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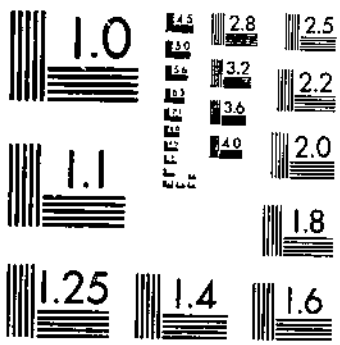

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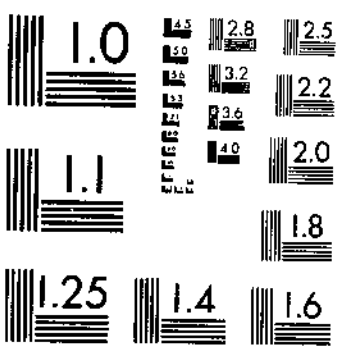

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EARLY DEVELOPMENTAL STAGES OF NEMATODES OCCURRING IN SWINE

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CONTENTS

INTRODUCTION

The object of this bulletin is to present the results of an investigation on the early developmental stages of nematodes of common occurrence in domestic swine. Observations on the stages in the definitive host of two of the nematodes, *Gongylonema pulchrum* and *Hyostrongylus rubidus*, are only briefly given, however, since little is known of these stages in these nematodes. The nomenclature of each parasite, its host relationship, geographic distribution, morphology, and bionomics of its early stages are discussed.

In addition to the scientific interest attaching to the new findings presented here, there is an economic aspect of considerable importance in that a knowledge of the life history and bionomics is essential in formulating control measures for these parasites, many of which are distinctly pathogenic.

Unless otherwise stated, all work was done in the laboratories of the Zoological Division at Washington, D. C.

[1] The material in this bulletin was presented as a thesis in January 1934 to the faculty of the Graduate School of the George Washington University in partial fulfillment of the requirements for the degree of doctor of philosophy. The writer is indebted to associates in the Zoological Division for suggestions and constructive criticisms in its preparation. R. E. Snodgrass, of the Bureau of Entomology and Plant Quarantine, supplied valuable information in connection with the study of the mode of encystment of *Gongylonema* larvae in the muscle of an insect host. E. A. Chapin, also of the same Bureau, identified various species of Coleoptera. Frank Smith, formerly professor at the University of Illinois, identified earthworms used in connection with studies of the life history of *Metastrongylus salmi*.

HISTORICAL RÉSUMÉ

The existing knowledge concerning the development and bionomics of swine nematodes has been accumulated within less than a century. One of the early life-history studies was that of *Trichinella spiralis*. Leidy (*1*)[2] in 1847 observed encysted larvae of *T. spiralis* in the body musculature of the pig; these encysted larvae were described, however, in 1835 by Owen (*85*) from similar findings in the human muscle. The cysts of *T. spiralis* were first found in human muscle by Tiedman in 1822 and by Peacock in 1828. According to Cobbold (*18*), Tiedman probably saw the calcified cysts of *T. spiralis*. Experiments on the transmission of *T. spiralis* were performed by Herbst (*46, 47*) in 1851 and 1852, Virchow (*2, p. 430*) in 1859, Leuckart (*58, 59*) in 1860 and 1866, and Zenker (*142*) in 1860.

The first investigation relative to the development of *Ascaris* eggs was undertaken by Schubart and Verloren in 1854, cited by Küchenmeister (*56*), on the dog ascarid, *Toxocara canis*. Richter in 1854, cited by Küchenmeister (*56*), and Davaine (*23*) in 1859, were apparently the first to note the development of the ascarid of man.

Numerous investigations have been carried out on the resistance of *Ascaris* eggs to various environmental conditions. The ascarids of man and of swine were reported to be resistant to low temperatures by Davaine (*23*), Leuckart (*60, v. 2, fig. 3*), Yoshida (*139*), Martin (*72*), Cram (*22*), and Raffensperger (*89*). The resistance of ascarid eggs to various chemicals has been noted by Galli-Valerio (*31*), Yoshida (*139*), Wharton (*136*), Kobayashi (*54*), and Ransom and Foster (*97*). The resistance of ascarid eggs to dryness has been recorded by Ross (*102*), Ransom and Foster (*97*), Martin (*73*), and others. Reports that ascarid eggs remain alive for long periods, even for several years, were published by Brown (*13*), Leuckart (*60*), Epstein (*24*), Morris (*78*), Ransom and Foster (*97*), Fülleborn (*28*), Martin (*73*), and others.

According to Linstow (*63*) in 1886, the myriapod *Julus guttulatus* and the closely allied species *Polydesmus complanatus* probably served as intermediate hosts for *Ascaris lumbricoides*. Stewart (*126, 127, 128, 129*) in 1916–18, reported that larvae of *A. lumbricoides* migrated to the lungs and appeared in the mouth or feces of the rat or mouse. He surmised that these larvae got on to the food of human beings from these hosts, and when swallowed with such food the larvae completed development in the intestine of the definitive host. Ransom and Foster (*95, 96*) in 1917 and 1919, and Ransom and Cram (*93, 94*) in 1921, contrary to Linstow and Stewart, demonstrated that the life history of *Ascaris* was direct.

One of the earliest investigations on the development of *Trichuris* eggs was made in 1858 on those of *T. trichiura* by Davaine (*23*). Leuckart (*60*) in 1876 showed that infection with *T. ovis* and *T. suis* followed after a feeding of embryonated eggs of these parasites to their respective hosts. Railliet (*91*) in 1884 obtained similar results with *T. vulpis* in dogs, and Grassi (*38*) in 1887 noted similar results with *T. trichiura* in man.

With reference to the life history of *Gongylonema pulchrum*, Stiles (*131*) in 1892, was of the opinion that this nematode was heteroxen-

[2] Italic numbers in parentheses refer to Literature Cited, p. 80.

ous. It was not until 1915 that Ransom and Hall (*98*) reported that dung beetles served as intermediate hosts for this parasite. They also noted that the life history of *Gongylonema* was strikingly similar to that of *Spiroptera obtusa* which, according to Leuckart (*60*) and Marchi (*71*), required the meal worm (larva of *Tenebrio molitor*) as the intermediate host.

The life histories of *Physocephalus sexalatus* and *Ascarops strongylina*, involving various species of dung beetles as intermediate hosts, were elucidated by the findings of Seurat (*115*, *116*, *117*), in 1913, 1915, and 1916.

The life histories of the swine lungworms *Metastrongylus elongatus* and *Choerostrongylus pudendotectus* were unsolved until recently. Leuckart (*60*) in 1876, believed these lungworms to be heteroxenous and suggested that an insect or snail might serve as the intermediate host. Several writers, namely, Von Linden and Zenneck (*62*) in 1915, Herms and Freeborn (*48*) in 1916, and Zebrowski (*140*, *141*) in 1922 and 1925, were of the opinion that the swine lungworm larvae developed into a free-living generation. It was not until 1929 that Hobmaier and Hobmaier (*49*, *50*) reported that earthworms served as intermediate hosts of *M. elongatus* and *C. pudendotectus*. Their work was confirmed by Schwartz and Alicata (*109*, *111*) in 1929 and 1931, and by Von Schuckmann and Zunker (*106*) in 1930. In the work reported in this bulletin the present writer has found that *Metastrongylus salmi*, another swine lungworm, also requires earthworms as intermediate hosts. Alessandrini (*3*) noted that long ago Cobbold considered earthworms as possible intermediate hosts.

The first observation on the preparasitic larval stages of *Stephanurus dentatus* was made in 1900 by Tayler (*133*), who gave a brief description of the hatching and morphology of these larvae. More extensive investigations on the developmental stages and bionomics of *S. dentatus* have been reported by Bernard and Bauche (*11*) in 1913 and 1914, Schwartz and Price (*112*, *113*, *114*) in 1928, 1929, and 1931, Ross and Kauzal (*100*, *101*) in 1929 and 1932, and Spindler (*124*, *125*) in 1931 and 1933.

There is very little literature relating to the life cycle of *Hyostrongylus rubidus*. Schang (*104*) in 1927 attempted to trace the larval development of this parasite, but from his description and illustrations it is evident that he had confused larvae of *H. rubidus* with those of free-living nematodes. Goodey (*37*), in the same year, gave a brief but accurate description of the preparasitic larval stages and bionomics of *H. rubidus*, in spite of the fact that he was dealing with very limited numbers of larvae. His finding showed that the preparasitic larval stages of *H. rubidus* were similar in general to those of other known strongyles.

Literature concerning the life history of *Oesophagostomum dentatum* is also very scarce. An accurate description of the preparasitic larval stages of this parasite and a discussion of their bionomics were given by Goodey (*36*) in 1924.

With reference to *Strongyloides ransomi*, Schwartz and Alicata (*110*), in 1930, noted that eggs derived from parasitic females developed either to strongyliform larvae or to free-living sexually mature worms, the progeny of the latter developing into strongyliform larvae capable of infecting the host.

GENERAL REMARKS ON LIFE HISTORIES OF GROUPS STUDIED

The nematodes known from swine include representatives of several large groups of parasitic nematodes. As would be expected in view of this fact, a considerable variety of life histories was found in the writer's investigation.

The nematodes discussed in this bulletin include seven families, and on the basis of life history they may be subdivided into the heteroxenous and monoxenous groups.

The heteroxenous group includes the families Spiruridae (represented by *Gongylonema pulchrum*, *Ascarops strongylina*, and *Physocephalus sexalatus*) and Metastrongylidae (represented by *Metastrongylus salmi*, *M. elongatus*, and *Choerostrongylus pudendotectus*).

The life history of the heteroxenous group may be of the following type: Adult male and female in definitive host; embryonated eggs outside of definitive host; first-, second-, and third-stage larvae in intermediate host; third- and fourth-stage larvae and adult males and females (fifth stage) in definitive host. Each larval stage except the first one is separated from the previous stage by a molt; two molts occur in the intermediate host, and two in the definitive host. The sheath of the second molt in the members of the Spiruridae is completely cast off, apparently because these larvae are protected in the body of the intermediate host by a cyst wall. In the third-stage larvae of the Metastrongylidae, the sheath of the second molt is retained.

The monoxenous group includes the families Ascaridae (represented by *Ascaris suum*), Trichuridae (represented by *Trichuris suis*), Trichostrongylidae (represented by *Hyostrongylus rubidus*), Strongylidae (represented by *Oesophagostomum dentatum* and *Stephanurus dentatus*), and Strongyloididae (represented by *Strongyloides ransomi*). Larvae of the monoxenous group show considerable variation in their mode of development before entering the host.

The eggs of the Ascaridae and Trichuridae are deposited with the feces of the host, and after becoming fully embryonated are capable of producing an infection when ingested by suitable hosts. The *Ascaris* embryo molts once while in the eggshell and is not infective until after this molt.

The life history of the Trichostrongylidae and Strongylidae families may be as follows: Adult male and female in host; segmenting eggs and first-, second-, and third-stage larvae outside of host; third- and fourth-stage larvae and adult males and females (fifth stage) in host. The preparasitic larval stages are separated by two molts, the sheath of the second molt being retained in most cases so that third-stage larvae are usually enclosed within a sheath. The parasitic stages also are separated by two molts.

The Strongyloididae group of nematodes has a heterogonous life history. In *Strongyloides ransomi*, larvae derived from eggs of parasitic females pursue one of two cycles of development, direct or indirect. In the direct cycle, the larvae develop as in the Strongylidae. In the indirect cycle, the larvae develop into free-living adults; the progeny of the latter develop as in the Strongylidae.

Throughout this bulletin the writer has followed for the most part the classification of the parasitic nematodes of vertebrates as given by Yorke and Maplestone (*138*). The lists of synonyms, hosts, and location and distribution of the various parasites discussed, have been taken for the most part from Hall (*42*, *43*).

