekomen Erstes Bandchen. Copenhagen. photostat
             copy of pp. 234-251 in Zool. Div. of John Crerar
             Library, Chicago.

Voge, M.
    1952.   Mesogyna hepatica n.g., n. sp. (Cestoda: Cyclo-
             phyllidea) from kitfox, Vulpes macrotis.   Tr. Am.
             Micr. Soc. 71:350-354.

    1955.   North American cestodes of the genus Mesocestoides.
             Univ. Calif. Pub. Zool. 59:125-156.

Voge, M.
1967a. Development in vitro of Mesocestoides (Cestoda) from oncosphere to young tetrathyridium. J. Parasitol. 53:78-82.

1967b. The post-embryonic developmental stages of Cestodes. In Dawes, Ben., ed. Advance in Parasitology. Vol. 5. Pp. 247-297. New York, N.Y., Academic Press, Inc.

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

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

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

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

Vojtková, L.
1963. Larvenstadien der Helminthen in den Amphibien der ČSSR. Germ. Sum. Česk. Parasitol. 10:171-185.

von Brand, T.
1966. Biochemistry of parasites. New York, N.Y., Academic Press.

Walter, H.
1866. Helminthologische Studien. 7. Ber. Offenbacker Ver. Naturk. (1865-1866), pp. 51-79.

Ward, Henry Baldwin.
1895. The parasitic worms of man and the domestic animals. Ann. Rep. Nebraska St. Bd. Agric. (1894). Pp. 225-348.

Wardle, R. and J. A. McLeod.
1952. The zoology of tapeworms. Minneapolis, Minnesota, Minnesota Press.

Watertor, Jean L.
    1967.   Intraspecific variation of adult Telorchis
            bonnerensis (Trematoda: Telorchiidae) in amphibian
            and reptilian hosts. J. Parasitol. 53:962-968.

Watson, M. L.
    1958.   Staining of tissue sections for electron micro-
            scopy with heavy metals. J. Biophys. Biochem.
            Cytol. 4:475-478.

Webster, J. D.
    1949.   Fragmentary studies on the life history of the
            cestode Mesocestoides latus. J. Parasitol. 3:83-
            90.

Wetzel, R. and G. Quittek.
    1940.   Über die Entwicklungsdauer (Präpatentperiode)
            der parasitischen Wurmer in Wirtstier. Arch.
            Wissensch. u. Prakt. Tierh. 75:336-369.

Wied, G. L. (ed.).
    1966.   Introduction to quantitative cytochemistry. New
            York, N.Y., Academic Press.

Witenberg, G. G.
    1934.   Studies on cestode genus Mesocestoides (with
            summaries in French, English and German). Arch.
            Zool. Ital. 20:467-509.

Yamaguti, S.
    1959.   Systema helminthum. Vol. 2, (Cestode). New York,
            N.Y., Interscience Publishers, Inc.

Zschokke, F.
    1885.   Über den Bau der Geschlechtswerkzeuge von Taenia
            litterata. Zool. Anz., Leipzig 198:380-384.

    1889.   Récherches sur la structure anatomique et
            histologique des cestodes. Mém. Inst. Nation.
            Genevois. 17:1-396, pls. 1-9, figs. 1-156.

ACKNOWLEDGEMENTS

Thirdly, to those persons who have had a direct influ-
ence in stimulating me to pursue a teaching/research career
I owe much. Among these, I particularly wish to thank the
late Professor William W. Everett, my former undergraduate
advisor and Chairman of the Biology Department at the Univer-
sity of Bridgeport, Connecticut, where I first taught; Dr.

APPENDIX A. TABLES

Table 1. Species, type hosts and host localities of Mesocestoides Vaillant, 1863 (Mesocestoididae Perrier, 1897)

Species	Type host
alaudae (Stossich, 1896)	Alauda arvensis
ambiguus (Vaillant, 1863)[c]	Viverra genetta
angustatus (Rudolphi, 1819)	Meles taxus
bassarisci MacCallum, 1921[c]	Bassariscus astutus
caestus Cameron, 1925[c]	Mellivora ratel
candelabrarius (Goeze, 1782)	Strix aluco L.
canis lagopodis (Rudolphi, 1810)	Alopex lagopus
carnivoricolis (Grundmann, 1956)	Taxidea taxus
cateniformis vulpis (Gmelin, 1790)	foxes (Vulpes)
charadrii Fuhrmann, 1909[b,c]	Tringa (Limonites) minuta
chrysaeti (Viborg, 1795)	Aquila chrysaeti
corti Hoeppli, 1925[a,c]	Mus musculus
dissimilis Baer, 1933	Myonax sanguineus
elongatus Meggitt, 1928	wolf (Canis)
imbutiformis (Polonio, 1860)	Anser ferus
jonesi Ciordia, 1955	Urocyon cinereoargenteus
kirbyi Chandler, 1944[a,c]	Canis latrans

[a]Species recognized by Voge, 1955

[b]Species recognized by Witenberg, 1934

[c]Species recognized by Wardle and McLeod, 1952

Table 1. (Continued)

Host localities
Trieste, Italy
North Africa
Europe
Mexico
N.E. Africa
Europe (?)
Europe
Utah, U.S.A.
Europe
Egypt
Europe
Colorado, U.S.A.
Africa
Egypt
Italy
Tennessee, U.S.A.
California, U.S.A.

Table 1. (Continued)

Species	Type host
latus Mueller, 1927[a,c]	_Mephitis minnesotae_
lineatus (Goeze, 1782)[a,b,c]	_Felis sylvestris_
litteratus (Batsch, 1786)[c]	fox (_Vulpes_)
longistriatus Setti, 1897	_Canis aureus_
macrocephala (Creplin, 1825)	_Anguilla vulgaris_
magellanicus Monticelli, 1889	not named
manteri Chandler, 1942[c]	_Lynx rufus_
margaretifera (Creplin, 1829)	birds of prey
mesorchis Cameron, 1925	_Vulpes feritatus_
michaelsenii Loennberg, 1896	_Canis azarae_
perlatus (Goeze, 1782)[b,c]	_Buteo buteo_ (L.)
polystriatus Setti, 1897	_Felis catus_
pseudocucumerina (Baillet, 1863)	dog (_Canis_)
pseudo-elliptica (Baillet, 1866)	dog (_Canis_)
tenuis (Creplin, 1829)	_Falco subbuteo_
tenuis Meggitt, 1931[c]	dog (_Canis_)
utriculiferus Walter, 1866	fox (_Vulpes_)
variabilis Mueller, 1927[c]	_Urocyon cinereoargenteus_

Table 1. (Continued)

Host localities

Minnesota, U.S.A.

Europe ("am Fuss des Brockens")

Europe

Erithrea

Europe

not named

Nebraska, U.S.A.

Europe and Asia

London Zoo (from Nepal)

Brazil

Europe (?)

Erithrea

Europe (?)

Europe

Europe (?)

Burma

Europe

U.S.A.

Table 2. Criteria used in differentiating species of
          Mesocestoides
          (Minima and maxima from various sources)[1,2]

M. species	Strobila Length	Strobila Width	Scolex Diameter	Sucker Diameter
lineatus	10^d-1600^e (2500^{3f})	to $3^d.0$	450^d-900	180^a-285^a
perlatus	100^d-350^g	0.6^g-2.5^d	400^g-800^d	240^gx50^g
charadrii	50^d	0.5^g	510^g	190^gx230^g
variabilis	50^a-300^f+	0.8^a-1.5	247^a-616^a	92^a-188^ax 99^a-208^a
corti	40^a-80^a	1.0^a	500^a-600^a	200^ax300^a
manteri	9^a-30^a	1^f-1.25^f	277^a-400^a	90^a-180^ax 126^a-210^a
bassarisci	35^a-60^a	$-^i$	297^a-420^a	90^a-126^ax 100^a-130^a
latus	19^a-1230^a	1.4^a-2.5^a	393^a-680^a	126^a-195^ax 128^a-272^a

[1]Superscript letters denote the following sources: a =
Voge (1955), b = Ciordia (1955), c = Grundman (1956), d =
Neveau-Lemaire (1936), e = Witenberg (1934), f = Wardle and
McLeod (1952), g = Joyeux and Baer (1936), h = Joyeux, Baer
(1932), i = no data.

[2]Strobila measurements in millimeters; all other measurements in microns.

[3]In error according to Mueller (1928), Witenberg (1934).
According to Mueller 1928 (in Witenberg, 1934), error by
Railliet perpetuated: 2500 mm. should be 250 mm.

Table 2. (Continued)

Testes		Cirrus pouch		Egg capsule	
Number	Diameter	Length	Width	Length	Width
25^f-100^d	40^f-81^a	126^a-300^d	90^a-144^a	359^f-680^d	-^{i}
20^d-70^g	32^h-35^h	170^g	80^g	120^g-330^h	-^{i}
-^{i}	-^{i}	140^g	70^g	-^{i}	-^{i}
32^a-86^a	36^a-86^a	81^a-180^a	59^a-103^a	239^a-447^a	215^a-454^a
35^a-60^a	26^a-60	100^a	60^a	350^a	-^{i}
20^a-45^a	29^a-65^a	71^a-140^a	54^a-110^f	-^{i}	245^f-275^f
24^a-35^a	-^{i}	80^a-98^a	-^{i}	-^{i}	-^{i}
31^a-117^a	52^a-92^a	81^a-180^a	63^a-120^a	285^a-408^a	200^a-297^a

Table 2. (Continued)

M. species	Strobila Length	Width	Scolex Diameter	Sucker Diameter
kirbyi	190^f-300^f	1.6^a-2.0^f	350^f-662	130^f-255^ax 207^a-300^f
jonesi	5.5^b-12^b	0.38^b-$.47^b$	190^b-270^b	130^b-220^bx 90 -120
carnivoricolis	62^c-112^c	0.60^c-$.85^c$	300^cx416^c	120^c
M. species wild, this study	12-2000+	0.9-2.6	234-649	85-213.6
M. species experimental this study	15-1153	0.74-1.86	266-628	75-159.6

Table 2. (Continued)

Number	Diameter	Cirrus pouch		Egg capsule	
		Length	Width	Length	Width
54^a-136^a	40^a-72^a	117^a-220^a	90^a-178^a	314^a-560^f	222^a-320^f
24^b-32^b	25^b-38^bx 48^b-35^b	66^b-135^b	33^b-75^b	105^b-183^b	80^b-144^b
25^c-35^c	24^c-32^cx 32^c-38^c	81^c(Ave)	61^c(Ave)	275^cx400^c	250^c
44-100	21-80	106-234	63.8-138	212-426	170-341
50-78	32-53	85.1-160	53-125	150-435	85-351

Table 3. Hosts recorded for adult and larval <u>Mesocestoides</u>[1]

	Number of species per genus	Habitat zones[2]							
		A	B	C	D	E	F	G	H
Mammalia (61 Genera, 108 Species)									
Marsupialia									
Dasyuridae									
Thylacinus	1	?						O	
Didelphidae									
Chironectes	1	O					Ø		
Didelphis	1	⊖					O		
Insectivora									
Erinaceidae									
Erinaceus	3		Ø	O					
Paraechinus	2		Ø	O					
Soricidae									
Crocidura	4		Ø	Ø				Ø	
Talpidae									
Desmana	1		Ø	Ø					
Talpa	1		Ø	O					
Chiroptera									
Vespertilionidae									
Miniopterus	1		Ø	O				O	O
Plecotus	1	O	Ø	O					
Vespertilio	1		Ø	O					

[1]Symbols denote the following: O = world distribution for host genus, ⊖ = hosts reported for adult Mesocestoides, Ø = hosts with tetrathyridia only, Ø = tetrathyridia and adult worms.

[2]Refer to map zones, Figure 1.

155

Table 3. (Continued)

	Number of species per genus	Habitat zones[2]							
		A	B	C	D	E	F	G	H
Primates									
Cercopithecidae									
Cercopithecus	1		∅				O		
Chaeropithecus	1		∅				O		
Hominidae									
Homo	1	⊖	⊖	⊖	O	O	O	O	O
Lemuridae									
Lemur	1						∅		
Lagomorpha									
Leporidae									
Lepus	1	O	∅	O			O		
Rodentia									
Cricetidae									
Arvicola	2		∅	∅					
Clethrionomys	2	O	∅	∅					
Cricetus[3] "hamster"	1	O	O	O					
Ellobius	1			∅					
Microtus	3	O	O	∅			O		
Ondatra	1	∅							
Peromyscus	2	∅				O			
Pitymys	1	O	∅	O					
Gliridae									
Dryomys	1		O	∅					

[3]Spread by man and possibly in an unnatural locality.

Table 3. (Continued)

	Number of species per genus	Habitat zones[2]							
		A	B	C	D	E	F	G	H
Muridae									
Acomys	1		ø	O			O		
Apodemus	4		ø	ø			O		
Micromys	1		ø	ø					
Mus	1	ø	ø	ø			O	O	
...Rattus[3]	2	O	ø	ø	O	O	O	O	O
Procaviidae									
Hyrax	1		Θ				O		
Sciuridae									
Citellus	2	O	ø	ø					
Eutamias	1	O		ø					
Marmota	1	O	ø	O					
Sciurus	1	O		ø		O			
Zapodidae									
Zapus	1	ø							
Carnivora									
Canidae									
Alopex	1	Θ	Θ	Θ					
Canis[3]	7	ø	ø	ø	O	O	O	O	Θ
Dusicyon	1					Θ			
Nyctereutes	1			ø					
Urocyon	2	Θ				O			
Vulpes	4(6)	Θ	ø	Θ		Θ	O		
Felidae									
Felis	9	Θ	ø	ø	O	O	Θ	O	O
Lynx	4	Θ	Θ	Θ			Θ		
Panthera	3	O		Θ		O	O	O	

157

Table 3. (Continued)

	Number of species per genus	Habitat zones[2]							
		A	B	C	D	E	F	G	H
Mustelidae									
Gulo	1	⊖	○	⊖					
Lutra	1	○	○	⊖		○	○	○	
Martes	3	○	⊖	∅				○	
Meles	2		∅	⊖					
Mellivora	1		⊖	○			○	○	
Mephitis	1(4)	⊖							
Mustella	5	⊖	∅	∅					
Spilogale	1(3)	⊖							
Taxidea	1	⊖							
Procyonidae									
Bassariscus	1	⊖							
Procyon	1	⊖				○			
Ursidae									
Ursus	1	○	○	⊖					
Viverridae									
Genetta	1		∅	○			○		
Herpestes	2		∅	○			○	○	
Artiodactyla									
Cervidae									
Capreolus	1		○	∅					
Odocoileus	1	∅				○			

Table 3. (Continued)

	Number of species per genus	Habitat zones							
		A	B	C	D	E	F	G	H
Aves (Birds) (34 Genera, 57 Species)									
Accipitriformes									
Aquila	6	O	Θ	Θ	--		O	O	
Astur	1	O	O	Θ	O	O	O	O	O
Buteo	3	O	Θ	O		O	O		
Circaetus	1		Θ	O			O		
Circus	3	O	Θ	O	O	O	O		
Falco (+ Cerchneis)	8	O	Θ	Θ	O	O	O	O	O
Milvus	4		Θ	∅			O	O	
Pseudogyps	1		O	Θ			O	O	
Terathopius	1						Θ		
Anseriformes									
Anser[3]	2	O	Θ	Θ	O	O	O	O	O
Clangula	1	Θ	O	O	O	O		O	
Mergus	1	Θ							
Ardeiformes									
Ardea	1	O	∅		O	O			
Charadriiformes									
Tringa (= Limonites)	1		Θ	O			O	O	
Columbiformes									
Goura	1			∅				O	
Cypseliformes									
Cypselus	1		∅	O			O	O	

Table 3. (Continued)

	Number of species per genus	Habitat zones							
		A	B	C	D	E	F	G	H
Galliformes									
Gallus	1	O	∅	O	O	O	O	O	O
Lyrurus	1			∅					
Meleagris	2	O	∅			O			
Numida	1		∅						
Pavo	1		∅						
Perdix	1	∅	∅						
Lariformes									
Larus	1	O	O	O		θ	O	O	O
Passeriformes									
Alauda	2		θ	θ					
Coleus	1		∅						
Corvus	2	O	∅	O		O	O	O	
Lanius	1	O	∅						
Passer	1	O	∅	O		O	O	O	
Pica	1	∅							
Turdus	1		O	∅			O	O	
Podicipediformes									
Aechmophorus	1	θ							
Strigiformes									
Asio	1	O	θ	O					
Strix	1	O	∅	O					
Lamprocolius	1		∅						