ABBREVIATIONS AND SYMBOLS USED IN ILLUSTRATIONS

adbp, anterior dorsal body papilla
ao, anal opening
amph, amphid
angl, anal ganglion
b, base
bc, buccal cavity
bcp, buccal capsule
clgl, cephalic lateral ganglion
cp, cervical papilla (deirid)
csdgl, cephalic subdorsal ganglion
csvgl, cephalic subventral ganglion
cstwl, cyst wall
cutb, cuticular bosses
dbp, dorsal body papilla
dgl, dorsal ganglion
esh, egg shell
epc, epithelial cell
es, esophagus
excnu, excretory cell nucleus
exgl, excretory gland
exp, excretory pore
exs, excretory sinus
fgp, female genital primordium
gc^1, first giant cell
gc^2, second giant cell
gc^3, third giant cell
gc^4, fourth (genital) giant cell
gp, genital primordium
gerc, germinal cell
gon, gonoduct
gub, gubernaculum
h, hook
insmu, insect muscle
int, intestine
lgl, lateral ganglion
lbgl, lumbar ganglion
lv, larva
mgp, male genital primordium
mthelev, mouth elevation
nr, nerve ring
orop, oral opening
ov, ovary
ovj, ovejector
p, papilla
pdbp, posterior dorsal body papilla
plgl, postero-lateral ganglion
prbcp, provisional buccal capsule
pres, procsophagus
ptes, postesophagus
pvgl, postero-ventral ganglion
rgd, rectal gland
rch, rachis
rvgl, retrovesicular ganglion
sv, seminal vesicle
sdgl, subdorsal ganglion
sdp, subdorsal papilla
sp, spicule
spa, spicule primordium
spr, spear
svgl, subventral ganglion
svp, subventral papilla
t, tail
tp, tail process
tel, telamon
ts, testis
ut, uterus
v, vulva
vdf, vas deferens
ve, vas efferens

MORPHOLOGICAL AND EXPERIMENTAL DATA

SPIRURIDAE

GONGYLONEMA PULCHRUM MOLIN, 1857

(Figs. 1–9)

Synonyms.—*Gongylonema filiforme* (?) Molin, 1857; *G. spirale* (?) Molin, 1857; *Filaria labialis* Pane, 1864; *Spiroptera scutata* Müller, 1869; *F. scutata* (Müller, 1864) Leuckart, 1873; *G. scutatum* (Müller, 1869) Railliet, 1892; *Myzonimus scutatus* (Müller, 1869) Stiles, 1892; *G. ursi* (?) (Dujardin, 1845) Neumann, 1894; *G. confusum* Sonsino, 1896; *G. subtile* Alessandrini, 1914; *G. hominis* Stiles, 1921; *G. ransomi* Chapin, 1922.

Hosts.—Definitive: Sheep, goat, ox, camel, fallow deer, buffalo, zebu, chevrotain, pig, wild boar, horse, donkey, bear(?), macaque, *Ateles* sp., *Pithecus entellus*, man, white rat, guinea pig, and rabbit. Intermediate: Coleoptera (*Aphodius coloradensis*, *A. distinctus*, *A. femoralis*, *A. fimetarius*, *A. granarius*, *A. rubeolus*,

A. vittatus, Blaps appendiculata, B. emondi, B. strauchi, Caccobius schreiberi, Oniticellus fulvus, Onthophagus hecate, O. pennsylvanicus, O. taurus(?), *Sphaerius* sp., and *Sphaeridium* sp.); and Orthoptera (*Blatella germanica*). Intermediate hosts reported for *Gongylonema* sp., probably *G. pulchrum: Aphodius haemorrhoidalis, Gymnopleurus* sp., *Blatta orientalis*, and *Periplaneta americana*. Accidental: Mammals; nematode larvae collected by A. McIntosh from the stomach wall of a mole (*Scalopus aquaticus aquaticus*) were identified by the writer as third-stage larvae of *Gongylonema* sp.

In addition to the above-mentioned intermediate hosts the following insects have been found by the writer to serve as intermediate hosts for *G. pulchrum: Aphodius lividus, Dermestes vulpinus, Parcoblatta* sp.

Location.—Adults in mucosa of esophagus, tongue, and oral cavity of definitive host; third-stage larvae in body cavity of intermediate host.

Distribution.—Africa, Asia, Australia, Europe, and North America (United States).

Description of Egg, Embryo, and Larval Stages

EGG

Egg elliptical in shape; shell about 3μ thick, with smooth surface (fig. 1, *A*). Under high-power magnification, a faint line can be made out at each pole, representing the operculum. In a series of measurements involving about 50 eggs, length 57μ to 59μ, width 30μ to 34μ. Each egg contains a well-developed embryo at time of oviposition.

EMBRYO

Embryos (fig. 1, *F*), obtained by crushing several eggs on a slide under a cover slip, 240μ to 280μ long by 13μ in maximum width. Embryo does not undergo additional development before being ingested by intermediate host. Morphology of embryo corresponds to that of young first-stage larva.

FIRST-STAGE LARVA

Shape and size.—Newly hatched larva slender and of same width for most of length. As it grows during this stage, posterior half grows more in width than does anterior half, giving larva a club-shaped appearance (fig. 2, *A* and *B*). Anterior end broadly rounded, posterior portion tapering slightly and ending in a rounded extremity. Size of larva depends on degree of development (table 1); before molting, first-stage larva sometimes attains a length of 540μ and a width of 38μ.

Cuticle.—Very thin, transparent, with very fine transverse striations; anterior end of ventral portion with 1 spine and 2 small hooks of various sizes arranged longitudinally (fig. 1, *B* and *C*). When viewed with oil-immersion lens, spine appears as a small shining body; anterior hook, about 1μ long; posterior hook most conspicuous, approximately V-shaped, about 3μ long; posterior to these hooks, cuticle armed with about 20 parallel rows of very minute spines encircling anterior portion of larva for a distance of about 16μ from anterior end; spines larger and more prominent on dorsal than on ventral surface (fig. 1, *B*); spines of posterior rows becoming gradually smaller, last row very difficult to see. Tail (fig. 1, *E*) with a row of about 8 to 10 small refringent points encircling tip; these structures were pointed out by Stiles (*131*) in embryonic forms, and this character is diagnostic for first-stage larvae.

Alimentary tract.—Oral opening leading into a transparent esophagus 167μ to 243μ long and extending to a distance of slightly less than one-half of length of worm. Intestine also transparent, apparently composed of about 5 anterior large cells extending about two-thirds of length of intestine, and a posterior group of about 6 cells connecting with a very short rectum (fig. 1, *D*).

Nervous system.—Difficult to determine in living specimens. After larva is stained with aqueous methylene blue, nerve ring appears as a band surrounding posterior third of esophagus, 45μ to 110μ from anterior end, and surrounded by several nuclei of nerve cells.

Excretory system.—Excretory pore, 60μ to 145μ from anterior end, leads into a short dilated excretory duct (fig. 1, *D*); excretory duct opens from a glandular excretory cell possessing a large nucleus.

Genital primordium.—In living specimens hardly distinguishable from large muscle cells of body wall.

Table 1 shows the rate of development of first-stage larvae of *Gongylonema pulchrum* in an intermediate host (*Blatella germanica*),

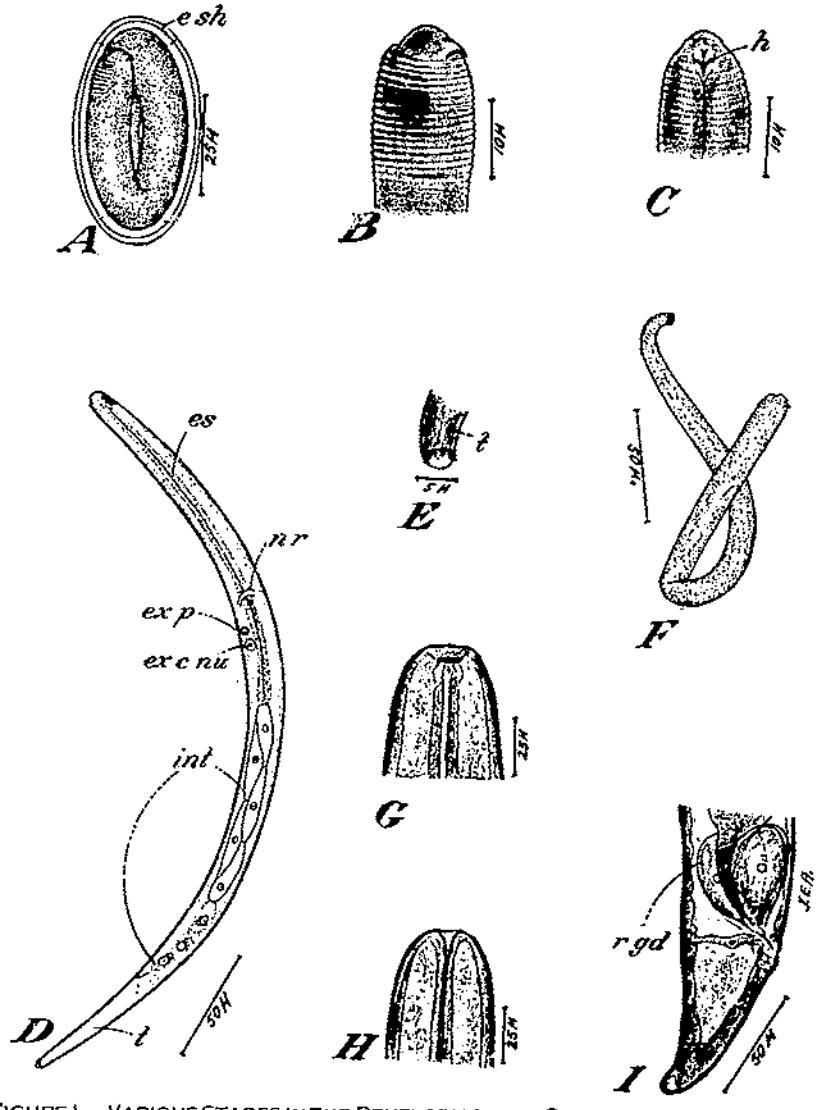

FIGURE 1.—VARIOUS STAGES IN THE DEVELOPMENT OF GONGYLONEMA PULCHRUM.

Embryo: *A*, Fully developed, in egg; *F*, fully developed, obtained by crushing the eggshell.
First-stage larva: *B*, Anterior end, lateral view; *C*, anterior end, ventral view; *D*, larva from intermediate host 4 days after experimental infection; *E*, tail, lateral view.
Second-stage larva: *G*, Anterior end, lateral view; *H*, anterior end, dorsal view; *I*, tail, lateral view.

the measurements having been made on different days after experimental infection.

TABLE 1.—*Principal measurements of 6 first-stage larvae of Gongylonema pulchrum at various periods of development in the roach*

Item	Period of development and measurements of larva no.—					
	1	2	3	4	5	6
Period of development.....days..	1	2	4	4	[1] 10	[1] 10
Length of body.....microns..	243	334	372	372	480	540
Maximum width of body.....do....	15	15	15	15	31	38
Length of esophagus.....do....	.....	107	178	172	190	243
Distance of nerve ring from anterior end.....do....	45	.....	60	58	.....	110
Distance of excretory pore from anterior end..do....	60	110	130	133	140	145
Length of tail.....do....	49	49	57	53	53	60

[1] Larva undergoing first molt.

SECOND-STAGE LARVA

Shape and size.—Young form similar in shape and size to older larva of first stage. As larva grows, it loses its club-shaped appearance and becomes more or less uniform in width, except for tapering anterior and posterior portions (fig. 2, *C*). Young specimen about 842μ long by 45μ wide; older specimen about 2.01 mm long by 53μ wide (table 2).

Cuticle.—Without armature or hooks at anterior portion and without refringent points encircling tip of tail (fig. 1, *I*); faint transverse striations present.