Table 3. (Continued)

	Number of species per genus	Habitat zones							
		A	B	C	D	E	F	G	H
Reptilia (24 Genera, 32 Species)									
Lacertilia (Lizards)									
Chalcides	1		∅	0					
Eumeces	1	∅	0	0		0			
Gerrhonotus	1	∅							
Lacerta	4	0	∅	0					
Leiopisma	1	∅		0		0	0	0	
Lialis	1			∅				0	
Mabuia	1	0	0	∅			0	0	
Sceloporus	1	∅							
Stellio	1		∅	0			0	0	
Tarentola	2		∅			0	0		
Ophidia (Snakes)									
Bungarus	1			∅				0	
Chrysopelea	1			∅					
Crotalis	1	∅							
Coronella	1		0	∅			0	0	
Elaphe	2	0	∅	0				0	
Hypsirhina	1							∅	
Myonax	1						∅		
Naja	1			∅				0	0
Natrix	2	0	0	∅					
Psammophis	1			∅				0	0
Rhabdophis	1			∅					
Simotes	2			∅					
Tropidonotus	1	0	∅	∅		0	0	0	
Zamenis	2	0	∅	0		0	0	0	

Table 3. (Continued)

	Number of species per genus	A	B	C	D	E	F	G	H
Amphibia (4 Genera, 6 Species)									
Anura (Frogs)									
Hyla	1	O	Ø	O		O	O	O	
Rana	2	Ø	Ø	O		O	O	O	
Urodela (Toads)									
Bufo	2	Ø	O	O		O	O		
Bombina	1		Ø						

Table 4. Summary of mammalian hosts naturally infected with adult <u>Mesocestoides</u>, 1962-1967

Host	Locality		Number examined	Number infected	Percent infected
	State	County			
<u>Didelphis</u>	Conn.	Fairfield	8	4	50.0
<u>marsupialis</u> L.		New Haven	5	2	40.0
	R.I.	Washington	1	0	-
	Va.	Giles	1	1	-
		Rockingham	3	0	00.0
	Iowa	Audubon	12	5	41.7
		Boone	4	2	50.0
		Dickinson	14	4	28.6
		Hamilton	1	1	-
		Ida	1	1	-
		Iowa	1	1	-
		Mahaska	3	0	00.0
		Marshall	1	1	-
		Poweshiek	4	1	25.0
		Sac	12	3	25.0
		Story	9	3	33.3
		Tama	3	0	00.0
		Union	7	1	14.3
		Warren	3	1	33.3
			93	31	33.3

163

Table 4. (Continued)

Host	Locality State	County	Number examined	Number infected	Percent infected
Canis familiaris L.	Conn.	Fairfield	3	0	-
	Iowa	Story	2	2	-
			5	5	-
Urocyon cinereoargenteus (Schreber)	Iowa	Story	1	1	-
Vulpes fulva (Desmarest)	Conn.	New Haven	3	0	00.0
	Va.	Rockingham	1	1	-
	Iowa	Audubon	10	0	00.0
		Dickinson	1	0	-
		Montgomery	5	0	-
		Poweshiek	1	0	-
		Story	5	0	00.0
		Union	4	0	00.0
		?	1	0	-
			31	1	03.2

Table 4. (Continued)

Host	Locality State	County	Number examined	Number infected	Percent infected
Mephitis					
mephitis Schreber	Iowa	Audubon	9	2	22.2
		Dickinson	12	3	25.0
		Mahaska	2	0	00.0
		Montogomery	1	1	-
		Poweshiek	7	4	57.1
		Sac	9	0	00.0
		Story	1	1	-
		Tama	7	3	42.9
		Warren	4	1	25.0
		Wright	8	1	12.5
		?	1	0	-
			61	16	26.2
Spilogale putorius (L)	Iowa	Dickinson	7	0	00.0
		Montogomery	2	0	00.0
		Poweshiek	3	2	66.7
		Wright	1	0	-
			13	2	15.4

Table 4. (Continued)

Host	Locality State	County	Number examined	Number infected	Percent infected
Procyon lotor L.	Conn.	Fairfield	2	1	50.0
		Hartford	1	1	-
		New Haven	2	0	00.0
	Iowa	Boone	1	0	-
		Dickinson	22	4	13.6
		Ida	1	1	-
		Mahaska	5	2	40.0
		Poweshiek	2	1	50.0
		Story	9	4	44.4
		Tama	7	5	71.4
		Union	10	7	70.0
		Wright	9	6	66.7
			71	32	45.1
		TOTAL	275*	85	30.9

*Does not include 7 species (79 individuals) of potentially positive hosts reported in the text of this study which were examined and found to be uninfected.

Table 5. Longevity of oncospheres within proglottids sub-
          jected to several environmental conditions
          (Based on examination of a minimum of three
          proglottids per day)

Humidity	Temperature in °C	Survival time (days)
Dry[1]	36	4
Dry	10	12
Damp[2]	10-15 (average 12)	13-51
Damp	- 5	49
Wet[3]	36	12
Wet	10	13

[1]Dry = exposed to room atmosphere without any added moisture.

[2]Damp = kept in most chamber but no direct contact with water.

[3]Wet = placed on wet filter paper in petri dish (covered).

Table 6. Longevity of oncospheres removed from parauterine
organs and subjected to several environmental
conditions
(From observations of oncospheres in eggs from
a minimum of five proglottids)

Conditions	Survival time (minutes)
Distilled water	0.05
3 Percent NaCl	1.0
Saline (0.65-0.9), pH 3-5 (with or without pepsin)	10-15
Saline (0.65-0.9), pH 8-10 (with trypsin)	30+/-5
Saline (0.65-0.9), pH 8 (in normal saline)	60-180
Amphibian intestinal juice	180-420
Dry at room temperature	0.05-1.5

Table 7. Invertebrates fed eggs of <u>Mesocestoides</u>[1,2]

Annelida

    <u>Lumbricus</u> sp. (earthworm)[b]

Mollusca[b]

    <u>Anguispira</u> sp. (tiger snail)

Arthropoda: Acarina

    Oribatid mites[c,d]

    Mesostigmatid mites

    Unidentified mites

Arthropoda: Crustacea

    <u>Cyclops</u>[a]

    <u>Oniscus</u> (sow or pill bugs)[b]

    <u>Hyalella</u> (scud, sideswimmer)

Arthropoda: Diplopoda (millipedes)[b]

Arthropoda: Chilopoda (centipedes)[b]

Arthropoda: Insecta

    Collembola (springtails)[b]

    Coleoptera (beetles)

        Staphylinidae (rove) adults and larvae

        Carabidae (ground) adults and larvae[b]

---

[1]Invertebrates previously reported to have been fed mesocestoidid eggs; letters in superscript position indicate a = Joyeux and Baer (1932), b = Webster (1949), c = Whittaker (personal communication), d = Soldatova (1944), e = Witenberg (1934), f = Henry (1927).

[2]Each group represented by several to many individuals.

Table 7. (Continued)

Stegobium sp. (spice)

Dermestidae (dermestids) adults and larvae

Tenebrio molitor[b] (mealworm) adults and larvae[3]

Nitidulidae (picnic)

Necrophorus (sexton)[3]

Harpalus sp.

Tribolium (confused flour)[b]

Coccinellidae (ladybird)

Onthophagus (dung)[3]

Silpha (silphids) adults and larvae

Curculionidae (weevils)

Hister sp.[b]

Orthoptera

Tettigoniidae (locusts)[b]

Gryllidae (field crickets)[b]

Gryllacrididae (camel crickets)[b]

Blattidae (cockroaches)[b,d,e]

Siphonaptera (flea) larvae

Diptera (flies) larvae[b,d,e]

---

[3]Those organisms which may represent positive infections; see Experimental Studies: Feeding Experiments, Invertebrate Hosts.

Table 8. List of vertebrate hosts fed tetrathyridia[1]

Hosts	Tet. source	Number fed	Time (days)[2]	Worms recovered
Birds				
1 Passer domesticus	R	5-10	38	0
1 Gallus gallus	R	5-10	39	0
1 Bubo virginianus	B	6-10	35	0
Mammals				
22 Didelphis marsupialis	R	6-10	8	0
Number infected:	R	6-10	8	0
10 (45.4 Percent)	R	5-10	48	0
	B	5-10	54	0
	B	5-10	56	0
	R	5-10	54	0
	R	1	34	0
	B	10	34	0
	Control			0
	Control			0
	Control			0

[1]Abbreviations denote the following: B = Bufo, e = early, G = gravid, I = immature, M = mature, O = negative, P = proglottids only, R = Rana.

[2]Time in days refers to time elapsed between feeding and examination of host, or between feeding and recovery of proglottids (P); O refers to absence of worm.

Table 8. (Continued)

Hosts	Tet. source	Number fed	Time (days)^2	Worms recovered
Mammals (continued)				
Didelphis marsupialis	R	5-10	27	4 Ge
	R	30-40	42	1 G (115 cm.)
	R	several +20	40-70	1 G
	R	20	11	2 I
	R	several	?	12 I
	R	several	34	0
	R	several	30	2 M
	R	many	60$^{\pm}$	1 I
	B	several	27	2 I
	B	several	30	4 M
	R	6-10	33	0
	B	6-10	37	0
	B	6-10	240	0
	B	6-10	8	2 I
2 Canis familiaris	B	6-10	33	3 M + G
Number infected:	R	30	8	2 Ie
2 (100 Percent)				
2 Canis latrans	B	6-10	38	2 G + I
Number infected:	R	several	55	0
1 (50 Percent)				
3 Vulpes fulva	B	6-10	38	0
Number infected:	B	6-10	34	8 G
1 (33.3 Percent)	R	many	65	0

Table 8. (Continued)

Hosts	Tet. source	Number fed	Time (days)[2]	Worms recovered
Mammals (continued)				
8 Felis catus	R	many	65	0
None infected	R	5	21	0
	R	10	21	0
	R	10	34	0
	R	10		
	B	5		
	R	100	51	0
	B	20-25	9	0
	B	20-25	9	0
	R/M	20	23	0
	R/M	15	23	0
28 Procyon lotor	B	6-10	37	P
Number infected:	B	6-10	23	P
18 (64.3 Percent)			24	P
			40	P
	B	6-10	28	P
			39	1 G
	B	6-10	35	P
			38	5 G
	R	20	1.5 Hrs.	Many Ie
	R	30	2.0 Hrs.	Many Ie
	R	20	23	Many G
	R	60	200	Many IMG
	R	100	140	
	R	many	1	

173

Table 8. (Continued)

Hosts	Tet. source	Number fed	Time (days)[2]	Worms recovered	
Mammals (continued)					
<u>Procyon</u> <u>lotor</u>	R	10	43	0	
	R	5	8	0	
	R	5	38	0	
	R	2	51	2	G
	Control			0	
	R	20-25	45+++	P	
	R	++++	240	45	Ie
	R	10	24	1	G
	R	negative) carcases)	-	0	
	R	10	34	4	I
	R	1	34	0	
	B	several	45	G	
			14	M	
	R	10	52	2	G
	B	5-10	30	1	M
			23	P	
	Control			0	
	Control			0	
	B/M	3	16	P	
			19	3	G
	Control			0	
	R	many	10	55	I
	R	1	39	0	
	R	1	53	0	
	R	1	36	1	M

174

Table 8. (Continued)

Hosts	Tet. source	Number fed	Time $(days)^2$	Worms recovered
Mammals (continued)				
9 Mephitis mephitis	R	8-10	17	9 I
Number infected:	B	5-10	30	P
5 (55.5 Percent)	B	5-10	30	1 G
	Control			0
	R	10	44	0
	R	1	50	0
	B	6-10	35	P
	B	6-10	39	0
1 Mustela vison	R	6-10	36	0
1 Homo sapiens	R	6-10		0
(36 year old man)	B	3		
1 Ondatra zibethicus (muskrat)	B	5-10	28	0
30 Mus musculus (laboratory mice)	R	5	47	0
	R	10	1	0
Number infected:	R	15	34	0
16 (53.3 Percent)	(12) R	5-15	-	0
	(17) R	several	0.5-238	Ie

TOTAL INFECTION: 53/110 = 48.2 Percent

Table 9. Histochemical staining: Parauterine organ[1]

Stains	Outer parauterine wall	Inner parauterine membrane
General		
Azan triple	B3,R1	R2
Protein		
HgBPB (Mazia's total)	-/2	-/1
van Gieson's (collagen)	-/2	-/1
Carbohydrate		
PAS (total	2/2	2/0-1
Best's (glycogen)	2/1	-/1
Acid mucopolysaccharides		
Colloidal iron	-/0	-/0
Alcian blue	-/0	-/0
Lipids		
Oil red O	-/1	-/1
Osmium tetroxide	1	1
Calcium		
von Kossa's	-/0	-/0
Iron		
Gomori's	-/0	-/0
DNA and/or RNA		
Feulgen (DNA)	-/1	-/1
Azure B (RNA)	-/2	-/1
after cold PCA	reduced stain in Azure B positive areas	
after hot PCA	no staining	
after RNase	as in cold PCA	
Vitelline material		
Brom phenol blue	no differentiation of vitelline material	
Egg shell (Malachite green)	0/0	0/0

[1]The following symbols are used in Tables 9 thru 13:
B = blue, R = red, s = scattered; 0, 1, 2, 3 = density of
color reaction from none to high; m/g = mature proglottid/
gravid proglottid; -/g = no data for mature proglottid;
m/- = no data for gravid.