Alimentary tract.—Oral opening leading into cavity surrounded by poorly developed buccal capsule, 36μ to 38μ long; capsule more distinct in older larva of this stage than in newly molted form; entrance to lumen of buccal cavity surrounded by a thin cuticularized ring, flattened laterally (fig. 1, *G* and *H*). Esophagus well developed, less transparent than that of previous stage, and occupying about one-half of body length; esophagus slender, more or less uniform in width in young larva of this stage, but in older larva becoming differentiated into procesophagus—an anterior, comparatively short slender muscular portion, 53μ to 240μ long—and postesophagus—a posterior and wider glandular portion, 441μ to 1,150μ long; esophagus opening into a long slender intestine composed of many cells having poorly defined walls; posterior portion of intestine opening into a large rectum surrounded by large rectal glands, 2 subventral and 1 dorsal.

Nervous system.—Nerve ring 109μ to 121μ from anterior end, distinctly visible as a band surrounding anterior portion of esophagus (fig. 2, *C*); details of nervous system most evident in late second-stage larva, and very similar to those of third-stage larva.

Excretory system.—As in previous stage. In living specimens, nucleus of excretory cell not so conspicuous as in previous stage, owing to greater thickness of cuticle; excretory pore opening 150μ to 200μ from anterior end.

Genital primordium.—This developing organ best seen in living specimens of older larva of this stage, appearing as a small ellipsoidal body, ventral in position, 53μ to 342μ from tip of tail, the distance depending on size of larva.

Table 2 shows rate of development of second-stage larvae of *Gongylonema pulchrum* in an intermediate host (*Blatella germanica*) the measurements having been made at different days after experimental infection.

TABLE 2.—*Principal measurements of 6 second-stage larvae of Gongylonema pulchrum at various periods of development in the roach*

Item	Period of development and measurements of larva no.—					
	1	2	3	4	5	6
Period of development.....days.	19	23	27	27	[1] 29	[1] 32
Length of body.....microns.	842	1,045	1,138	1,497	1,990	2,010
Maximum width of body.....do..	45	46	53	53	53	53
Length of procesophagus.....do	.....	.....	53	60	220	240
Length of postesophagus.....do	.....	.....	441	780	810	1,150
Total length of esophagus.....do....	300	501	494	840	1,030	1,390
Distance of nerve ring from anterior end.....do....	.....	.....	.....	109	114	121
Distance of excretory pore from anterior end..do..	160	171	174	.....	152	200
Distance of genital primordium from posterior end microns.	53	.....	180	.....	342	.....
Length of tail.....do....	68	83	88	98	93	105

[1] Larva undergoing second molt.

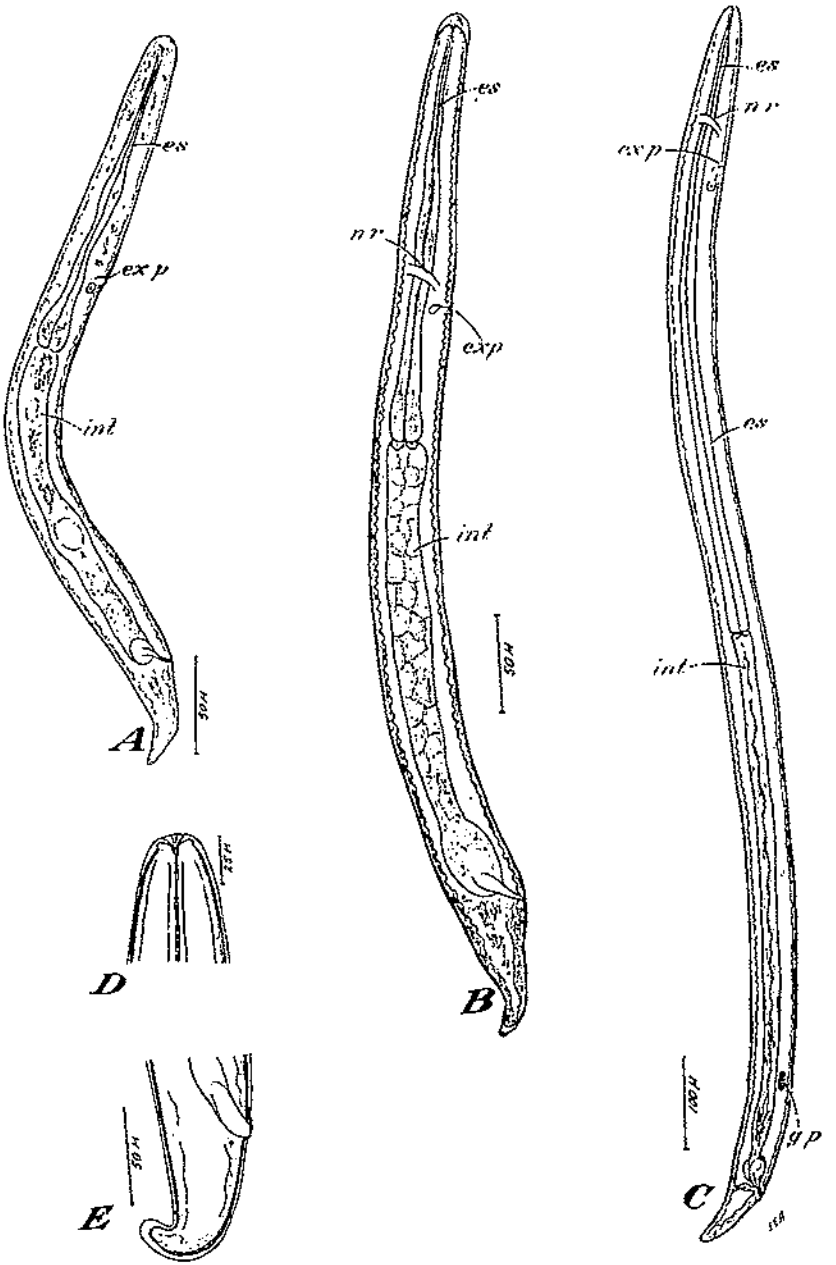

FIGURE 2.—FIRST- AND SECOND-STAGE LARVAE OF GONGYLONEMA PULCHRUM.

First-stage larva: *A*, Lateral view; *B*, undergoing first molt.
Second-stage larva: *C*, Lateral view; *D*, anterior end of larva undergoing second molt; *E*, posterior end of larva undergoing second molt.

THIRD-STAGE LARVA

Shape and size.—Body of larva slender and of same width for most of length, tapering slightly at anterior portion and rather abruptly posterior to anus.

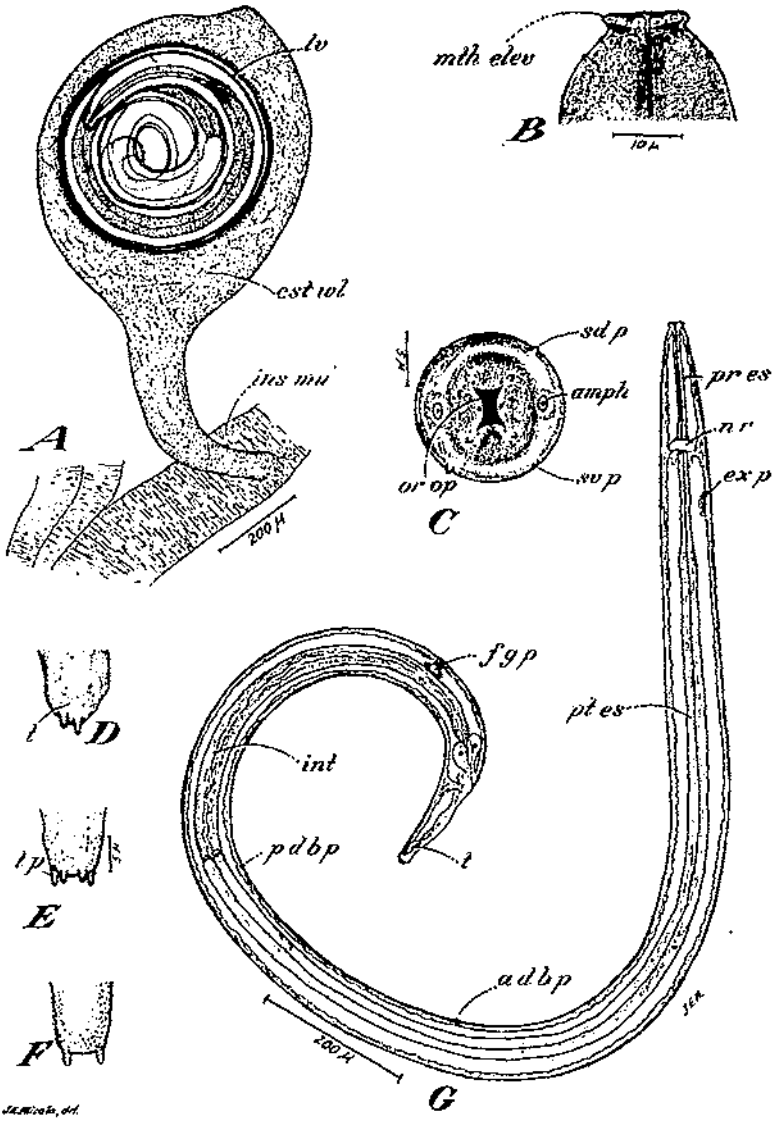

FIGURE 3.—THIRD-STAGE LARVAE OF GONGYLONEMA PULCHRUM.

A, Larva encysted in musculature of a roach (*Blatella germanica*); *B*, anterior end of larva, ventral view; *C*, anterior end, en face view; *D*, posterior end showing digitiform processes, lateral view; *E*, posterior end showing the usual four digitiform processes, ventral view; *F*, posterior end showing digitiform processes; *G*, lateral view of larva.

Lateral border of mouth projecting outward and elevated above surrounding surface of head (fig. 3, *B*); head surrounded by outer circle of 2 subdorsal and 2 subventral papillae and 2 lateral amphids; also an inner circle of smaller papillae, 2 pairs subdorsal, 2 pairs subventral, and 1 pair lateral (fig. 3, *C*); 2 small lateral

cervical papillae (deirids) projecting from cuticle slightly posterior to midway between anterior body extremity and nerve ring; 2 additional papillae on dorsal surface of larva, the anterior one slightly postequatorial and the posterior near region of base of esophagus (figs. 3, *G* and 4, *G*). Tail conical, usually terminating in 4 small digitiform processes, 2 subdorsal and 2 subventral, the subventrals in some specimens scarcely visible or entirely lacking (fig. 3 *D*, *E*, and *F*); visible

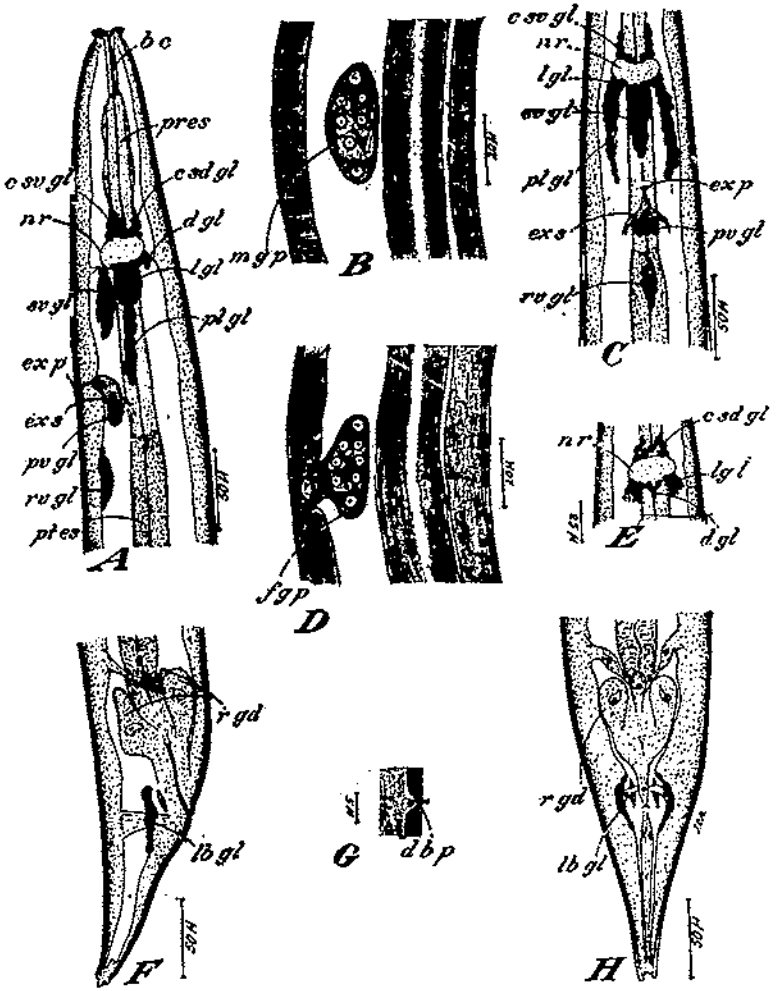

FIGURE 4.—THIRD-STAGE LARVAE OF GONGYLONEMA PULCHRUM.