Table 9. (Continued)

Viscid content of egg mass	Calcareous corpuscles	Caudal appendage	Egg shell	Oncospheral hooks
B2,R1-3	R3	R3/R3	B1	-/0
1-2 -/0	0-3 -/0-2	2-3 -/1	-/2 -/0	-/2 -/0
0-3 -/1	-/0 -/0-1	-/2 -/1	-/3 -/2	-/0 -/0
-/0 -/1	-/0-1 -/1-2	-/0 -/0	-/0 -/0	-/0 -/0
-/0 0	-/0-1 0	-/2 1	-/0 0	-/0 0
-/0	-/3	-/0	-/0	-/0
-/0	-/1	-/0	-/0	-/0
-/0 -/2	-/0 -/0	-/2 -/1	-/0 -/0	-/0 -/0
0/0	0/0	0/0	0/0	0/0

Table 10. Histochemical staining: Uterus

Stains	Outer glandular cells	Inner lining
General		
Azan triple	R1/R1	R3/R3
Protein		
HgBPB (Mazia's total)	-/2	-/3
van Gieson's (collagen	0/0	0-1/1
Carbohydrate		
PAS (total)	2/3	0-1/3
Best's (glycogen)	-/1	-/0
Acid mucopolysaccharides		
Colloidal iron	-/0	-/0
Alcian blue	-/0	-/0
Lipids		
Oil red O	-/0	-/0
Osmium tetroxide	-/0	-/0
Calcium		
von Kossa's	-/0	-/0
Iron		
Gomori's	-/0	-/0
DNA and/or RNA		
Feulgen (DNA)	2/2	1/0
Azure B bromide (RNA)	2/3	0/0
after cold PCA	reduced stain in Azure b positive areas	
after hot PCA	no staining	
after RNase	as in cold PCA	
Vitelline material		
Brom phenol blue	no differentiation of vitelline material	
Egg shell (Malachite green)	0/2	0/0

Table 10. (Continued)

Egg shell	Oncospheral hooks	Uterine content
Bl	0/0	-
-/2 -/0	-/2 -/0	-/0-1 -/0
3 -/2	-/0 -/0	-/0-1 -/0
-/0 -/0	-/0 -/0	3/1 3/1
-/0 -/0	-/0 -/0	-/0 -/0
-/0	-/0	-/0
-/0	-/0	-/0
0/0 -/0	0/0 -/0	0/0 0/0
0/0	0/0	0/0

Table 11. Histochemical staining: Male and female organs
(exclusive of parauterine organ and uterus)

Stains	Ovary	Ovarian membrane
General		
Azan triple	R3/-	B2/B2
Protein		
HgBPB (Mazia's total)	3/-	0/-
van Gieson's (collagen)	0-1/0	1/1
Carbohydrate		
PAS (total)	0/-	3/-
Best's (glycogen)	0/-	3/-
Acid mucopolysaccharides		
Colloidal iron	0/-	0/-
Alcian blue	0/0	0/0
Lipids		
Oil red O	0/0	0/0
Osmium tetroxide	0/0	0/0
Calcium		
von Kossa's	0/0	0/0
Iron		
Gomori's	0/0	0/0
DNA and/or RNA		
Feulgen (DNA)	3/-	0/-
Azure B bromide (RNA)	3/-	0/-
after cold PCA	reduced stain in azure B positive areas	
after hot PCA	no staining	
after RNase	as in cold PCA	
Vitelline material		
Brom phenol blue	no differentiation of vitelline material	
Egg shell (Malachite green)	0/-	0/-

Table 11. (Continued)

Vitellaria	Vitelline membrane	Testes
R3/-	B2/B2	R1/-
2/-	0/-	3/-
0/-	1/-	1/-
2/-	3/-	0/-
1/-	3/-	0-1/-
0/-	0/-	1/-
0/0	0/0	0/0
0/0	0/0	0/0
0/0	0/0	0/0
0/0	0/0	0/0
0/0	0/0	0/0
1/-	0/-	3/-
3/-	0/-	3/-
0/-	0/-	0/-

Table 12. Histochemical staining: Parenchyma and associated
structures

Stains	Subtegumental parenchyma	Extra-medullary parenchyma
General		
Azan triple	R3-1/R1	B2/B2
Protein		
HgBPB (Mazia's total)	2-3/2-3	0/0
van Gieson's (collagen)	0-1/0-1	1/1
Carbohydrate		
PAS (total)	2-3/2-3	3/3
Best's (glycogen)	2/2	3/3
Acid mucopolysaccharides		
Colloidal iron	0/0	0/3
Alcian blue	0/0	1-2/0
Lipids		
Oil red O	0/0	-/3
Osmium tetroxide	0/0	2/2
Calcium		
von Kossa's	0/0	0/0
Iron		
Gomori's	0/0	0/0
DNA and/or RNA		
Feulgen (DNA)	2/2	1/1
Azure B bromide (RNA)	3/3	2/1^s
after cold PCA	reduced stain in azure B positive areas	
after hot PCA	no staining	
after RNase	as in cold PCA	
Vitelline material		
Brom phenol blue	no differentiation of vitelline material	
Egg shell (Malachite green)	0/0	0/0

Table 12. (Continued)

Muscle bands and fibers	Calcareous corpuscles	Lining of excretory ducts
R3/R3	R1-3/R1-3	B2/B2
3/3 0/0	0-3/0-3 0-1/0-3	1/1 1/1
$1-2^S/2^S$ 1-2/2	0/0 0/0-2	0/0 0/0
0/0 0/0	0/0-1 0/3	0/0 1/1
-/0 0/0	-/0-1 0-1/0	-/0 0/0
0/0	3/3	0/0
0/0	1/1	0/0
0-1/0 0-1/0	0/0 0/0	0-1/1 0-1/0-1
0/0	0/0	0/0

:

Table 13. Histochemical staining: Tegument

Stains	Outer layer
General	
Azan triple	B-R3/B-R3
Protein	
HgBPB (Mazia's total)	1/3
van Gieson's (collagen)	2/3
Carbohydrate	
PAS (total)	3/3
Best's (glycogen)	2/2
Acid mucopolysaccharides	
Colloidal iron	1/0
Alcian blue	2/1
Lipids	
Oil red O	0/0
Osmium tetroxide	0/0
Calcium	
von Kossa's	0/0
Iron	
Gomori's	0/0
DNA and/or RNA	
Feulgen (DNA)	0/0
Azure B bromide (RNA)	0/0
after cold PCA	-
after hot PCA	-
after RNase	-
Vitelline material	no differential staining
Egg shell (Malachite green)	0/0

Table 13. (Continued)

Middle layer	Inner layer
B-R3/B-R3	B3/B3
2/3	1/3
1/0	2/1-2
2/1	0/0
0/0	0/0
0/0	0/0
1/0	0/0
0/0	0/0
0/0	0/0
0/0	0/0
0/0	0/0
0/0	0/0
0/0	0/0
-	-
-	-
-	-
0/0	0/0

Table 14. Measurements of adult <u>Mesocestoides</u> from experimentally infected hosts[1]

Host	Age[2]	Strobila Length	Width	Scolex Diameter	Sucker Diameter
Didelphis	M	85	.702	361.8	149
Procyon	G	620	1.06	489.4	106.4
	M	-	-	287.3	74.5
	G	-	1.74	-	-
Mephitis	G	500+	1.60	372.4	159.6
	I	10	-	628.4	159.6
Canis canis	G	680	1.86	532	85.1
Vulpes	G	310	1.60	404.3	124.0
Procyon	G	620	1.40	425.6	74.5
Canis latrans	G	260	0.74	426.0	74.5
Mephitis	G	350+	1.74	425.6	117.0

[1]Strobila measurements in millimeters; all other measurements in microns.

[2]Abbreviations: I = immature, M = mature, G = gravid.

Table 14. (Continued)

Testes		Cirrus pouch		Egg capsule	
Number	Diameter	Length	Width	Length	Width
60	53.2	-	95.8	-	-
58	50.3	149.0	74.5	319.2	223.4
-	-	-	-	-	-
55	74.5	149 & 138.3	75.0	383.0	351.1
-	53.2	159.6	127.6	212.8	85.1
-	-	-	-	-	-
76	31.9	127.7	74.5	435.4	279.9
78	45.0	127.7	74.5	319.2	255.4
72	42.6	106.4	63.8	180.9	180.9
50	40.1	85.1	532.0	149.0	127.7
55	42.6	138.3	63.8	266.0	244.7

Table 15. Measurements of adult Mesocestoides from naturally infected hosts[1]

Host	Age[2]	Strobila Length	Strobila Width	Scolex Diameter	Sucker Diameter
Didelphis	G	600	2.20	529.0	192.2
Didelphis	M	-	1.03	639.2	213.6
Procyon	G	530	1.62	585.3	202.0
Didelphis	M	-	1.87	596.4	160.2
Didelphis	G	-	2.05	-	-
Didelphis	G	-	1.31	596.1	160.7
Didelphis	G	400	1.49	372.4	170.2
Mephitis	G	-	1.15	-	-
Procyon	M	-	0.81	234.1	85.1
Didelphis	M	-	1.43	649.0	212.8
Didelphis	G	-	0.93	497.6	191.5
Procyon	G	700	-	-	-
Procyon	G	650	1.40	311.0	117.04
Procyon	G	-	1.87	528.7	127.7
Procyon	G	590	1.40	-	-
Procyon	G	-	1.56	-	-
	M	25	0.287	266.0	95.8
	M	50	0.532	372.4	117.0
Procyon	G	2000	1.75	393.7	127.7
Procyon	G	1200	2.6	-	-

[1]Strobila measurements in millimeters; all other measurements in microns.

[2]Abbreviations: M = mature, G = gravid.

Table 15. (Continued)

Testes		Cirrus pouch		Egg capsule	
Number	Diameter	Length	Width	Length	Width
100±	80.1	192.0	128.2	426.1	341.3
70	53.4	149.0	106.4	neg.	neg.
46	53.2	223.1	117.0	341.0	255.1
-	-	-	-	-	-
-	-	213.2	127.1	426.2	351.0
70	53.2	106.4	63.8	244.7	234.1
80	53.1	127.7	74.5	212.8	170.2
44	31.9	234.1	138.3	373.2	311.0
-	31.9	-	-	-	-
-	31.0	-	-	-	-
60	53.2	148.9	106.4	266.0	260.0
-	-	-	-	425.6	319.2
68	53.2	170.2	127.7	266.0	297.9
-	-	149.0	74.5	319.2	319.2
-	-	159.6	74.5	287.3	244.7
63	42.6	149.0	85.1	308.6	308.0
56	21.3	-	-	-	-
64	31.9	106.4	53.2	-	-
65	42.2	212.8	95.8	404.3	340.5
-	-	-	-	-	-

APPENDIX B. ILLUSTRATIONS

ABBREVIATIONS USED

C	cirrus	PC	parauterine cells
CA	caudal appendage	PG (?)	proteinaceous granules
CC	calcareous corpuscle	PO	parauterine organ
CP	cirrus pouch	PU	primary uterus
D	degenerating reproductive complex	S	scolex
E	egg	ST	sperm tails
EB	excretory bladder	T	testes
ED	excretory duct	TC	transverse excretory canal
EM	egg mass	TR	tail remnant
ER	endoplasmic reticulum	U	uterus
G	glycogen	UC	uterine component of parauterine organ
H	hooks		
IL	inner lining of parauterine organ	V	vitellaria
L	lipid material	VE	vas efferens
M	mitochondria	VG	vagina
MB	muscle bands	W	wall of parauterine organ
MC	myelin configurations	Z	zygote
MV	microvilli		
O	ovary		
OD	developing oncosphere		
P	proglottid		
PA	parenchymal cell		

PLATE I

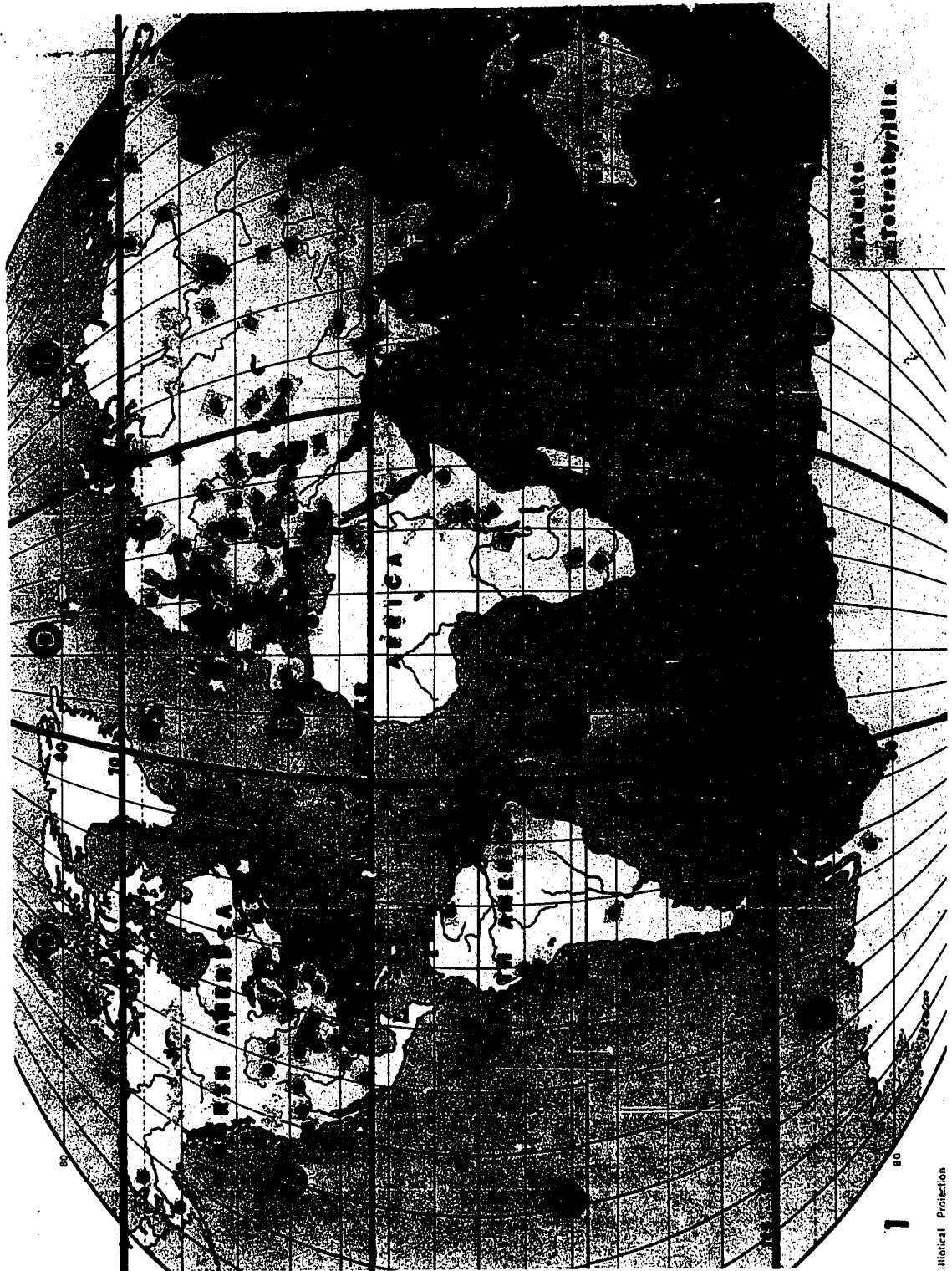Figure 1. World distribution of larval and adult <u>Mesocestoides</u>.

PLATE II

Figure 2. Comparison of taxonomic criteria used in determination of ten currently
accepted species of <u>Mesocestoides</u>.

20+

10

8

7

6

5

4

3

2

1

a = LINEATUS
b = PERLATUS
c = CHARADRII
d = VARIABILIS
e = CORTI
f = MANTERI
g = BASSARISCI
h = LATUS
i = KIRBYI
j = JONESI
k = CARNIVORICOLIS

STROBILA
LENGTH
IN mm. x100

STROBILA
WIDTH
IN mm.

SCOLEX
DIAMETER
IN μ x 100

SUCKER
DIAMETER
IN μ x 100

TESTES
NUMBER
x 100

TESTES
DIAMETER
x 100

CIRRUS POUCH
LENGTH
IN μ x 100

CIRRUS POUCH
WIDTH
IN μ x 100

EGG CAPSULE
LENGTH
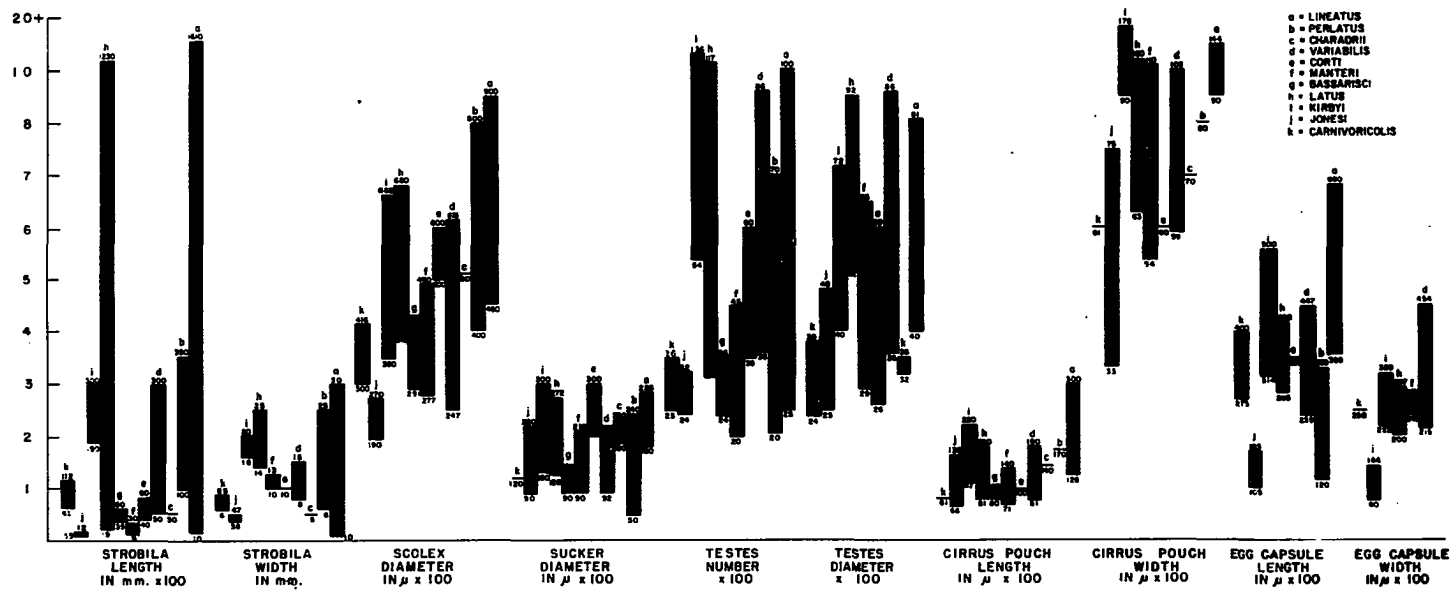IN μ x 100

EGG CAPSULE
WIDTH
IN μ x 100

PLATE III

Figure 3. Measurements used in identifying reported species of <u>Mesocestoides</u>
compared with similar measurements from mesocestoidids examined in
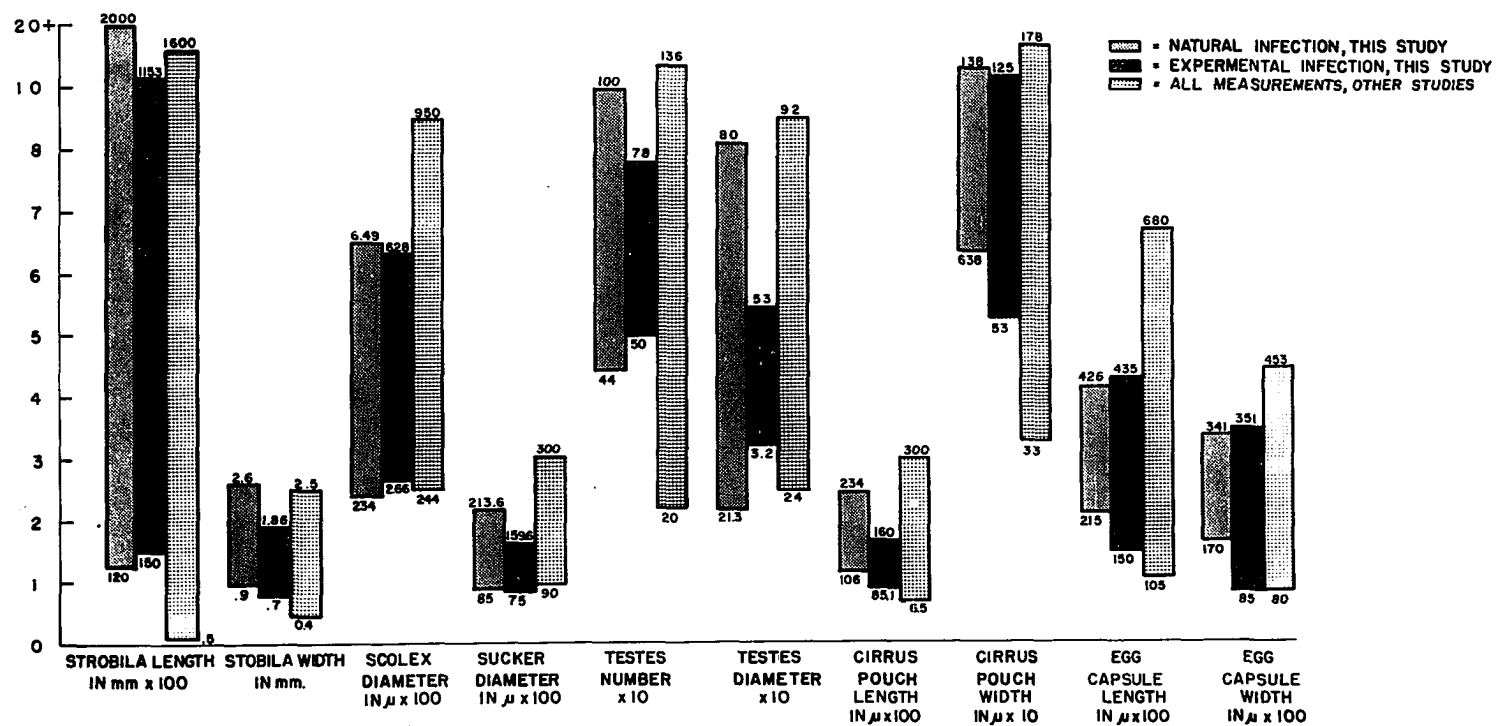this study.

20+

10

8

7

6

5

4

3

2

1

0

- NATURAL INFECTION, THIS STUDY
- EXPERMENTAL INFECTION, THIS STUDY
- ALL MEASUREMENTS, OTHER STUDIES

| STROBILA LENGTH IN mm x 100 | STOBILA WIDTH IN mm. | SCOLEX DIAMETER IN μ x 100 | SUCKER DIAMETER IN μ x 100 | TESTES NUMBER x 10 | TESTES DIAMETER x 10 | CIRRUS POUCH LENGTH IN μ x 100 | CIRRUS POUCH WIDTH IN μ x 10 | EGG CAPSULE LENGTH IN μ x 100 | EGG CAPSULE WIDTH IN μ x 100 |

Strobila length: 2000, 1153, 1600, 120, 180, .5

Stobila width: 2.6, 1.86, 2.5, .9, .7, 0.4

Scolex diameter: 6.49, 6.28, 950, 234, 2.66, 244

Sucker diameter: 213.6, 1.596, 300, 85, 75, 90

Testes number: 100, 78, 136, 44, 50, 20

Testes diameter: 80, 53, 92, 21.3, 3.2, 24

Cirrus pouch length: 234, 160, 300, 106, 85.1, 6.5

Cirrus pouch width: 138, 125, 178, 638, 53, 33

Egg capsule length: 426, 435, 680, 215, 150, 105

Egg capsule width: 341, 351, 453, 170, 85, 80

PLATE IV

Figure 4. Gravid <u>Mesocestoides</u> <u>lineatus</u> from experimentally
infected opossum. Scale shown in millimeters.

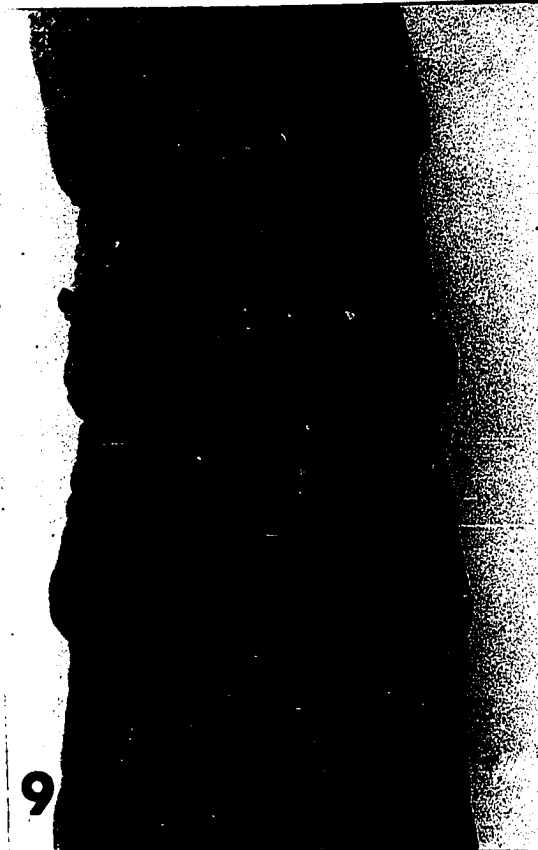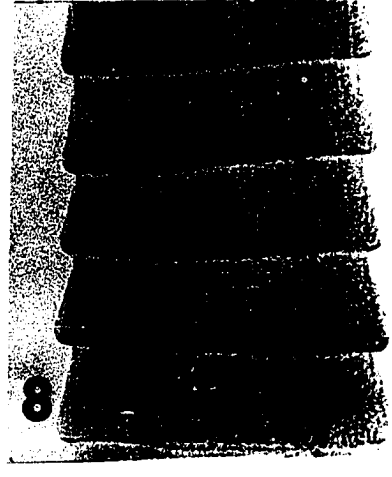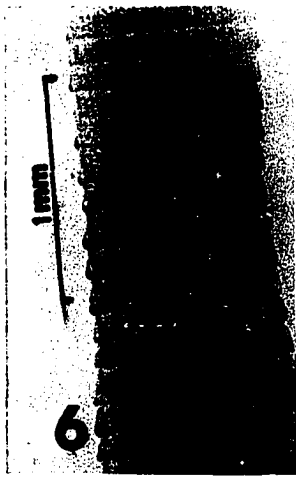Figure 5. Mature segment of <u>M</u>. <u>lineatus</u> showing parauterine
cells.
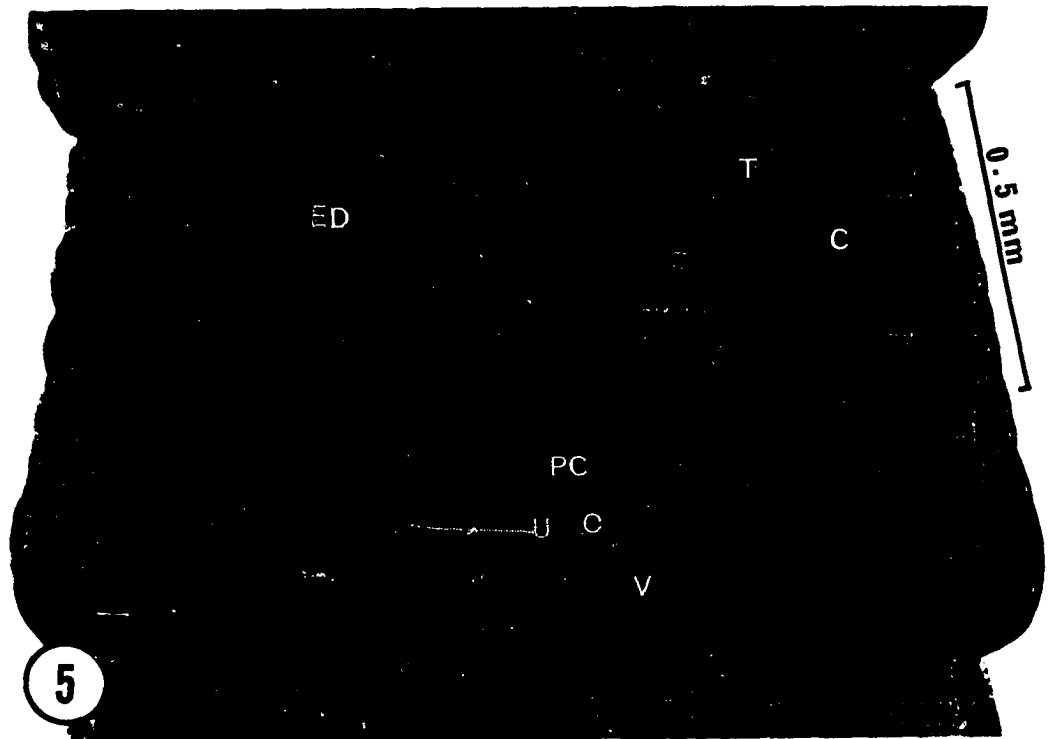
PLATE V

Figures 6 thru 10. Selected strobilar regions of M.
lineatus from naturally infected skunk
(Mephitis mephitis) showing progressive develop-
ment of reproductive organs. (All drawn to same
scale as shown in Figure 6.).

Figure 6. Young proglottids with genital
primordia.

Figures 7 thru 10. Progressive development of
mature proglottids. Note trapezoidal
shape of proglottids.
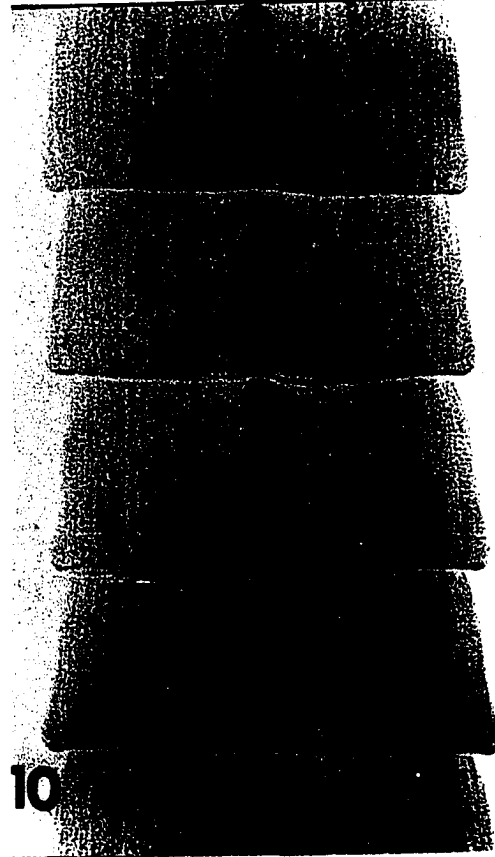
PLATE VI

Figures 11 thru 14. [Continuation of] Selected strobilar
            regions of M. lineatus from naturally infected
            skunk (Mephitis mephitis) showing progressive
            development of reproductive organs. (All
            drawn to same scale as shown in Figure 11.).

     Figure 11. Late mature proglottids. Note early
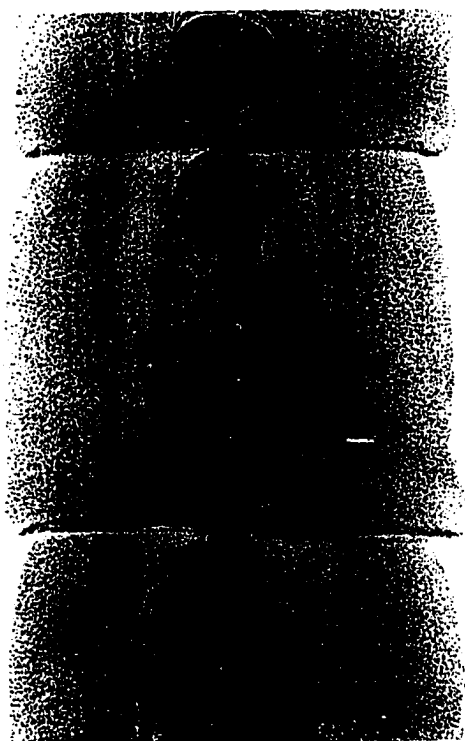   development of parauterine organ.
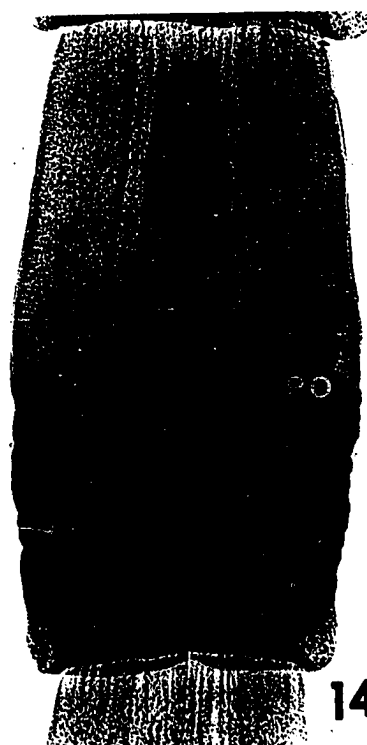
     Figures 12 thru 14. Stages in development of
                 gravid proglottids.

11
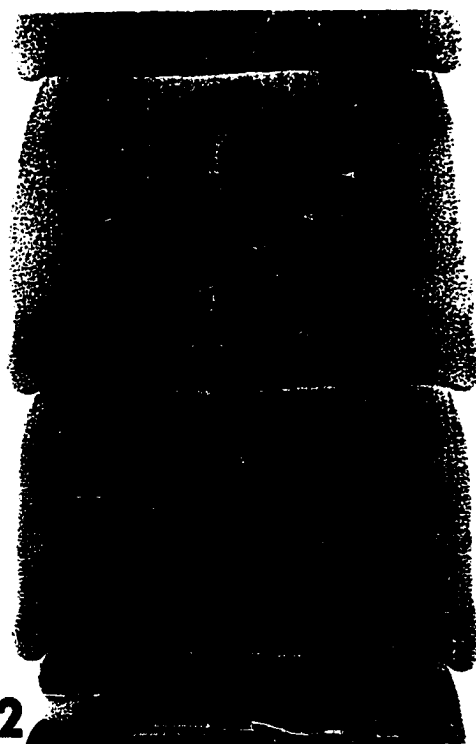
12

13

14

PLATE VII

Figure 15. Larvae (possibly mesocestoidid developmental
            stages) taken from haemocoel of the dung
            beetle, Onthaphagus hecate, collected from
            the wild but fed eggs of Mesocestoides in
            the laboratory.