A, Anterior end, lateral view; *B*, portion of larva showing male genital primordium, lateral view; *C*, anterior portion showing features of nervous system, ventral view; *D*, portion of larva showing female genital primordium attached to body wall, lateral view; *E*, region of nerve ring, dorsal view; *F*, posterior portion of larva, lateral view; *G*, portion of larva showing a dorsal body papilla; *H*, posterior portion of larva, ventral view.

processes about 2μ long. Larvae from 1.9 to 2.45 mm long by 50μ to 68μ wide; according to Seurat (*117*), larvae from 3.4 to 4.2 mm long by 72μ to 77μ wide; according to Ransom and Hall (*99*), larvae 1.9 mm long by 60μ wide.

Cuticle.—With prominent transverse striations.

Alimentary tract.—Oral opening, in en face view, elongated dorsoventrally, rectangular, with concave margins (fig. 3, *C*); aperture leading into a slender mouth cavity. In optical section, walls of mouth cavity appear as two long rods

differing slightly in length, the dorsal rod about 26μ long and the ventral one about 28μ long; width of these rods, about 2.5μ. Esophagus about three-fourths as long as body, differentiated into a procesophagus, 258μ to 308μ long, and a postesophagus 1.07 to 1.26 mm long. Intestine a short tube occupying about one-fifth of body length, connected posteriorly to rectum. Rectum about 35μ long, surrounded by 3 large rectal glands, 2 subventral and 1 dorsal.

Nervous system.—Readily visible, especially in specimens stained in acid carmine. Nerve ring appears as thick ring encircling esophagus, 114μ to 136μ from anterior end; according to Seurat (*117*), nerve ring 140μ to 160μ, according to Ransom and Hall (*99*), 125μ, from anterior end. Anterior to nerve ring, 4 strands of well-stained nuclei present, probably representing the nuclei of 2 subdorsal and 2 subventral cephalic ganglia (fig. 4, *A*, *C* and *E*); nuclei of cells of the lateral ganglia not observed. Posterior to nerve ring, 2 lateral ganglia, each connected posteriorly to another group of cells, the posterolateral ganglia; dorsally, one nucleus probably representing the cell of the dorsal ganglion; ventrally, the subventral ganglia fused into one large group of cells (fig. 4, *C*). Posterior to excretory sinus, the posteroventral ganglia represented by seven cells of which only the nuclei are visible; slightly posterior to excretory pore, a group of cells forming the retrovesicular ganglia (fig. 4, *A* and *C*) are present; surrounding the anal region and extending posteriad to the rectal glands, a small group of nuclei representing the cells of the lumbar ganglia (fig. 4, *F* and *H*).

Excretory system.—As in previous stage. Excretory pore 190μ to 228μ from anterior end; according to Seurat (*117*), excretory pore 215μ to 250μ according to Ransom and Hall (*99*), 210μ, from anterior end. Excretory duct opening into a triangular excretory sinus possesses a large nucleus in its walls (fig. 4, *C*).

Genital primordium.—As observed by the writer, male genital primordium (fig. 4, *B*) elliptical in shape, 30μ to 34μ long by 10μ to 15μ wide, located on ventral side between body wall and intestine, 345μ to 375μ from posterior end of body, and composed of 2 large epithelial cells enclosing a group of about 6 or 7 nuclei of germinal cells. Female genital primordium (fig. 4, *D*) somewhat elliptical, 30μ long by 10μ wide, attached to ventral side of body wall as noted by Seurat (*118*, *119*); attachment by means of a large cell about 8μ long located 260μ to 275μ from tip of tail. Measurements given in table 3 indicate that the female genital primordium is closer to the posterior end of the larva than is that of the male.

Table 3 shows the measurements of third-stage larvae of *Gongylonema pulchrum* in an intermediate host (*Blatella germanica*).

TABLE 3.—*Principal measurements of 10 third-stage larvae of Gongylonema pulchrum at various periods of development in the roach*

Item	Period of development and measurements of—									
	Male no.—					Female no.—				
	1	2	3	4	5	1	2	3	4	5
Period of development.....days..	32	32	38	38	42	32	32	36	38	42
Length of body.....millimeters..	1.90	2.08	2.20	2.25	2.27	2.05	2.10	2.28	2.30	2.45
Maximum width of body....microns..	53	53	53	53	53	50	60	64	53	68
Length of buccal cavity.....do....	28	26	20	28	28	26	28	28	28	30
Length of procesophagus.....do....	285	266	273	281	281	268	258	300	266	308
Length of postesophagus..millimeters..	1.07	1.26	1.17	1.12	1.20	1.07	1.09	1.20	1.25	1.26
Distance of nerve ring from anterior end.....microns..	114	124	136	.....	120	130	130	130	136	136
Distance of excretory pore from anterior end.....microns..	190	228	220	215	210	220	228	220	226	220
Distance of cervical papillae from anterior end.....microns..	76	80	86	86	90	94	80	88	94	102
Distance of dorsal body papillae from posterior end:										
Anterior papilla.....microns..	900	.....	1,000	1,080	1,100	1,050	.....	.....	.....	1,092
Posterior papilla.....do....	575	.....	700	710	780	700	.....	.....	.....	890
Distance of genital primordium from posterior end.....microns..	375	375	375	.....	345	.....	260	260	275	285
Length of tail.....do....	98	114	114	114	98	95	114	114	114	106

DIFFERENCES IN FIRST THREE STAGES

The outstanding differential features of the first three developmental stages of *Gongylonema pulchrum* are as follows:

First-stage larva.—Cuticle at anterior end provided with 1 spine and 2 hooks longitudinally arranged; posterior to these hooks, about 20 parallel rows of very minute spines encircling the cephalic portion; tip of tail blunt, surrounded by a row of small refringent processes.

Second-stage larva.—Cuticle without armature present as in previous stage; anterior and posterior ends bluntly rounded.

Third-stage larva.—Cuticle as in second stage; lateral border of mouth raised above surrounding surface of head and projecting outward; tail provided with 4 small digitiform processes, 2 subventral and 2 subdorsal, the subventral ones frequently only barely visible or entirely lacking.

DEVELOPMENT IN INTERMEDIATE HOST

Eggs of *Gongylonema pulchrum* were obtained by chopping up gravid female worms in a few drops of distilled water. This material was then transferred to small pieces of bread and introduced into a 300-cc Erlenmeyer flask in which there were placed about a dozen roaches (*Blatella germanica*). The roaches had been previously starved for about 24 hours. The top of the flask was closed with a layer of cheesecloth. In order to learn the approximate time required for larval development, the roaches were allowed to feed on the infested bread for about 5 hours, after which they were transferred to a clean flask containing food not contaminated with eggs.

Roaches dissected about 24 hours after they had ingested the eggs already had empty eggshells in their crops and intestines, an observation which was reported by Ransom and Hall (*98, v. 2*) in their first extensive paper on the life history of this parasite. At this time there were also found several newly hatched larvae still adhering to the wall of the crop and apparently ready to invade the body cavity of the roach. No larvae were found in the lumen or wall of the intestine, a fact which shows that possibly hatching took place in the crop and that the larvae found their way into the body cavity by piercing the wall of the crop. About 48 hours after ingestion of eggs, first-stage larvae were found in the body cavity of the roach, usually more numerous in the thoracic region than elsewhere. At the end of about 15 days, the wandering larvae had doubled their original size and appeared very plump. At this time the first cuticle had begun to loosen at the anterior and posterior ends (fig. 2, *B*). These observations are in agreement with the findings of Ransom and Hall (*98, v. 2*), who state that these larvae were on the verge of a molt in the intermediate host 2 weeks after experimental infestation. The first molt did not take place, however, until about the nineteenth day, when some larvae are already in the second stage.

The second-stage larvae also wandered in the body cavity, especially in the abdominal region, where they increased considerably in length. In about 27 days the larvae were 1.13 to 1.5 mm long by 53μ wide. At this time they usually penetrated the muscles of the body wall, especially those of the ventral portion of the abdomen. In heavy infestations the larvae may invade the walls of the crop and intestine and the muscles of the coxae. Partial encystment has been observed to precede the second molt. Larvae undergoing the second molt were found 29 to 32 days after experimental infection,

and larvae which had completed the second molt and were in the third stage were found 32 days after infection. These findings agree with those of Ransom and Hall (*98, v. 2*), who state that at the end of about a month the larvae were encysted, that is, in the final larval stage. The writer's observations are also in agreement with those of Lucker (*68*), who, in dissecting experimentally infested roaches 34 days after infection, found encysted larvae which produced an infestation when fed to pigs.

Encysted *Gongylonema* larvae were studied both by gross dissection and in cross sections of infested roaches. The observations made indicated that each encysted larva was embedded within the sarcoplasm surrounding the muscle fiber. The nuclei in the sarcoplasm surrounding the cyst wall of the larva were very distinct in stained sections (figs. 5 and 6). As the cyst became well formed it was some-

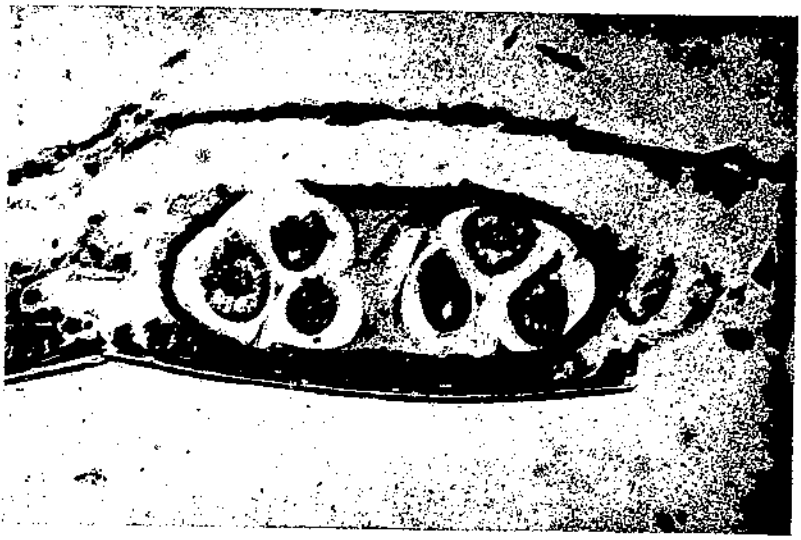

FIGURE 5.—Cross section of a roach (*Blatella germanica*), showing *Gongylonema* larva encysted in the body wall.

times pushed out into the body cavity until its attachment to the muscle was merely by a thin strand (fig. 3, *A*). At this time most of the nuclei in the cyst wall became degenerated, so that in stained sections of these cysts only a few nuclei were visible at the place of the attachment of the cyst. The outer portion of the cyst did not stain well and had the appearance of cloudy degeneration as observed in vertebrate tissue.

DEVELOPMENT IN DEFINITIVE HOST

The study of the third and fourth stages in the definitive host was advantageously carried out in the guinea pig, an experimental animal which can be easily and thoroughly examined post mortem. Though this animal is not a normal host for *Gongylonema*, it is safe to assume that the same mode of larval development and migration takes place in it as in the more usual hosts, namely, cattle, sheep, and swine.