Figure 16. Enlarged view of one larva shown in Figure 15.
            Note numerous calcareous corpuscles scattered
            throughout the body.

Figure 17. Faecal mass from an infected opossum
            (Didelphis virginiana) showing typical
            surficial position of Mesocestoides proglottids.

Figures 18 and 19. Unarmed cysticercoids from the
            mesenteries and tracheal tubules of a sextant
            beetle (Necrophorus orbicolis). (Both figures
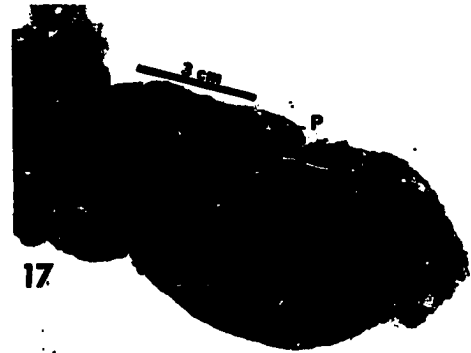   drawn to scale shown in Figure 18.).

15

16

17

18

19

PLATE VIII

Figure 20. Longitudinal section of scolex of M. lineatus attached to villi of lower small intestine of experimentally infected mouse, 134 hours post-feeding.

Figure 21. Section of scolex of M. lineatus attached to duodenum of experimentally infected raccoon, 44 days post-infection. (Same scale as Figure 20.).

Figures 22 thru 29. Tetrathyridia from various vertebrate hosts. (All but Figure 25 are at same scale as that shown in Figure 22.).

   Figures 22 thru 24. Tetrathyridia from Rana pipiens showing variations in size.

   Figure 25. Smallest tetrathyridium (0.32 mm. diameter) recovered from a naturally infected R. pipiens.

   Figure 26. Tetrathyridium from naturally infected Bufo cognatus.

   Figure 27. Tetrathyridium from naturally infected B. americanus.

   Figure 28. Tetrathyridium from experimentally infected mouse previously fed tetrathyridia from a naturally infected R. pipiens.

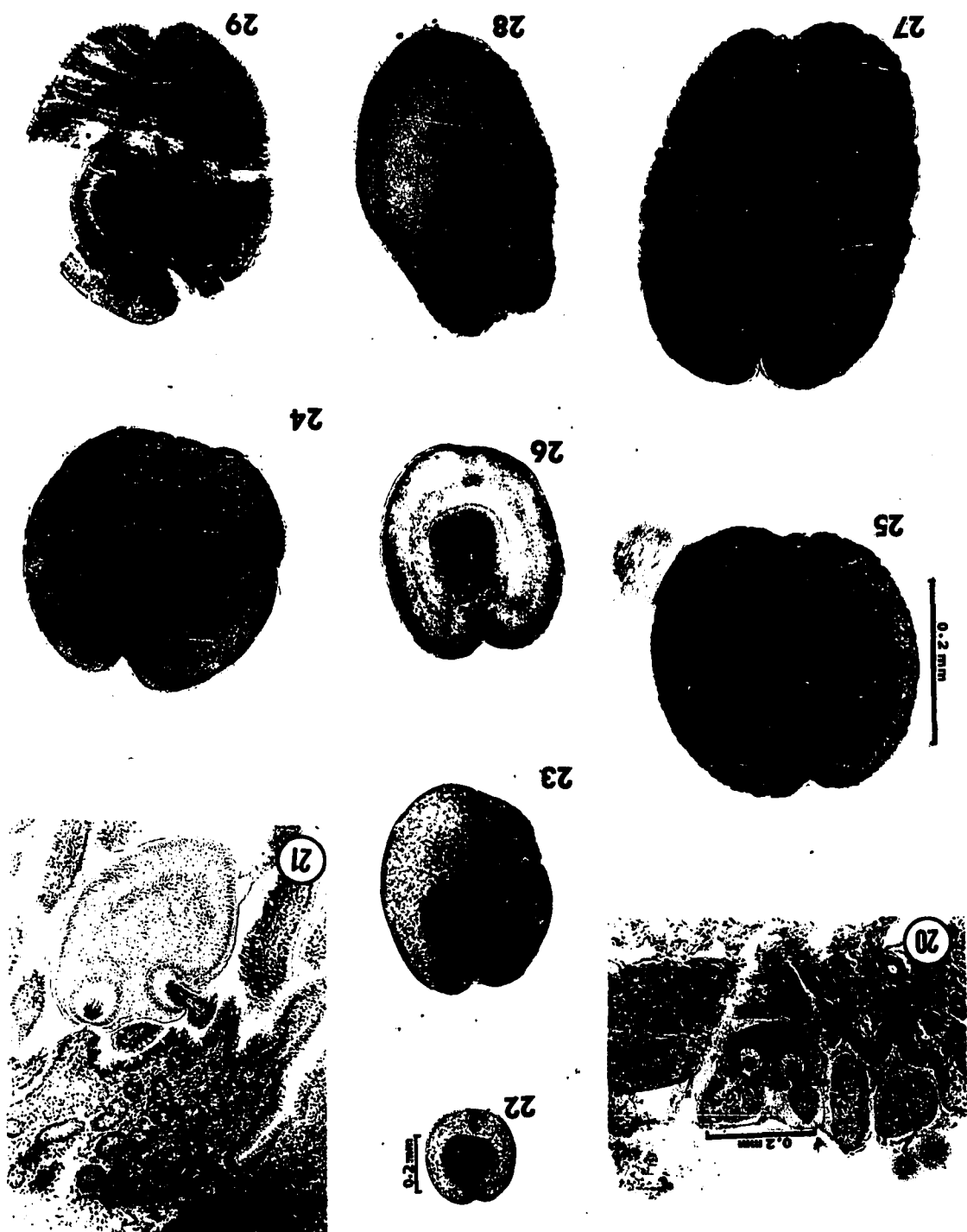   Figure 29. Tetrathyridium (U.S.N.M. Coll. No. 54520) from liver of naturally infected shrew (Crocidura oliveri).

PLATE IX

Figure 30.   Egg mass of Mesocestoides lineatus teased
             from parauterine organ, phase contrast.

Figure 31.   Egg-encased oncospheres of M. lineatus from
             egg mass.   Note calcareous corpuscles.

Figure 32.   Oncosphere of M. lineatus emerging from egg
             shell.   Note oncospheral hooks.

Figure 33.   Oncospheres of M. lineatus on intestinal
             mucosa of Rana pipiens, 5 hours post-feeding.
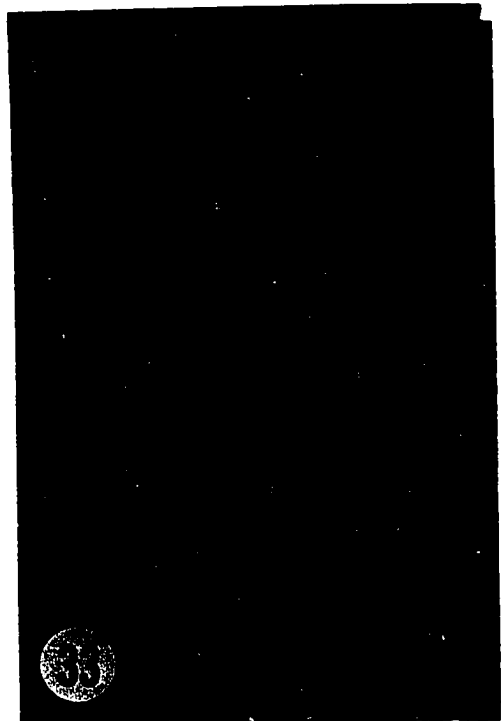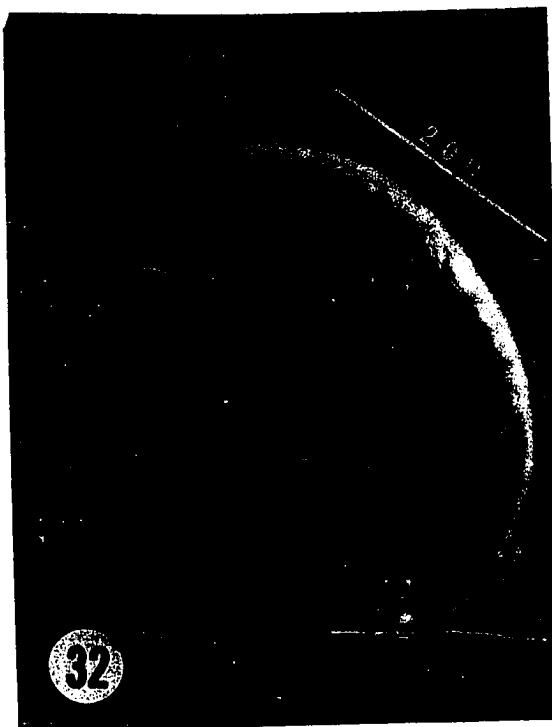   (Same scale as shown in Figure 31.).
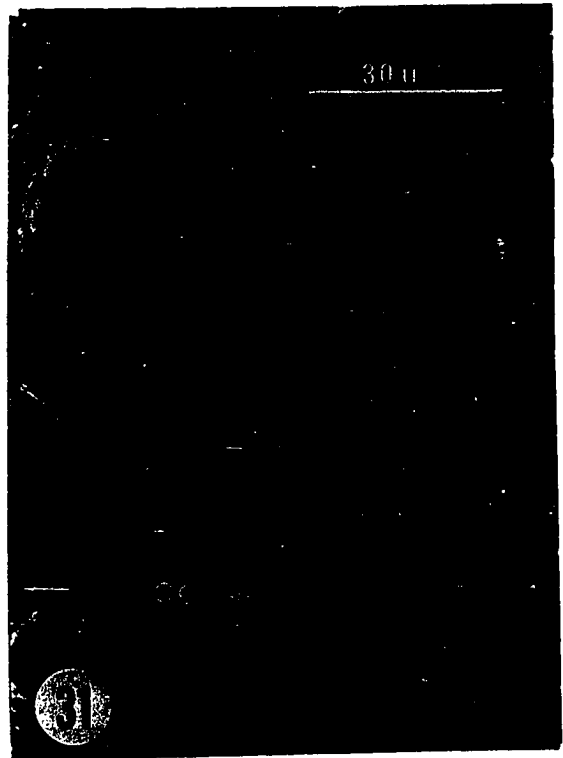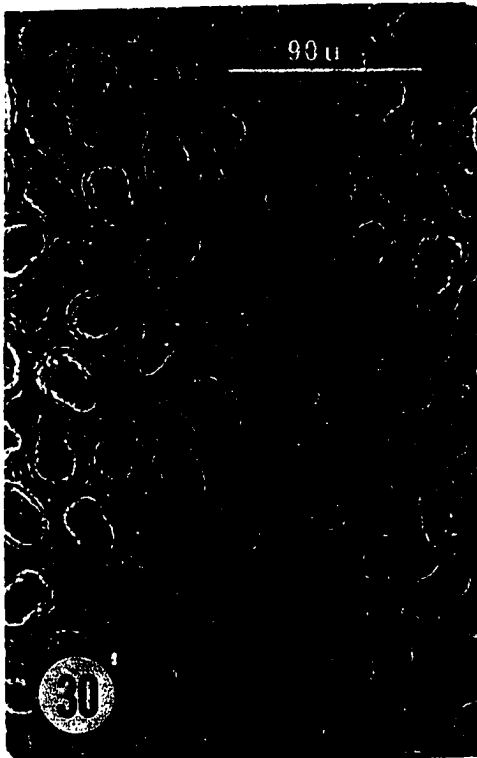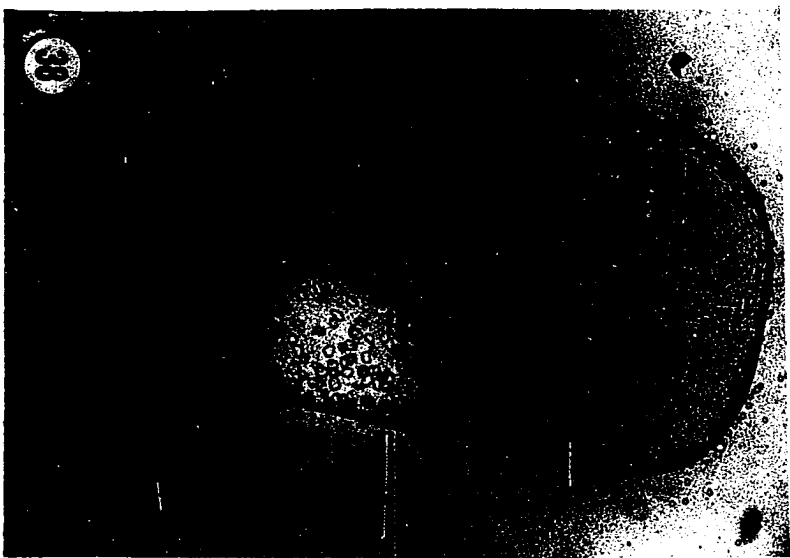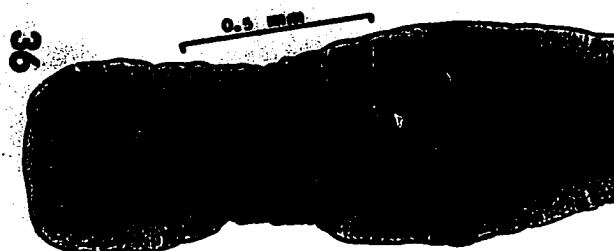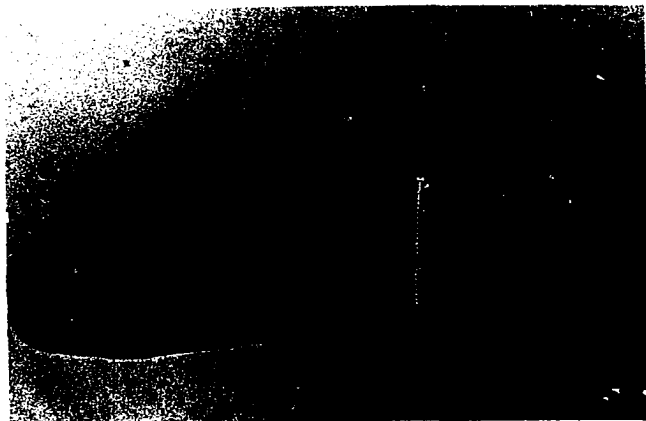
PLATE X

Figure 34. Early strobilation in young <u>M</u>. <u>lineatus</u> from
experimentally infected mouse, <u>15</u> hours post-
feeding.

Figure 35. Posterior end of young adult M. <u>lineatus</u> from
experimentally infected dog, <u>8</u> hours post-
feeding.

Figure 36. Early strobilation in young <u>M</u>. <u>lineatus</u> from
duodenum of experimentally <u>infected</u> raccoon,
8 hours post-feeding. (Same scale as shown
in Figure 34.).

Figure 37. "Tail" remnant of young strobilate <u>M</u>. <u>lineatus</u>
from experimentally infected <u>raccoon</u>, <u>14</u> days
post-feeding.

Figure 38. Scolex of very young <u>M</u>. <u>lineatus</u> recently
separated from the <u>tetrathyridium</u>. Note
caudal point of attachment (tail remnant) and
numerous calcareous corpuscles. (Same scale
as shown in Figure 35.).

PLATE XI

Figures 39 thru 52. Developmental stages of <u>Mesocestoides</u>
        <u>lineatus</u> from tetrathyridium to young non-
        strobilate adult. (All photographs taken at
        scale shown in Figure 39.).

Figure 39.   Tetrathyridium from <u>Rana</u> <u>pipiens</u>
              showing numerous calcareous
              corpuscles (alizarin red prepara-
              tion).

Figure 40.   Tetrathyridium from stomach of
              experimentally infected mouse, 1/2
              hour post-feeding. Body in initial
              stage of degeneration.

Figure 41.   Tetrathyridium from stomach of
              experimentally infected raccoon,
              9 hours post-feeding.

Figure 42.   Later stage in body degeneration of
              tetrathyridium from stomach of
              experimentally infected mouse, 1
              hour post-feeding.

Figures 43 thru 44.   Stages in release of scolex
              from tetrathyridial body taken from
              stomach of experimentally infected
              mouse, 1 hour post-feeding.

Figure 45.   Tetrathyridium with scolex everted
              from body; from experimentally
              infected mouse, 1/2 hour post-feeding.

Figure 46.   Tetrathyridial body following release
              of scolex; from experimentally
              infected raccoon, 1-1/2 hours post-
              feeding.

Figure 47.   Very young <u>M. lineatus</u> showing in-
              vaginated scolex and "tail"; from
              experimentally infected raccoon,
              1-1/2 hours post-feeding.

Figures 48 and 49.   Extended young worms from
              small intestine of mouse, 30 minutes
              post feeding.

PLATE XI (continued)

Figure 50. Evaginated scoleces of young M.
lineatus recovered from small
intestine of experimentally infected
raccoon, 8 hours post-feeding.

Figure 51. As above (Figure 50), but from
opossum.

Figure 52. Early young adults recovered from
small intestine of experimentally
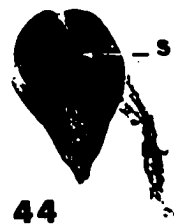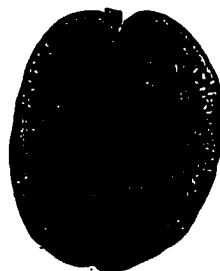infected mouse, 37 hours post-
feeding. Note absence of "tail".

PLATE XII

Figures 53 thru 56. Young adult M. lineatus from various
vertebrate hosts. (All but Figure 54 at same
scale as Figure 53.).

Figure 53. From naturally infected red fox
(Vulpes vulpes).

Figure 54. From experimentally infected dog,
8 hours post-feeding.

Figure 55. From experimentally infected mouse,
157 hours post-feeding.

Figure 56. From an intestine of naturally
infected skunk (Mephitis mephitis).

53

0.4 mm

54

56

55

PLATE XIII

Figures 57 thru 61. Variations in scoleces of
Mesocestoides lineatus.

Figure 57. Scolex from naturally infected
skunk. Note anterior notch in
sucker.

Figure 58. Scolex from naturally infected
raccoon.

Figure 59. Scolex from naturally infected
skunk. (Same scale as shown in
Figure 58.).

Figure 60. Scolex from naturally infected
raccoon. Note posterior notch
in sucker. (Same scale as shown
in Figure 57.).

Figure 61. Scolex from experimentally
infected raccoon. (Same scale
as shown in Figure 57.).

57　58　59

60
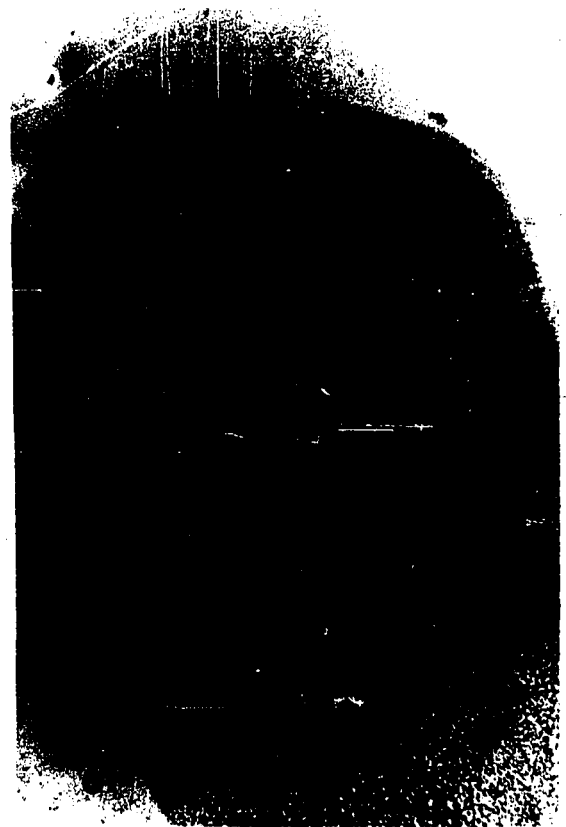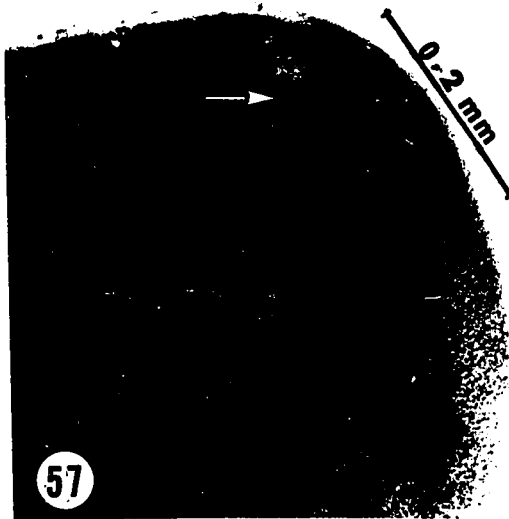
PLATE XIV

Figure 62.   Typical egg of M. lineatus, phase contrast,
             30 x 40 microns.

Figure 63.   Distribution of calcareous corpuscles in young,
             mature M. lineatus (air-dried preparation
             cleared in methyl salicylate).

Figures 64 and 65.   Parauterine organ and caudal appendage
             of M. lineatus from a raccoon. (Same scale as
             in Figure 63.).

        Figure 64. Note S-shaped caudal appendage.
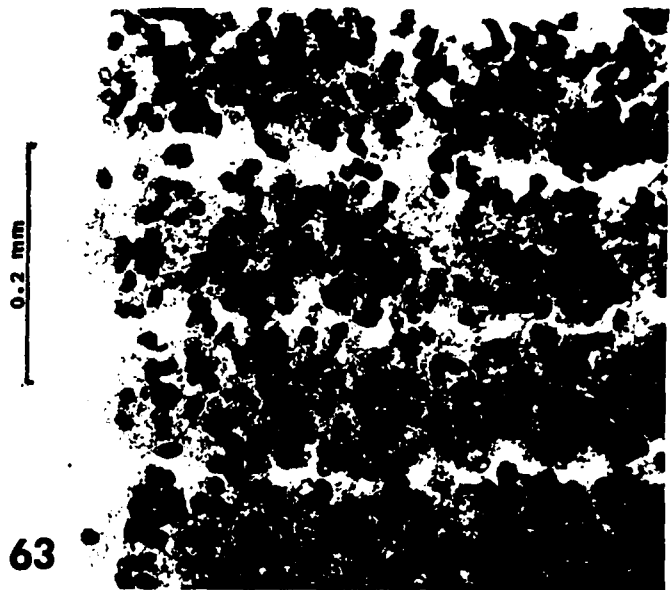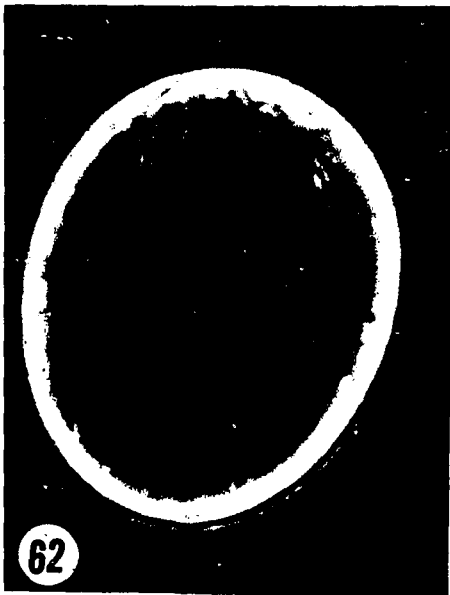
        Figure 65. Note straight caudal appendage.
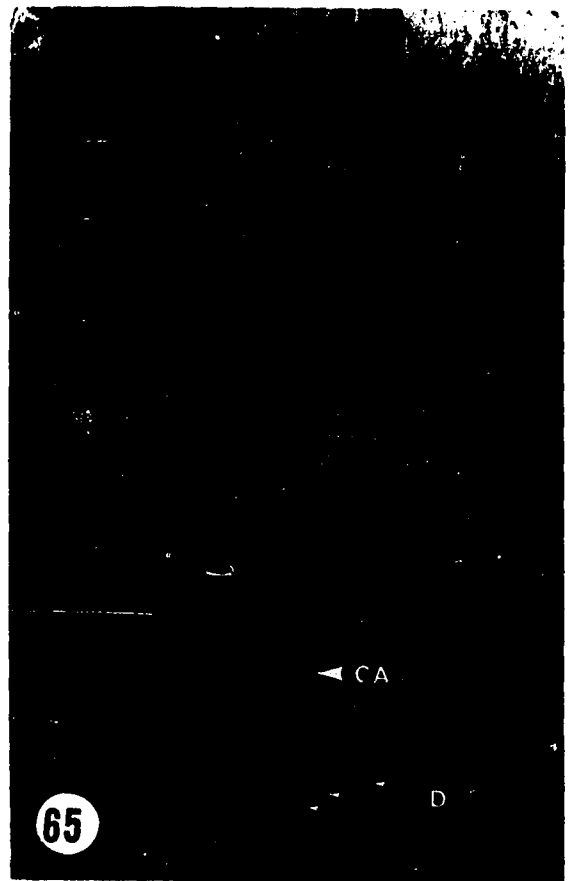
PLATE XV

Figure 66. Variations in shape (square, rectangular) or
gravid proglottids of M. lineatus from experi-
mentally infected opossum.

Figure 67. Rectangular proglottids from experimentally
infected opossum.

Figure 68. Wall of parauterine organ showing its fibrous
nature, photographed from life, phase contrast.

Figures 69 thru 71. Abnormalities in Mesocestoides
lineatus (Figures 69 and 70 are from naturally
infected raccoons; Figure 71 is from an experi-
mentally infected opossum). (Same scale as
shown in Figure 66.).

Figure 69. Incomplete transverse division of
segment (arrow).

Figure 70. Bifurcate strobila.

Figure 71. Reversal and doubling of reproductive
organs.

Figure 72. Parauterine organ showing extension of uterine
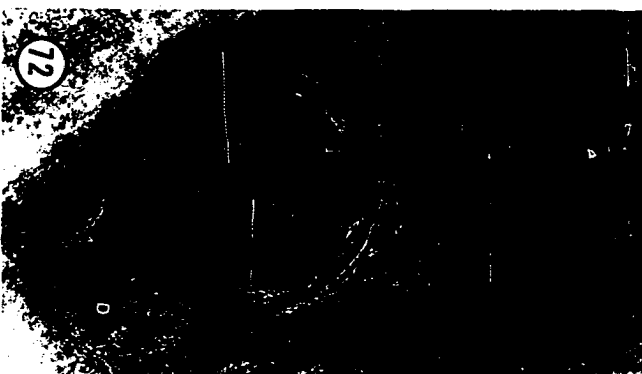component through parauterine wall. (Same scale
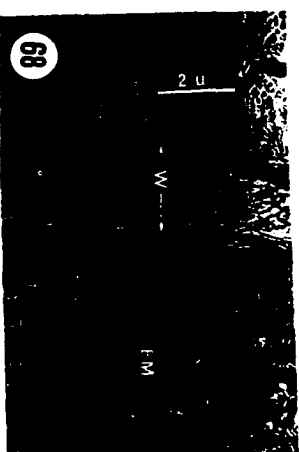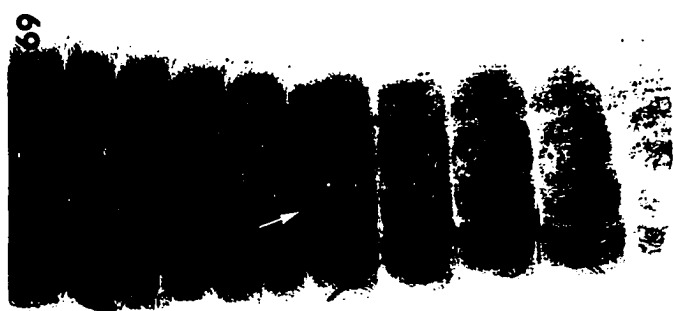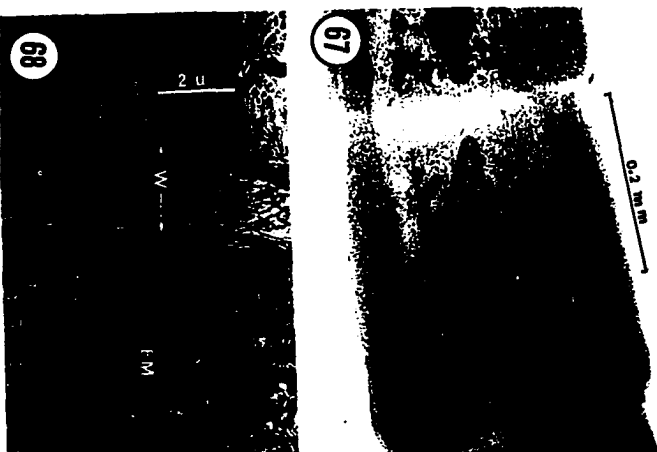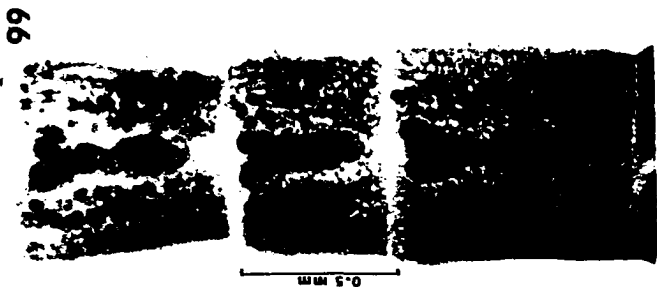as shown in Figure 67.).

PLATE XVI

Figures 73 thru 75. Sections of tetrathyridia from naturally
        infected frogs (Rana pipiens).

        Figure 73. Tetrathyridia in situ, wall of small
                    intestine.

        Figure 74. Multiple cyst from brachial region.
                    (Same scale as shown in Figure 73.).

        Figure 75. Enlargement of tetrathyridium shown
                    in Figure 73. (Same scale as shown
                    in Figure 76.).

Figure 76. Whole mount of tetrathyridium from naturally
        infected toad (Bufo cognatus). Note excretory
        bladder.

73

74

75

76

PLATE XVII

Figures 77 thru 82. Representative cross-sections through
          mature proglottid of M. lineatus, osmium
          tetroxide preparations of hand-cut thick
          sections, phase contrast. (All to same scale
          as shown in Figure 82.).

          Figure 77.   Section immediately posterior to
                       developing parauterine organ.

          Figure 78.   Section through developing
                       parauterine organ.

          Figure 79.   Section through mid-region of
                       proglottid at level of cirrus.

          Figure 80.   Section through anterior region of
                       uterus.

          Figure 81.   Section through anteromost region
                       of uterus. Note accumulation of
                       lipids around uterus.