Tables 4 and 5 show the measurements of third- and fourth-stage larvae in different periods of development. As shown in table 4, in 12 days after infection the larvae still in the third stage had practically doubled their length. In a guinea pig killed 9 days after experimental infection, there were found larvae at the beginning of

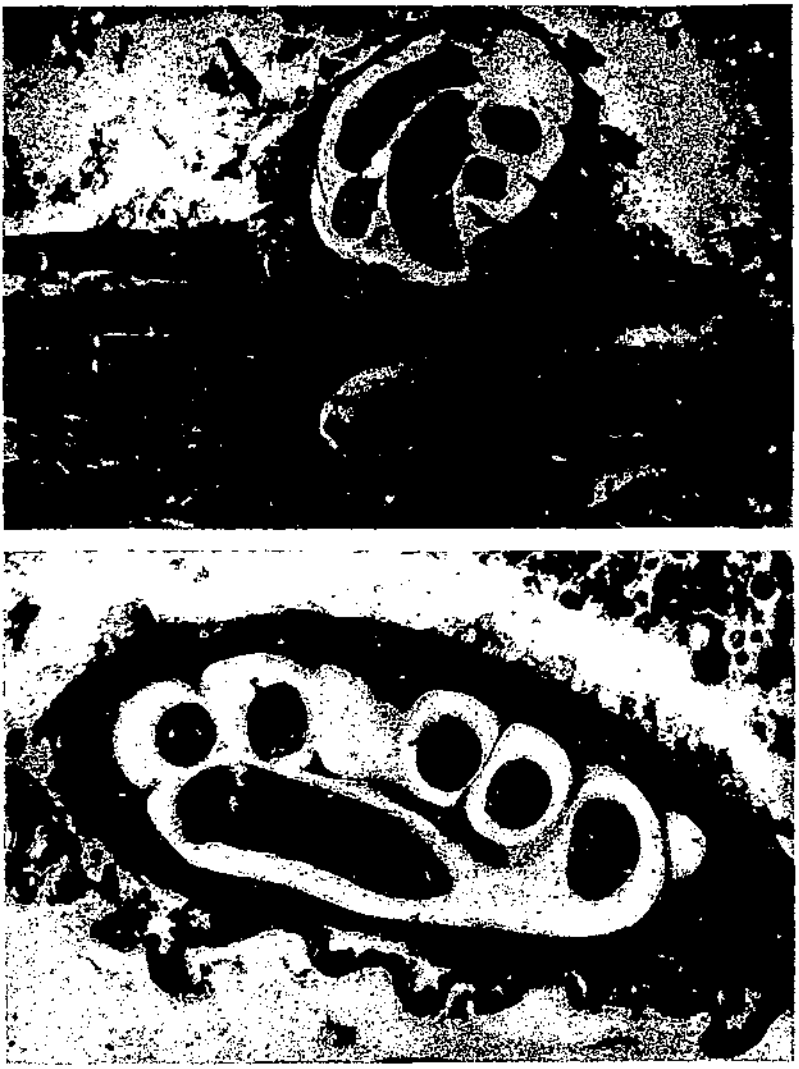

FIGURE 6.—Sections of a roach (*Blatella germanica*) showing *Gongylonema* larva encysted in the musculature.

the third molt (fig. 7, *A–D*). The most outstanding morphological feature of these larvae, as shown in figure 7, is the development of the genital primordium. In the males, the genital system appeared as a slender tube about 550μ long, with its posterior portion about ready to connect with the rectum; in the females, a short ovejector and two divergent uteri were present.

TABLE 4.—*Measurements of 6 third-stage larvae of Gongylonema pulchrum in various periods of development in guinea pigs*

Item	Period of development and measurements of—					
	Male no.—		Female no.—			
	1	2	1	2	3	4
Period of development.....days	3	[1] 9	[1] 8	3	[1] 9	[1] 12
Length of body.....millimeters	2.80	7.80	2.57	2.58	3.70	4.45
Maximum width of body.....microns	76	78	73	70	83	91
Length of buccal cavity.....do	38	38	38	38	38	38
Length of procsophagus.....do	343	312	243	240	326	304
Length of postesophagus.....millimeters	1.61	1.78	1.03	1.70	1.87	2.10
Distance of nerve ring from anterior end.....microns	.....	171	136	148	167	167
Distance of excretory pore from anterior end.....do	.....	.....	235	180	281	304
Distance of cervical papillae from anterior end...do	.....	.....	106	.....	.....	121
Distance of genital primordium from posterior end microns	452	.....	310	.....	421	450
Length of tail.....do	106	156	106	129	152	152

[1] Larva undergoing third molt. [2] Hours.

TABLE 5.—*Measurements of 9 fourth-stage larvae of Gongylonema pulchrum in various periods of development in guinea pigs*

Item	Period of development and measurements of—								
	Male no.—				Female no.—				
	1	2	3	4	1	2	3	4	5
Period of development.....days	12	16	[1] 27	[1] 31	12	16	27	[1] 27	[1] 31
Length of body.....millimeters	4.60	7.70	11.00	12.00	5.85	8.90	15.50	18.00	20.00
Maximum width of body.....microns	.....	90	90	121	90	114	121	121	156
Length of buccal cavity.....do	38	45	45	45	38	42	45	53	45
Length of procsophagus.....do	295	..	304	402	350	360	402	487	456
Length of postesophagus.....millimeters	1.76	....	2.40	2.55	2.48	2.40	2.65	3.36	3.00
Distance of nerve ring from anterior end microns	152	174	184	220	180	205	197	281	256
Distance of excretory pore from anterior end microns	268	.....	334	364	349	..	387	440	468
Distance of cervical papillae from anterior end microns	115	120	...	130	118		136	144	156
Distance of dorsal body papillae from posterior end:									
Anterior papilla.....millimeters	2.75	4.80	6.20		3.10	5.30	10.8	.....	.....
Posterior papilla.....do	1.60	2.60	3.98	.	2.25	3.10	5.3	.....	.....
Distance of vulva region from posterior end microns	..	...	...		540	826	1,170	1,200	1,200
Length of tail.....do	121	167	178	...	144	159	179	167	182

[1] Larva undergoing fourth molt.

In a guinea pig killed 12 days after infection, some larvae already in the fourth stage were found. Measurements of these larvae are given in table 5. The outstanding features of this stage are the following: The anterior end of the larvae retains to some extent lateral and outward directed elevations (fig. 7, *F* and *G*) similar to those present in the previous stage. The cuticle in young fourth-stage larvae does not show bosses on the anterior portion, but these structures are gradually formed as the larvae grow older (fig. 7, *H*). In males, the posterior portion of the vas deferens is already connected with the rectum, and at the time of the fourth molt males have well-developed spicules and caudal papillae have formed (fig. 7, *J*). The females have a long ovejector with one uterus extending anteriorly and the other posteriorly (fig. 7, *L*). The tails of both sexes at this stage are rounded and lack the digitiform appendages found in larvae of the previous stage. Fourth-stage larvae grow considerably in length, the males apparently not growing so long as the females. In 27 to 31 days after infection, the males are 11 to 12 mm long,

whereas the females are 18 to 20 mm long. At this time both males and females undergo the fourth or last molt, and this cuticle is not actually cast off until 37 days after infection. The fifth stage, which represents adult forms, is easily differentiated from the previous

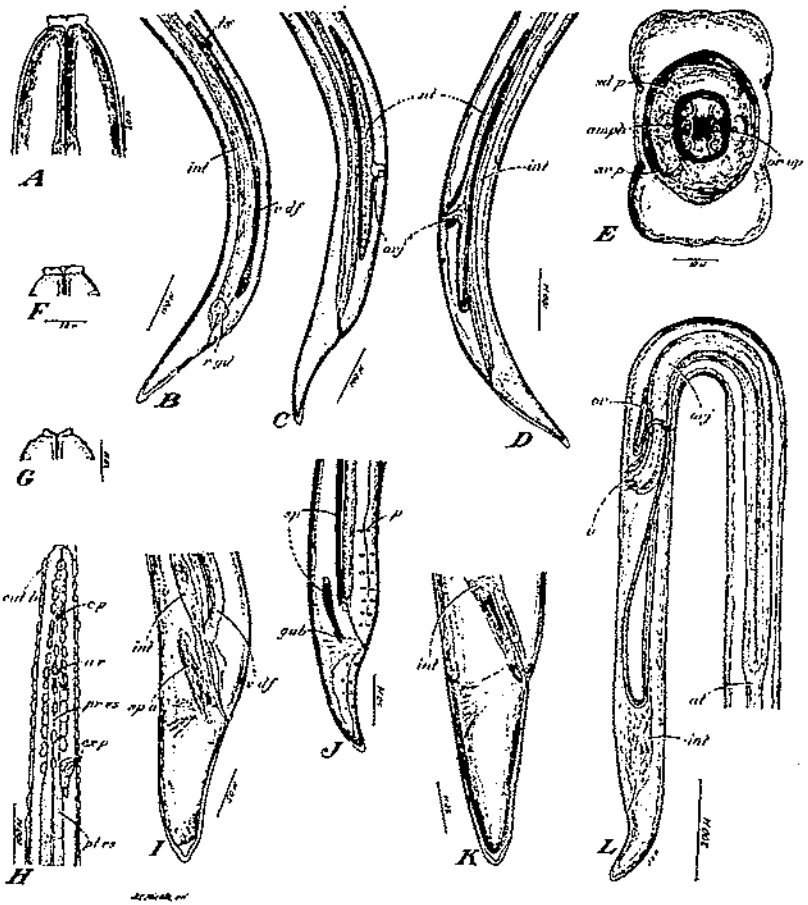

FIGURE 7.—THIRD- AND FOURTH-STAGE LARVAE OF GONGYLONEMA PULCHRUM.

Third-stage larva: *A*, Anterior end, undergoing third molt; *B*, posterior portion of male undergoing third molt; *C*, *D*, posterior portion of female undergoing third molt.

Fourth-stage larva: *E*, Anterior end, en face view; *F*, anterior end, lateral view; *G*, anterior end, ventral view; *H*, anterior portion; *I*, posterior portion of male on verge of fourth molt; *J*, posterior portion of male in fourth molt; *K*, posterior portion of larva in fourth molt; *L*, region of vulva of larva undergoing fourth molt.

stage; the females have the vulva opening to the outside and the male's caudal alae have several preanal and postanal papillae.

After the last molt, these worms grow considerably. One adult male worm obtained from the esophagus of a guinea pig 70 days after experimental infection had the following measurements; Length, 32 mm; maximum width, 140μ; length of procesophagus and postesophagus, 577μ and 4.5 mm, respectively; length of right and left spicules, 121μ and 8.5 mm, respectively. Two young adult female

worms just beginning oviposition, also obtained from the esophagus of the guinea pig referred to above, had the following measurements: Length, 55 and 60 mm; maximum width, 235μ; length of proesophagus,

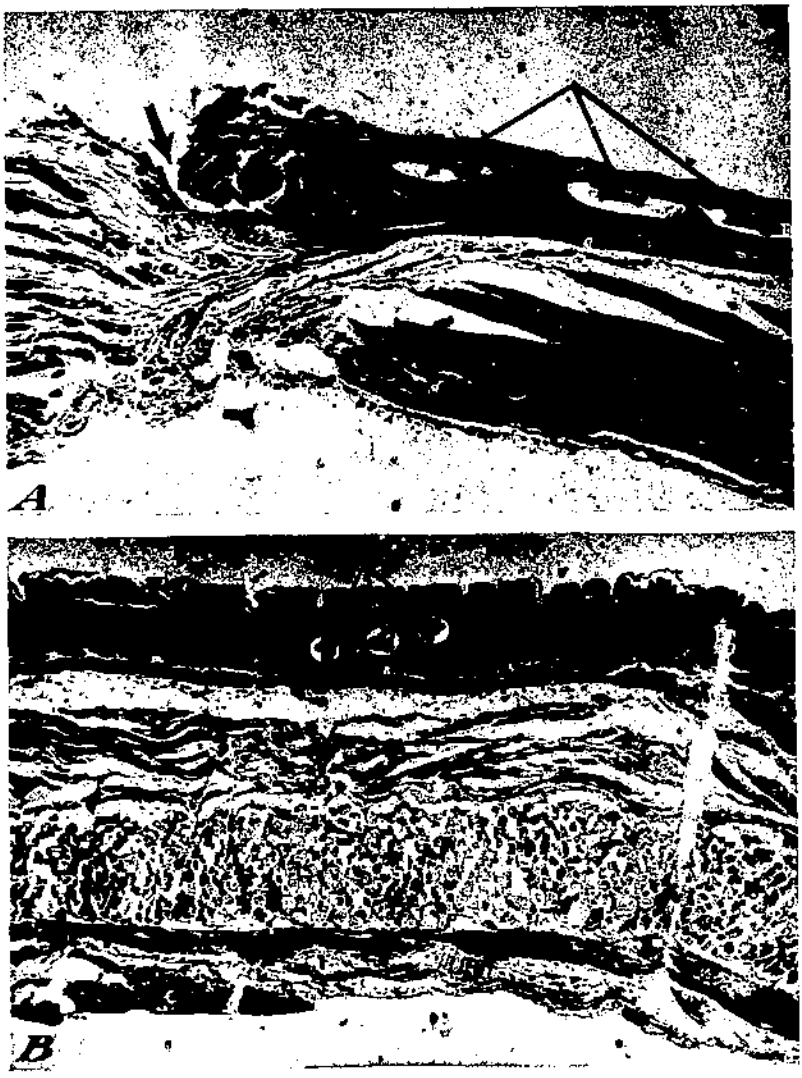

FIGURE 8.—*A*, Longitudinal section of gastroesophageal junction of a guinea pig 18 hours after experimental infection with larvae of *Gongylonema pulchrum*, showing larvae embedded in epithelium of esophagus. Large arrow points out the possible mode of entrance of the larvae into the esophagus; *B*, cross section of esophagus of a guinea pig 18 hours after experimental infection showing a larva embedded in the epithelium.

655μ and 672μ; length of postesophagus, 5 and 5.2 mm; length of tail, 280μ and 292μ.

MIGRATION OF LARVAE IN DEFINITIVE HOST

During the month of April 1933, encysted third-stage larvae of *Gongylonema pulchrum*, obtained from experimentally infected

cockroaches, were fed with the aid of a pipette to five guinea pigs weighing from 135 to 150 g. These guinea pigs were kept without food for about 24 hours before the infective larvae were fed. A description of these tests and the results are as follows:

Guinea pig 1 was given 9 infective larvae and was killed one-half hour later. The tongue, esophagus, and stomach were then dissected and removed from the body. The stomach was opened and washed in a Petri dish containing physiologic saline solution. Most of the stomach was cut into pieces about 2 cm square, and each piece was pressed between two glass slides and examined with the aid of a binocular microscope. The esophagus, together with a portion of the anterior part of the stomach, was examined as a whole. Press preparations were also made of the tongue, palate, and oral linings. By this method larvae were easily detected whenever they were present. Seven larvae were recovered from this guinea pig, 2 larvae being embedded in the esophageal wall about 2 mm from the gastroesophageal junction, 1 larva in the cardiac portion of the stomach wall, and 4 excysted larvae in the stomach contents. The other 2 larvae which had been fed were not found.

Guinea pig 2 was fed 12 infective larvae and was killed 1 hour later. Most of the larvae were found at the junction between the stomach and esophagus, 1 larva in the esophageal wall slightly above this junction, and 2 in the wall of the esophagus about 2 mm from the junction. No larvae were found in the stomach wall or its contents or in the tongue or oral lining. The other larvae which had been fed to the guinea pig were not found.

Guinea pig 3 was fed 12 infective larvae and was killed 18 hours later. Six larvae were found embedded in the wall of the esophagus, scattered about 1 to 3 mm from the gastroesophageal junction, and 1 larva was found about 2 cm from this junction. No larvae were found in the stomach wall or its contents. The esophagus was sectioned (fig. 8) at the points where embedded larvae were found. Figure 8, *A*, shows a larva embedded in the epithelium of the esophagus not far from the gastroesophageal junction, and figure 8, *B*, shows a larva also in the epithelium of the esophagus; the latter larva was located about 2 cm from the base of the esophagus of the guinea pig. The other larvae which had been fed to the guinea pig were not found.

Guinea pig 4 was fed 45 infective larvae and was killed 3 days later. Three larvae were found about 3 cm from the base of the esophagus, and 5 larvae in the posterior portion of the tongue; other larvae were probably present in this region, but were not sought for as the writer was concerned only with the portions of tissues infested. No larvae were found in the stomach wall or its contents.

Guinea pig 5 was fed 45 infective larvae and was killed 10 days later. Two larvae were found 4 cm from the base of the esophagus, several larvae in the walls and lateral portions of the tongue, and 1 larva in the wall of the hard palate. No larvae were found in the stomach or its contents.

These observations indicate that excysted larvae of *Gongylonema pulchrum* excyst in the stomach of guinea pigs and may invade the esophagus within one-half hour after feeding. The observations suggest also that the path of entry of these larvae to the esophagus is through the tissue at the junction of the stomach and esophagus, as the larvae are usually most numerous in that area. This may be

due to a lack of resistance of this tissue or to a chemotaxis or other tropism. In figure 8, *A*, the large arrow points out the possible mode of entrance of the larvae into the esophagus. Larvae may migrate to the esophagus also by first entering the wall of the stomach, but this possibility is apparently rare as in only 1 out of 5 feedings was even 1 larva found in the stomach wall.

After the larvae have penetrated the esophagus, they migrate upward and invade the walls of the oral cavity 3 days after experimental feeding. These larvae may migrate promptly to any part of the walls of the tongue (fig. 9), palate, or lining of the buccal cavity. In observations other than the ones given, larvae have been found to develop to maturity in any of the tissues which have been mentioned as being invaded. It is of interest to note that the wandering larvae are found only in the epithelium of the structures invaded (figs. 8 and

FIGURE 9.—Section of tongue of a guinea pig showing *Gongylonema pulchrum* larvae embedded in the epithelium, 31 days after experimental infection.

9). No extensive lesions have been found associated with infestations with these larvae. This is in agreement with the opinion of Ransom and Hall *(98, v. 2)* who, in comparing this worm with *Gongylonema neoplasticum* (described by Fibiger and Ditlevsen *(25)*, in 1914, as inducing the production of neoplastic changes in stomachs of rats) state that *G. scutatum* (=*G. pulchrum*) can be regarded as probably an innocuous parasite.

OBSERVATIONS ON EFFECTS OF ENVIRONMENT

RESISTANCE OF EGGS TO LOW TEMPERATURES

On February 17, 1933, several thousand eggs of *Gongylonema pulchrum* were mixed with sterile sand and transferred to 3 small glass tubes about 5 cm high and 8 cm in diameter. Enough water was added to this mixture to give the sand a moist appearance. These tubes were plugged with cotton and placed in two 1-pint fruit

jars, 2 tubes being put in 1 jar and 1 in another jar; the tubes were held straight on the bottom of the jar by fitting absorbent cotton around the tubes. In order to prevent drying of the sand, water was added to the jars to a height of about 3 cm. The jars were then covered with moist paper hand towels and a few holes were made at the top of the paper with the aid of a dissecting needle. The jar containing two tubes was placed outdoors (Washington, D. C.) under shelter so as to prevent rain or snow from falling upon it. On June 17, 1933, 4 months later, one tube was removed from the jar. Some of the eggs were examined and found to contain viable embryos. The eggs were then separated from the sand with the aid of a screen, placed on a piece of bread, and fed to six roaches. About 40 days later the roaches were examined, and all were found to contain *Gongylonema* larvae encysted in the body cavity.

Data in connection with the outside temperatures [3] during the 4 months' exposure of the eggs are as follows: Minimum, —6.6° C.; maximum, 37.7°; total time during which temperature was 0° or lower, 66 hours, and from 1 to 10°, 1,002 hours. The eggs were thus kept in cold and sometimes freezing temperatures 37 percent of the entire period of outdoor exposure without being killed.

LONGEVITY OF EGGS AT ROOM TEMPERATURE

The jar containing one tube was kept indoors at a temperature of from 22° to 24° C. The tube was examined in 4 months and was found to contain eggs with viable embryos. The eggs were separated from the sand and fed to six roaches in the same manner as in the experiment previously described. The roaches were examined 40 days later and were found to contain encysted *Gongylonema* larvae.

ASCAROPS STRONGYLINA (RUDOLPHI, 1819) ALICATA AND McINTOSH, 1933

(Figs. 10-11)

Synonyms.—*Spiroptera strongylina* Rudolphi, 1819; *S. strongyliformis* de Blainville, 1828; *Filaria strongylina* (Rudolphi, 1819) Schneider, 1866; *Arduenna strongylina* (Rudolphi, 1819) Railliet and Henry, 1911; *Habronema strongylina* (Rudolphi, 1819) Ostertag, 1932.

Hosts.—Definitive: Swine, rabbit, wild boar, cattle, guinea pig. Intermediate: Coleoptera (*Aphodius rufus, A. castaneus, Gymnopleurus* sp., *Scarabaeus* sp.) and Odonata (*Anax parthenope*). Accidental: Mammals, birds, and reptiles for third-stage larvae; Alicata and McIntosh (*9*) have found these larvae encysted in the stomachs of bats captured in Washington, D. C.

In addition to the above intermediate hosts, the following insects have been found by the present writer to serve as intermediate hosts for *A. strongylina: Passalus cornutus* and *Aphodius granarius*

Location.—Adults in stomach of definitive host; third-stage larvae in body cavity of intermediate host, and wall of intestine or mesentery of accidental host.

Distribution.—Africa (Algeria), Asia (Cochin China, India, Philippines, Turkestan), Australia, Europe (France, Germany, Hungary, Italy, Rumania), Central America (Nicaragua, Panama), North America (United States), South America (Argentina).

DESCRIPTION OF EGG, EMBRYO, AND LARVAL STAGES

EGG

Egg somewhat elliptical in shape, slightly flattened at each pole (fig. 10, *A*); shell about 2μ thick, with numerous small punctations on surface; under high-

[3] Temperature records obtained from the U. S. Weather Bureau in the District of Columbia.

power magnification each pole with a small pluglike thickening. In a series of measurements involving about 50 eggs, length 41μ to 45μ, width 22μ to 26μ;

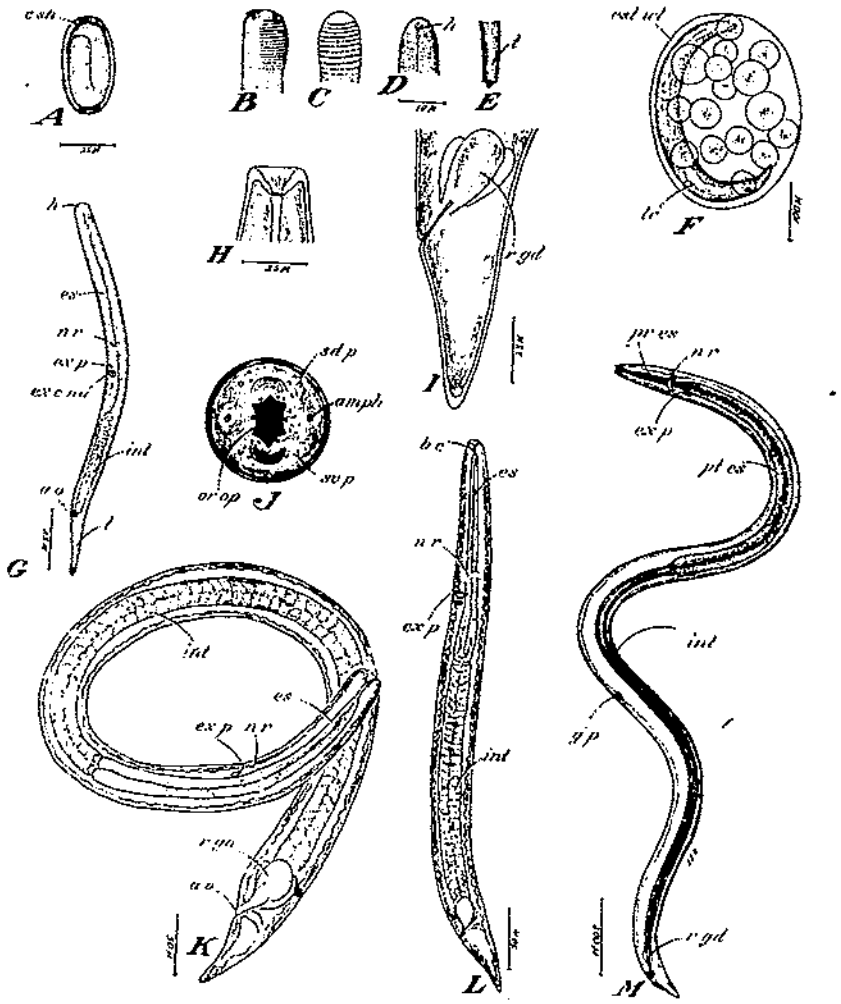

FIGURE 10.—VARIOUS STAGES IN THE DEVELOPMENT OF ASCAROPS STRONGYLINA.