          Figure 82.   Section through anteromost region
                       of proglottid showing longitudinal
                       excretory duct and transverse
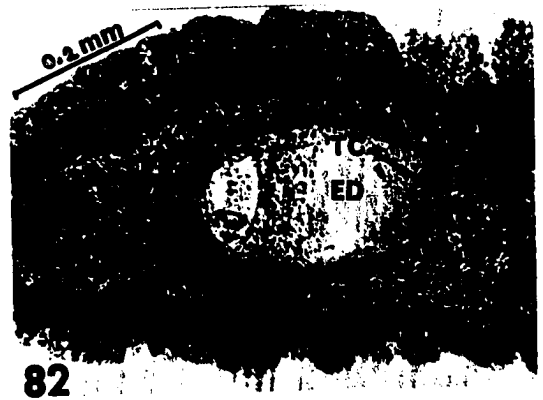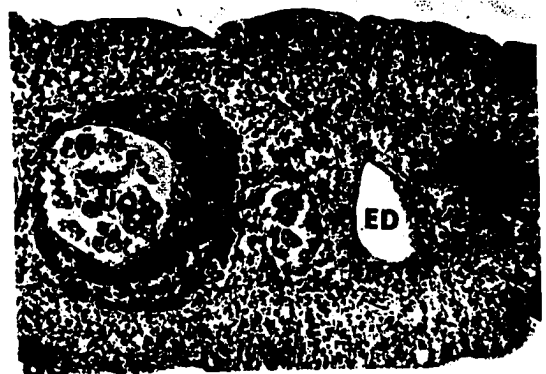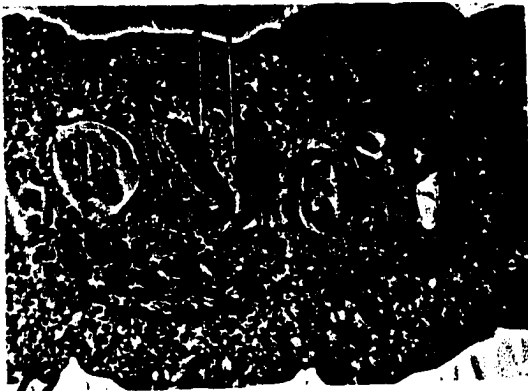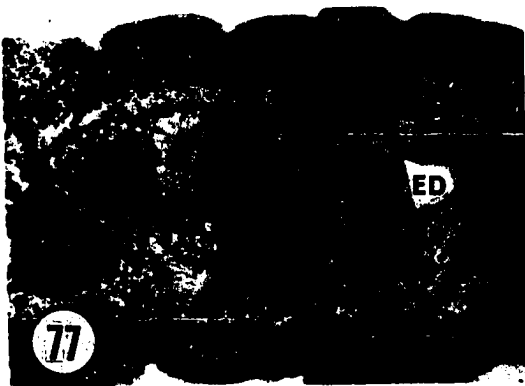   excretory canal.

PLATE XVIII

Figure 83.   Section of gravid parauterine organ, phase contrast.

Figure 84.   Enlargement of wall of parauterine organ shown in Figure 83.

Figure 85.   Electron micrograph of wall of parauterine organ from region similar to that shown in Figure 84.

Figure 86.   Enlarged portion of parauterine wall region shown in Figure 85. (Areas A, B, and C may be seen at greater magnification in Figures 94, 95, and 97, respectively.).
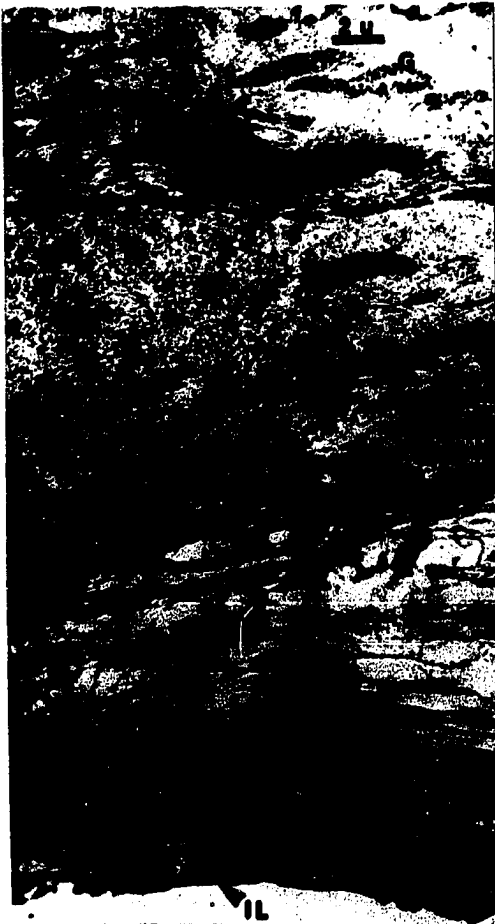
PLATE XIX

Figures 87 thru 92. Selected cross-sections through
            posterior region of mature proglottid to show
            early development of parauterine organ in
            relation to associated reproductive structures.
            Phase contrast, sections cut at 2 microns (see
            also Figures 105, 107, and 109). (All at same
            scale as shown in Figure 87.).

       Figure 87. Section through region of ovary
                   showing vagina and primary uterus
                   (see also Figures 105, 107, and
                   109).

       Figures 88 thru 92. Progressive sections from
                   posterior to anterior through the
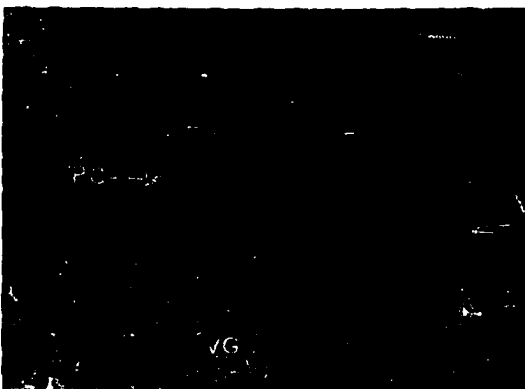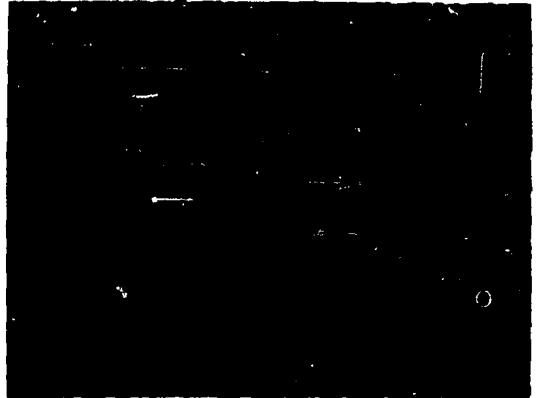   early developing parauterine organ.

PLATE XX

Figure 93. Montage of early mature proglottid of
Mesocestoides lineatus showing very early
development of parauterine organ and typical
early parauterine cells as seen in Figures
87 through 92.

PLATE XXI

Figures 94 thru 97. High magnification electron micro-
graphs of wall of parauterine organ.

Figure 94. Region of parauterine wall shown in
Figures 86, area A.

Figure 95. Region of parauterine wall shown in
Figure 86, area B. (Same scale as
shown in Figure 94.).

Figure 96. High magnification view of lamellar
region of parauterine wall.

Figure 97. Region of parauterine wall shown in
Figure 86, area C. (Same scale as
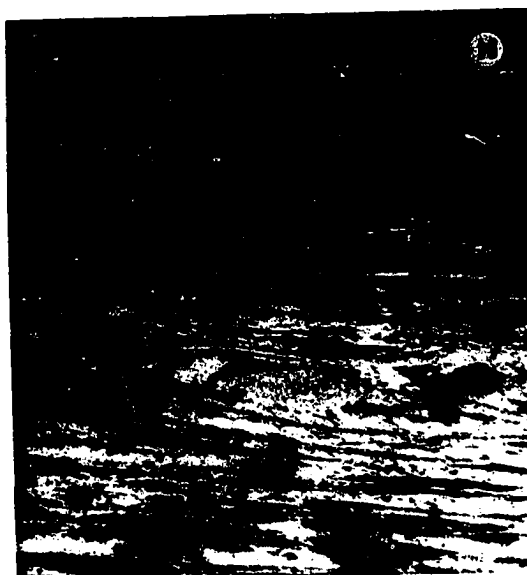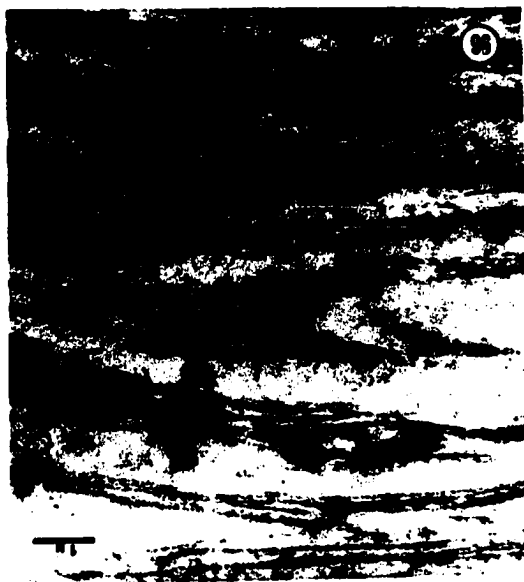shown in Figure 96.).

PLATE XXII

Figure 98. Free-hand drawing of montage (Figure 99) show-
ing general relationship of the three major
reproductive structures in the region of the
parauterine organ.

Figure 99. Montage of the area of the developing
parauterine organ in mature proglottid (see
also Figure 87).

98   PU

99

PLATE XXIII

Figure 100.   Montage of the area of the developing
parauterine organ in an early gravid
proglottid. Area extends from the egg mass
to the parenchymal muscle bands. Letters
A-D refer to views at higher magnifications
of the areas seen in Figures 101 thru 104.

Figure 101.   Section showing medullary muscle fibers
associated with parauterine organ. See area
A, Figure 100.

Figure 102.   See area B, Figure 100.

Figure 103.   Outer limit of parauterine organ at the junc-
tion with parenchyma. See area C, Figure 100.
(Same scale as shown in Figure 101.).

Figure 104.   Early embryo of M. lineatus within the
parauterine organ. See area D, Figure 100.
Note cellular products (myelin configurations
and mitochondria) in surrounding medium. (Same
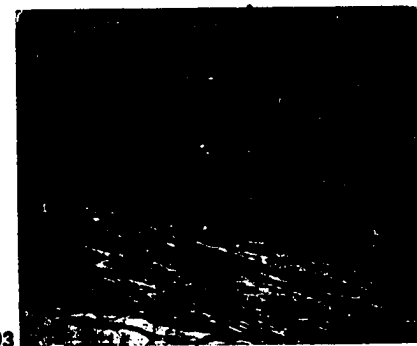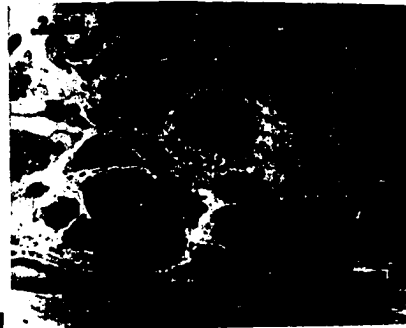scale as shown in Figure 101.).

PLATE XXIV

Figures 105, 106, and 109. Enlargements of areas illus-
trated in Figure 87.

      Figure 105. Portion of ovary in region of
                   developing parauterine organ.

      Figure 107. Vagina.

      Figure 109. Uterus. Note myelin figures
                   associated with zygote.

Figure 106. Active region of parauterine organ surrounding
egg mass. Note presence of myelin configura-
tions (MC), mitochondrion (M), and endoplasmic
reticulum (ER).

Figure 108. Parenchymal region medial to cortical muscle
layer of M. lineatus showing muscle bands
(MB), secretory products (glycogen, lipids,
and proteins), vas efferens (VE) through
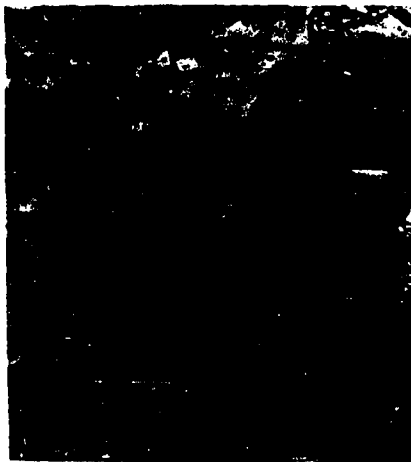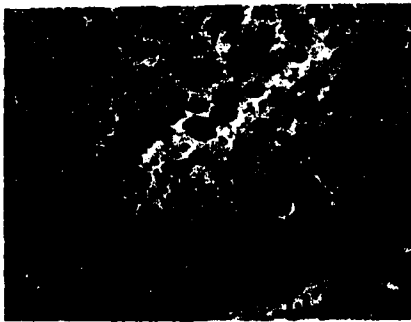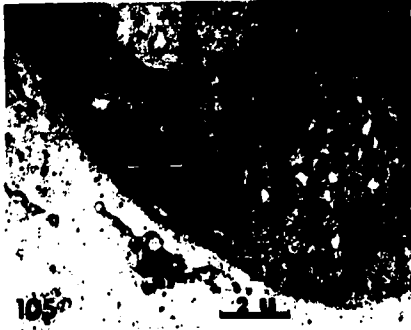sperm tails (ST).

PLATE XXV

Figure 110. Single and multiple tetrathyridial cysts of
Mesocestoides lineatus in pelvic region of
naturally infected Rana pipiens. (Scale in
millimeters).

Figure 111. Tetrathyridial cysts as in Figure 110 but
attached to mesentery and intestine (see also
Figures 73 and 75). Note large cyst contain-
ing numerous individuals a section of which
is shown in Figure 74. (Scale in millimeters).
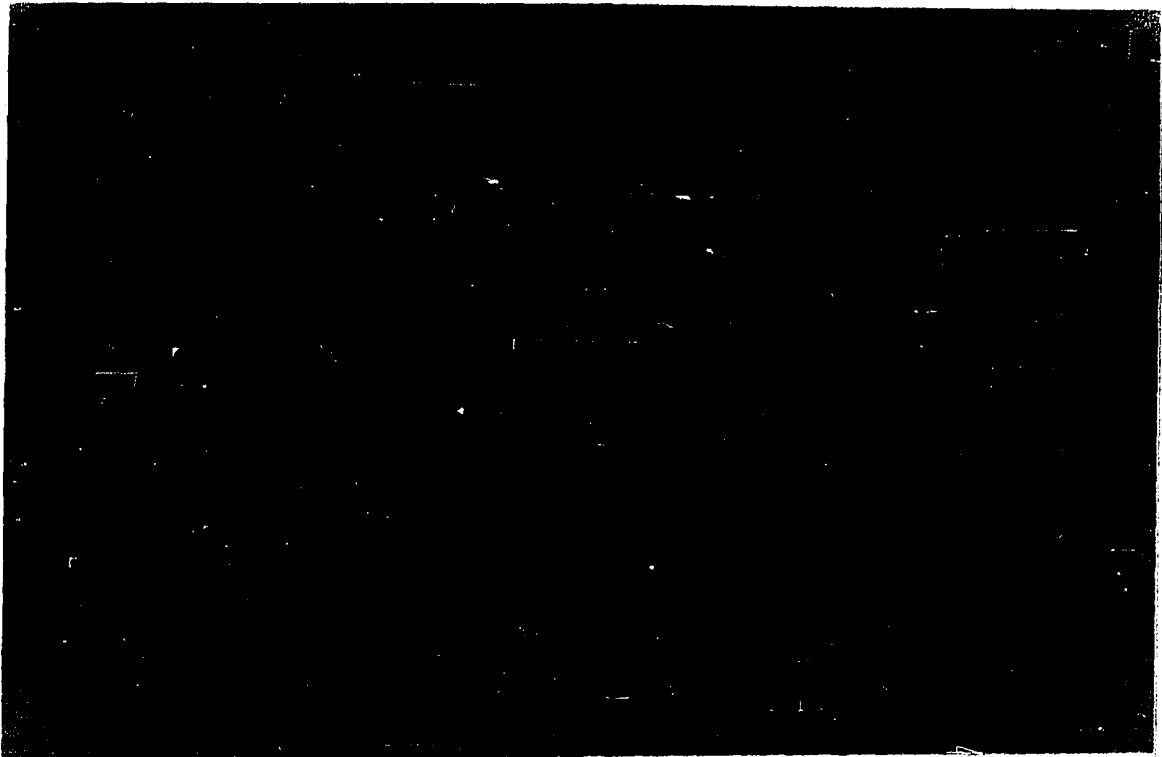
PLATE XXVI

Figures 112 thru 119. Histochemically stained sections of mature and gravid proglottids of Mesocestoides lineatus. (see also Figures 120 thru 139).