A, Egg, containing fully developed embryo.
First-stage larva. *B*, Anterior end, lateral view; *C*, anterior end, dorsal view; *D*, anterior end, ventral view; *E*, tail; *F*, encysted larva; *G*, larva recovered from an intermediate host 3 days after experimental infection; *L*, larva undergoing first molt.
Second-stage larva: *H*, Anterior end of larva undergoing second molt, lateral view; *I*, posterior end of larva undergoing second molt, lateral view; *K*, lateral view of larva.
Third-stage larva. *J*, Anterior end, en face view; *M*, lateral view of larva.

according to Foster (*36*), length 34μ to 39μ, width 20μ. Each egg contains a well-developed embryo at time of oviposition.

EMBRYO

Embryos, obtained by crushing several eggs on a slide under a cover slip, 110μ to 115μ long by 7.5μ in maximum width. Embryo does not undergo further development before being ingested by intermediate host. Morphology of embryo corresponds to that of young first-stage larva.

FIRST-STAGE LARVA

Shape and size.—Newly hatched larva slender and of same width for most of length (fig. 10, *G*). As it grows during this stage, posterior half of body grows more in width than does anterior half, giving larva a club-shaped appearance. Anterior end broad and rounded, posterior portion tapering slightly; tip of tail ending in characteristic short conical structure about 1.5μ long (fig. 10, *E*). Size of larva depends on degree of development (table 6); before molting, first-stage larva sometimes attains a length of 530μ and a width of 35μ.

Cuticle.—Very thin, transparent, and with very fine transverse striations; ventral portion of anterior extremity with two hooks of different sizes, arranged longitudinally (fig. 10, *D*). When viewed with oil-immersion lens, the most anterior hook appears very minute; posterior hook most conspicuous, approximately V-shaped, about 1.7μ long; posterior to these hooks, cuticle armed with about 17 parallel rows of very minute spines encircling anterior portion of larva for a distance of about 16μ from anterior end; spines larger and more prominent on dorsal than on ventral surface (fig. 10, *B*, *C*, and *D*); spines of posterior rows becoming gradually smaller, the last row being very difficult to see.

Alimentary tract.—Oral opening leading into a long, transparent esophagus 80μ to 190μ in length and extending about one-half of length of worm, length of esophagus depending on degree of development in intermediate host. Intestine transparent, apparently composed of many cells; intestine connecting posteriorly with a very short rectum surrounded by three rectal glands.

Nervous system.—Difficult to determine in living specimens. In larva stained with aqueous methylene blue, nerve ring shows as a band encircling esophagus, 50μ to 75μ from anterior end, and surrounded by several nuclei of nerve cells.

Excretory system.—Excretory pore 60μ to 95μ from anterior end, leading into a short dilated duct, this duct opening into a large excretory cell possessing a large nucleus (fig. 10, *G* and *L*).

Genital primordium.—Hardly distinguishable from large muscle cells of body wall.

Table 6 shows the rate of development of first-stage larvae of *Ascarops strongylina* in an intermediate host (*Aphodius granarius*), the measurements having been made on different days after experimental infection.

TABLE 6.—*Principal measurements of 5 first-stage larvae of Ascarops strongylina at various periods of development in dung beetles*

Item	Period of development and measurements of larva no.				
	1	2	3	4	5
Period of development.....days.	3	4	4	10	[1] 17
Length of body.....microns	158	160	160	163	530
Maximum width of body.....do	9	9	9	10	35
Length of esophagus.....do	80	95	85	88	190
Distance of nerve ring from anterior end.....do	50	50	55	55	75
Distance of excretory pore from anterior end.....do	60	68	70	72	95
Length of tail.....do	26	26	30	30	49

[1] Larva undergoing first molt.

SECOND-STAGE LARVA

Shape and size.—Young form similar in shape and size to older larva of first stage. As larva grows, it loses its club-shaped appearance and becomes more or less uniform in width, except for tapering anterior and posterior portions (fig. 10, *K*). Tip of tail rounded, having lost the conical structure with shedding of first cuticle. These specimens about 720μ to 1,650μ long by 41μ to 62μ wide, depending on degree of development (table 7).

Cuticle.—Without armature in anterior portion; faint transverse striations present.

Alimentary tract.—Oral opening leading into a buccal capsule 35μ to 38μ long; capsule more distinct in older larva of this stage than in newly molted forms. Esophagus slender, 210μ to 436μ long, occupying about one-third of body length,

more or less uniform in width in young larva of this stage, but in older ones becoming differentiated into procsophagus—an anterior, comparatively short slender portion—and postesophagus—a posterior, wide portion about five times as long as procsophagus; esophagus opening into a long slender intestine composed of many cells having poorly defined walls; posterior portion of intestine opening into a large rectum surrounded by large rectal glands, 2 subventral and 1 dorsal in position.

Nervous system.—Nerve ring 80μ to 144μ from anterior end; details of nervous system most evident in late second-stage larvae and very similar to those of third-stage larvae.

Excretory system.—Structure as in previous stage; opening of excretory pore 80μ to 167μ from anterior end of body.

Genital primordium.—Small ellipsoidal body, ventral in position, 362μ to 530μ from tip of tail, the distance depending on size of larva; best seen in living specimens of older larva of this stage.

Table 7 shows the rate of development of second-stage larvae of *Ascarops strongylina* in the intermediate host (*Aphodius granarius*), the measurements having been made on different days after experimental infection.

TABLE 7.—*Principal measurements of 7 second-stage larvae of Ascarops strongylina at various periods of development in dung beetles*

Item	Period of development and measurements of larva no.—						
	1	2	3	4	5	6	7
Period of development.....days..	10	19	19	22	22	1 23	1 28
Length of body.....microns..	720	725	920	1,310	1,328	1,375	1,650
Maximum width of body.....do..	41	41	45	53	55	55	62
Length of buccal cavity.....do..				35	35	35	38
Length of esophagus.....do....	240	210	245	380	400	395	436
Distance of nerve ring from anterior end microns	102	80	110		120	110	144
Distance of excretory pore from anterior end microns	102	80	110	125	130	120	167
Distance of genital primordium from posterior end.. microns					362	400	530
Length of tail .....do	57	57	60	68	83	80	83

1 Larva undergoing second molt.

THIRD-STAGE LARVA

Shape and size.—Body slender, of same width for most of its length, tapering slightly anteriorly and rather abruptly posterior to anus (fig. 10, *M*); anterior end with characteristic dorsoventral liplike elevations (fig. 11, *C*). Head surrounded by outer circle of 2 large and 2 smaller subdorsal papillae, with corresponding subventral papillae; 2 lateral amphids present; an inner circle of smaller papillae, 1 pair subdorsal, 1 pair subventral, and 1 pair lateral (fig. 10, *J*); 2 small lateral asymmetrical cervical papillae (deirids) present; papilla on right and left sides, 159μ to 195μ and 91μ to 132μ from anterior end, respectively. Tail conical, terminating in a characteristic small smooth knob 7μ to 8μ long (fig. 11, *G*). Larvae 1.91 to 2.32 mm long by 53μ to 91μ wide; according to Seurat (*117*), 1.9 mm long by 80μ wide.

Cuticle.—With prominent transverse striations.

Alimentary tract.—In en face view, oral opening is somewhat hexagonal and elongated dorsoventrally, the aperture leading into a slender mouth cavity. In optical section, walls of mouth cavity appear as 2 rods, each 53μ to 70μ long. Esophagus about one-third as long as body, differentiated into a procsophagus 114μ to 200μ long, and a posteosphagus 590μ to 800μ long. Intestine about two-thirds as long as body, connected posteriorly with rectum. Rectum 30μ to 40μ long, surrounded by 3 large rectal glands, 2 subventral and 1 dorsal in position.

Nervous system.—Readily visible, especially in specimens stained in acid carmine. Nerve ring appears as a thick ring encircling the esophagus 129μ to 152μ from anterior end; according to Seurat (*117*), 154μ from anterior end. General

structure of nervous system (fig. 11, *C*, *E*, *F*, *G*, *H*), similar to that of third-stage larvae of *Gongylonema pulchrum*, except that in *Ascarops strongylina* the cells of subventral ganglia, ventral and posterior to nerve ring, are divided into two groups.

Excretory system.—In general, as in previous stages. Excretory pore 150μ to 205μ from anterior end, its duct opening into a triangular excretory sinus, the sinus wall possessing a single large nucleus.

Genital primordium.—As in *Gongylonema pulchrum*, sex can be differentiated at this stage. Male genital primordium (fig. 11, *B*) 15μ to 18μ long by 9μ to 11μ

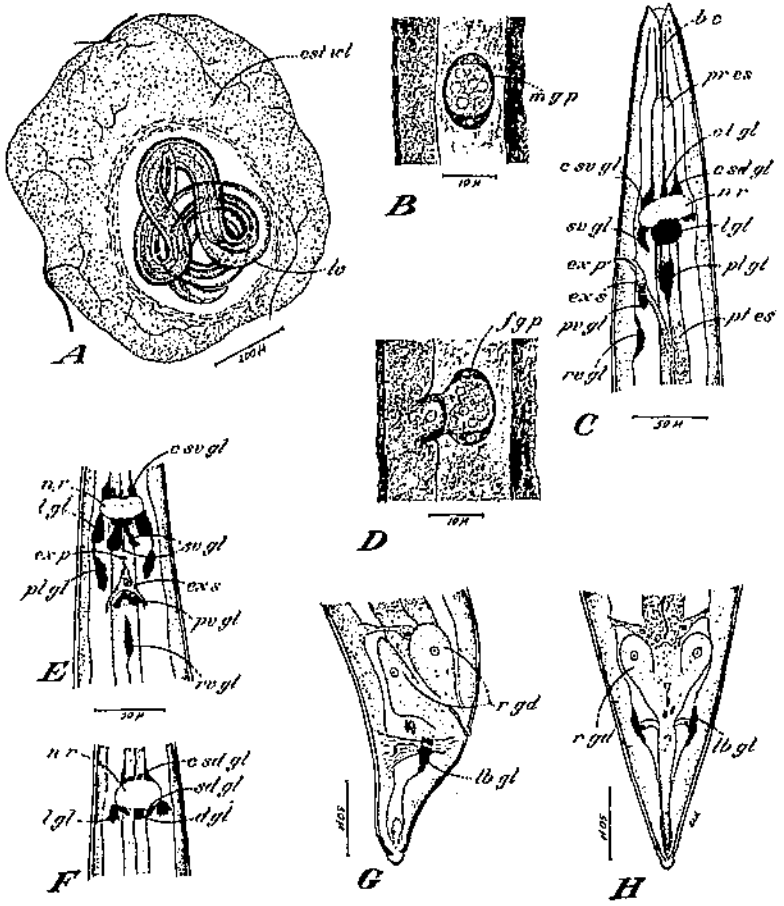

FIGURE 11.—THIRD-STAGE LARVAE OF ASCAROPS STRONGYLINA.