Figure 112. Gravid proglottid, azan triple stain. Note caudal appendage and extension of uterus into parauterine organ.

Figure 113. Enlarged view of parauterine organ region shown in Figure 112.

Figure 114. Mature proglottid, azure B bromide stain. Note deep blue areas of RNA concentration. (Same scale as shown in Figure 113.).

Figure 115. Gravid proglottid, as in Figure 114. (Same scale as shown in Figure 112.).

Figure 116. Mature proglottid, PAS stain. Note quantity and distribution of carbohydrate (purple-blue). (Same scale as shown in Figure 113.).

Figure 117. Gravid proglottid, as in Figure 116. Note low concentration of carbohydrate in parauterine organ wall. (Same scale as shown in Figure 113.).

Figure 118. Mature proglottid, Best's carmine stain for glycogen (red). Note that almost all carbohydrate deposits seen in Figures 116 and 117 are glycogen. (Same scale as shown in Figure 113.).

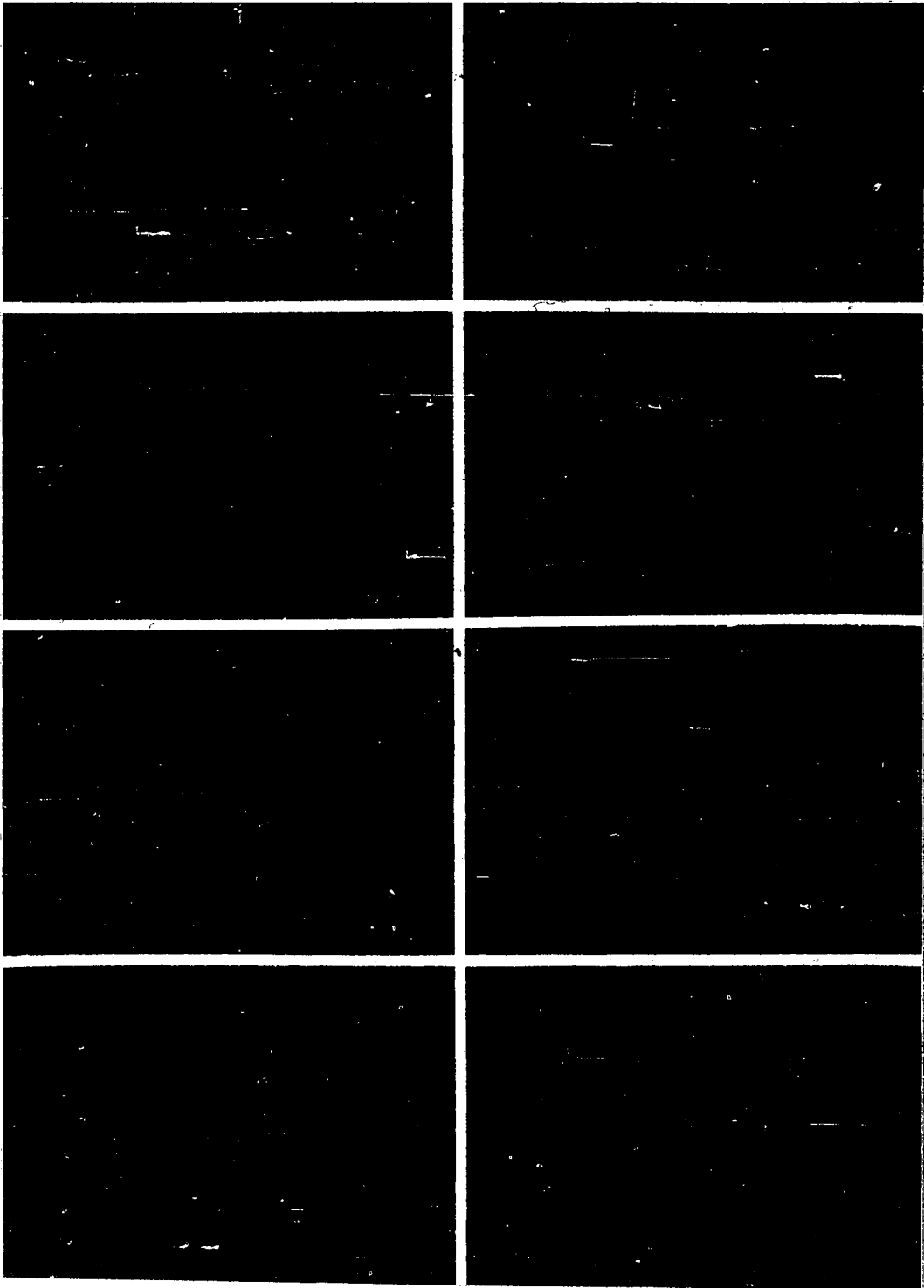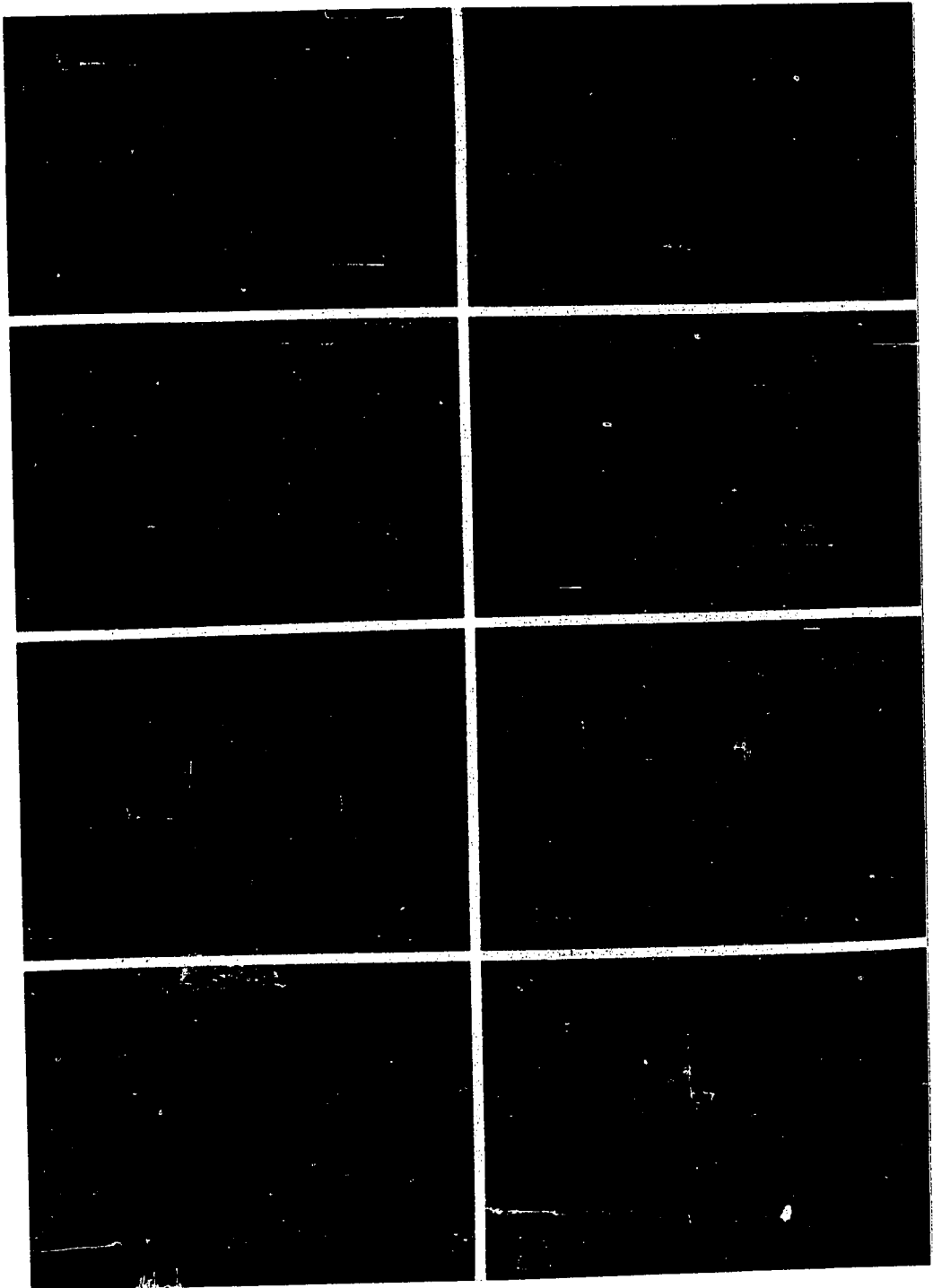Figure 119. Gravid proglottid, as in Figure 118. (Same scale as shown in Figure 113.).

PLATE XXVII

Figures 120 thru 127. [Continuation of] Histochemically stained sections of mature and gravid proglottids of **M. lineatus**.

Figure 120. Parenchyma of gravid proglottid, Gomori's stain for iron. Note Fe^{++} (blue color) in calcareous corpuscle. (Retouched)

Figure 121. Eggs with embryo stained with malachite green for quinone tanned scleroproteins. Note green color (positive reaction, retouched) of oncospheral hooks and its absence from shell material. See also uterine gland cells of Figure 138.

Figure 122. Tegumental and parenchymal region of gravid proglottid, van Gieson's collagen stain. Note collagenous tissue (reddish, retouched) of innermost tegumental layer. (Same scale as shown in Figure 120.).

Figure 123. Excretory duct of gravid segment, van Gieson's stain for collagen. Note strong concentration (reddish, retouched) in inner lining (arrow). (Same scale as shown in Figure 120).

Figure 124. Mature proglottid, oil red O lipid stain. Note heavy deposition (red-orange) around reproductive organs. (see also Figures 77 thru 82 and Figures 126 and 127).

Figure 125. Portion of egg capsule of para-uterine organ from gravid proglottid stained with azure B bromide. Note high nucleic acid concentration (blue) within embryos. (Same scale as shown in Figure 120.).

Figure 126. Gravid proglottid, oil red O stain for lipid. Note exceptionally heavy deposition (red-orange) around uterus.

Figure 127. Enlargement of area of parauterine organ seen in Figure 126. See also Figures 77 thru 82 and 124. (Same scale as shown in Figure 124.).

PLATE XXVIII

Figures 128 thru 135. [Continuation of] Histochemically
stained sections of mature and gravid proglot-
tids of M. lineatus. Figures 128 and 135 at
same scale; Figures 129 thru 134 at scale
shown in Figure 129.).

Figure 128. Gravid proglottid, feulgen stained.
Note almost total absence of nuclei
from parauterine organ tissue.

Figure 129. Gravid proglottid, as in Figure
128, lower magnification. Note
the numerous nuclei in developing
embryos.

Figure 130. Gravid proglottid, von Kossa's
stain for Ca^{++}. Note distribution
of calcareous corpuscles which
appear dark brown to black.

Figure 131. Mature proglottid, as in Figure
130.

Figure 132. Gravid proglottid, colloidal iron
stain (AFIP) for acid mucopoly-
saccharides (blue). Note presence
in calcareous corpuscles (CC) of
parenchyma.

Figure 133. Mature proglottid, as in Figure
132. Note exceptionally strong
reaction (blue) in vagina and
outer layer of tegument.

Figure 134. Gravid proglottid, Mazia's total
protein stain (red). Note con-
tinuation of uterine lining
through parauterine organ and
terminating posteriorly as the
caudal appendage.

Figure 135. Gravid proglottid, as in Figure
134. Highly enlarged view of
the uterine-parauterine organ
junction seen in Figure 134.
Note that inner lining of uterus
is continuous with inner lining
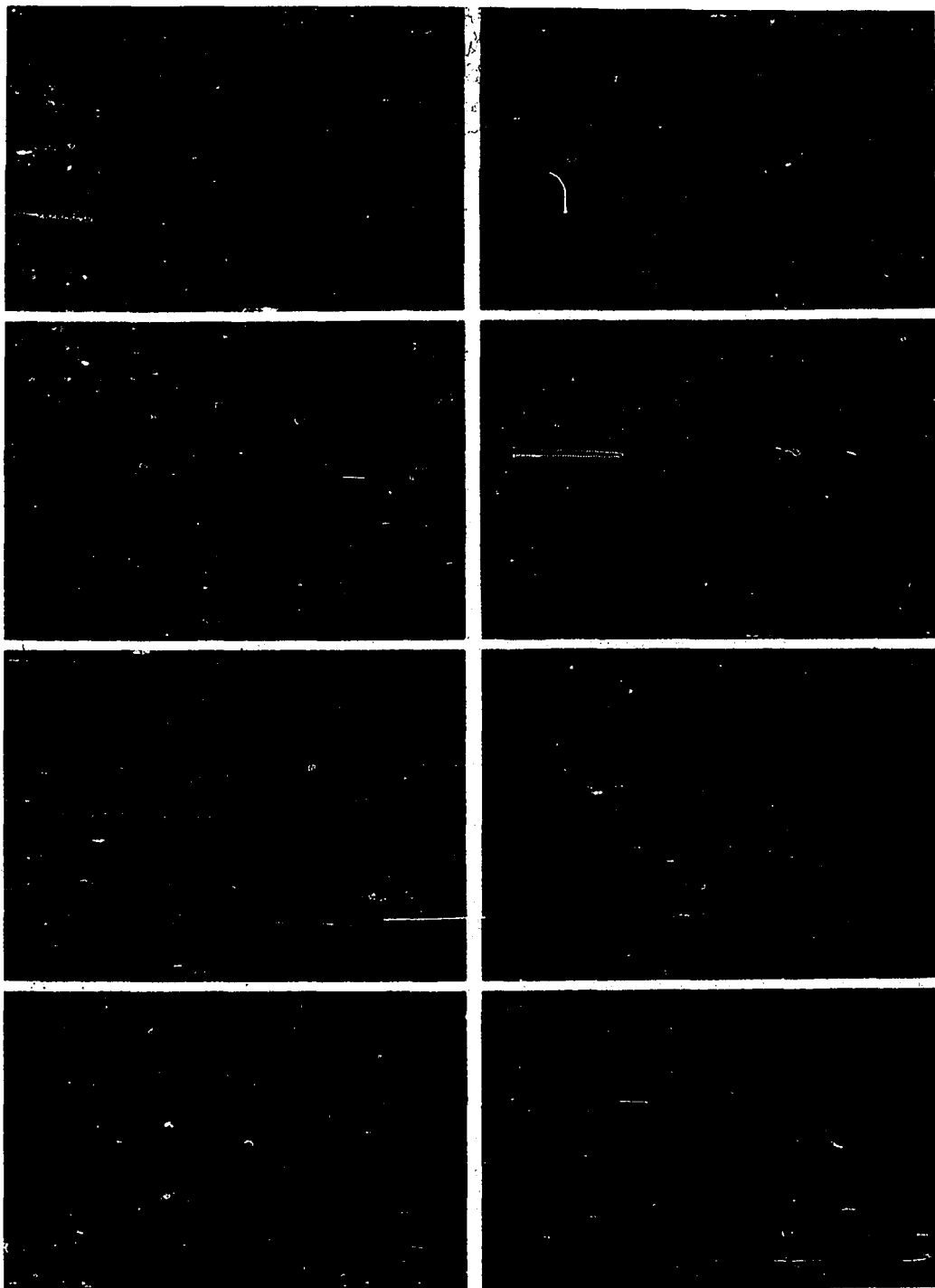of parauterine organ (IL).

PLATE XXIX

Figures 136 thru 139. [Continuation of] Histochemically
stained sections of mature and gravid pro-
glottids of M. lineatus.

Figure 136.   Region of parauterine organ in a gravid
segment, colloidal iron stain (AFIP) for
acid mucopolysaccharides (blue and collagen
(red).

Figure 137.   As in Figure 136 but at lower magnification.
Note collagenous nature (red) of parauterine
organ wall and its relationship to associated
structures (caudal appendage and uterus).

Figure 138.   Gravid proglottid, malachite green stain for
quinone tanned scleroproteins. Note strong
reaction (green) in outer uterine gland cells.
Compare with oncospheral hooks seen in Figure
121.

Figure 139.   Gravid proglottid, stained with azure B bromide
for DNA and RNA. (Deep blue areas are rich in
RNA:) (Same scale as shown in Figure 137.).