A, Encysted larva; *B*, portion of larva showing the male genital primordium; *C*, anterior portion of larva, lateral view; *D*, portion showing the female genital primordium attached to body wall, lateral view; *E*, anterior portion of larva showing features of nervous system, ventral view; *F*, region of nerve ring, dorsal view; *G*, posterior portion of larva, lateral view; *H*, posterior portion, ventral view.

wide, located ventrally between body wall and intestine, 600μ to 750μ from posterior end of body, composed of 2 large epithelial cap cells enclosing about 7 germinal cells. Female genital primordium also elliptical, 15μ to 18μ long by 9μ to 11μ wide, attached to body wall on ventral side by means of a large cell (fig. 11, *D*) 700μ to 835μ from tip of tail.

Table 8 shows the measurements of third-stage larvae of *Ascarops strongylina* in an intermediate host (*Aphodius granarius*).

TABLE 8.—*Principal measurements of 9 third-stage larvae of Ascarops strongylina at various periods of development in dung beetles*

Item	Period of development and measurements of larvae								
	Male no.—					Female no.—			
	1	2	3	4	5	1	2	3	4
Period of development.....days	29	29	35	35	40	35	35	40	40
Length of body.....millimeters	1.91	1.97	2.11	2.20	2.30	1.97	2.00	2.30	2.32
Maximum width of body.....microns	55	60	65	72	83	53	60	76	91
Length of buccal cavity.....do	53	53	60	64	60	45	.....	60	70
Length of procsophagus.....do	132	114	179	125	200	171	165	133	174
Length of postesophagus.....do	590	600	600	710	296	600	600	760	800
Distance of nerve ring from anterior end.....do	120	136	144	144	152	144	136	144	152
Distance of excretory pore from anterior end.....microns	182	190	190	195	205	150	150	159	205
Distance of cervical papillae from anterior end:									
Right papilla.....microns	182	180	.....	175	190	.....	159	170	195
Left papilla.....do	95	106	.....	106	126	.....	91	106	132
Distance of genital primordium from posterior end.....microns	670	600	750	700	700	700	700	820	835
Length of tail.....do	83	76	96	87	83	76	91	83	83

DIFFERENCES IN FIRST THREE STAGES

The outstanding differential features of the first three developmental stages of *Ascarops strongylina* are as follows:

First-stage larva.—Cuticle at anterior end apparently provided with 2 longitudinally arranged hooks; posterior to these hooks, about 17 parallel rows of very minute spines encircling the cephalic portion of larva; tip of tail conical.

Second-stage larva.—Cuticle without such armature as in previous stage; anterior and posterior ends bluntly rounded.

Third-stage larva.—Cuticle as in second-stage larva; anterior end with 2 dorsoventral elevations; tip of tail possessing a smooth knoblike process.

DEVELOPMENT IN INTERMEDIATE HOST

Eggs of *Ascarops strongylina* were obtained by chopping up gravid female worms in a few drops of distilled water. This material was transferred to small pieces of blotting paper and introduced into small glass tubes about 2 cm in diameter and 5 cm in height. In each of these tubes, there were placed six dung beetles (*Aphodius granarius*). The beetles had been collected from sheep manure, and when many of them were dissected previous to infection none were found to harbor a natural infestation with *Ascarops strongylina*. Glass tubes containing eggs and beetles were kept outdoors under shelter.

Beetles (*Aphodius granarius*), dissected 24 hours after they had been exposed to infective eggs, contained a few first-stage larvae in the abdominal portion of the body cavity. Young larvae, 1 to 2 days after infection, were 158μ to 160μ long by 9μ wide. About 15 days after infection, most first-stage larvae were found encysted in the walls of the Malpighian tubules. These cysts were usually spherical and thin-walled, and, besides the larva, a cyst contained several rounded bodies, possibly fat cells (fig. 10, *F*). At the end of about 17 days, several larvae were noted undergoing the first molt (fig. 10, *L*), and 2 days later several larvae were already in the second stage. Larvae undergoing the second molt (fig. 10, *H, I*) were found 28 days after experimental infection, and third-stage larvae

were found in beetles dissected 1 day later. During the development of the larva the cyst increased in size, being about 524μ to 936μ in its greater diameter and 420μ to 700μ in its lesser diameter when fully developed. Completely formed cysts were usually found free in the abdominal portion of the body cavity of the beetle, frequently being interlaced superficially by small tracheal tubules of the insect (fig. 11, *A*).

Observations on Effects of Environment

Resistance of Eggs to Low Temperatures

On February 17, 1933, about 1,000 eggs of *Ascarops strongylina* were mixed with sterile sand, and the mixture was placed in two small glass tubes. Enough water was added to this mixture to give the sand a moist appearance. These tubes were plugged with cotton and placed in a refrigerator. On March 9, 20 days later, the tubes were removed from the refrigerator. The eggs were separated from the sand, placed on pieces of blotting paper, and the blotting paper and eggs put in a tube containing several dung beetles (*Aphodius granarius*). These beetles were examined at intervals of from 10 to 20 days after their exposure to infestation, and each was found to contain several young larvae of *Ascarops strongylina*.

The temperature range during the 20 days' exposure in the refrigerator was as follows: Minimum, −4° C.; maximum, 2°. The total time during which the temperature was 2° was 48 hours; 1°, 24 hours; 0°, 48 hours; −1°, 24 hours; −2°, 216 hours; −3°, 24 hours; −4°, 76 hours. The eggs were thus kept at freezing or below-freezing temperatures for 85 percent of the entire period of refrigerator exposure without being killed.

Longevity of Eggs at Room Temperature

On February 17, several thousand eggs of *Ascarops strongylina* were mixed with sterile sand and put in two small glass tubes. These tubes were plugged with cotton and placed in a pint fruit jar. Water was put in the fruit jar in order to retain moisture in the jar and thus prevent drying of the sand. The jar was covered with moist paper hand towels, and a few small holes were made through the paper at the top of the jar. These jars were kept at room temperature (22° to 24° C.) until June 18, 1933, about 4 months, when the tubes were removed from the jar, and the eggs were separated from the sand and fed to dung beetles (*Aphodius granarius*). These beetles were examined 10 and 20 days later and each was found to contain several larvae of *Ascarops strongylina*. These observations show that eggs of *Ascarops strongylina* at room temperature are able to survive for a period of about 4 months.

PHYSOCEPHALUS SEXALATUS (MOLIN, 1860) DIESING, 1861

(Figs. 12–13)

Synonyms.—*Spiroptera sexalata* Molin, 1860; *S. strongylina suis labiata* Molin, 1860; *Filaria sexalata* (Molin (?), 1860) Perroncito, 1891; *S. strigis* (Linstow, 1877) Seurat, 1915; *Habronema sexalata* (Molin, 1860) Ostertag, 1932.

Hosts.—Definitive: Swine, wild boar, white-lipped peccary, tapir, cattle, horse, ass, dromedary. Intermediate: Coleoptera (*Canthon laevis, Geotrupes douei, G. stercorarius, G. stercorosus?, Gymnopleurus sturmi, G. sinnatus, Onthophagus bedeli, O. hecate, O. nebulosus, Phanaeus carnifex, P. vindex, Scarabaeus*

sacer, *S. variolosus*). Accidental: Mammals, birds, reptiles, and amphibians for third-stage larvae; the writer (*4*) has found these larvae encysted in the stomachs of bats captured in Washington, D. C.

In addition to the above intermediate hosts, the following Coleoptera have been found by the writer to serve as intermediate hosts for *P. sexalatus*: *Ataenius cognatus* and *Passalus cornutus*.

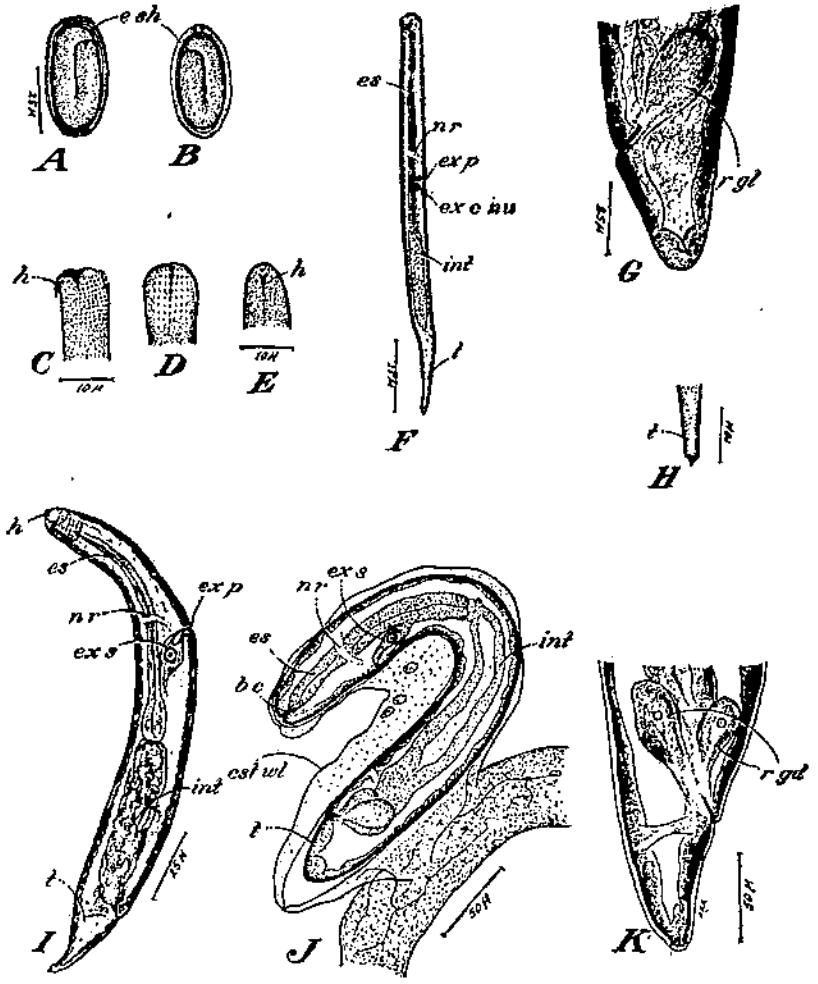

FIGURE 12.—VARIOUS STAGES IN THE DEVELOPMENT OF PHYSOCEPHALUS SEXALATUS

Egg: *A*, With fully developed shell and embryo; *B*, showing appearance of shell before it is fully formed.
First-stage larva: *C*, Anterior end, lateral view; *D*, anterior end, dorsal view; *E*, anterior end, ventral view; *F*, larva from intermediate host 2 days after experimental infection, lateral view; *H*, tail; *I*, larva from intermediate host 12 days after experimental infection; *J*, larva undergoing first molt.
Second-stage larva: *G*, Tail, lateral view; *K*, posterior portion of larva undergoing second molt.

Location.—Adults in stomach of definitive host; third-stage larvae in body cavity of intermediate host, and in wall of intestine or mesentery in accidental host.

Distribution.—Africa (Algeria, Madagascar), Asia (Indo-China), Australia, Europe (Italy, Germany, Rumania), Central America (Panama), North America (United States), South America (Brazil).

Description of Egg, Embryo, and Larval Stages

EGG

Egg (fig. 12, *A*) similar to that of *Ascarops strongylina*. Ciurea (*17*) described a pluglike protuberance at one pole of the egg; Foster (*26*) was unable to confirm Ciurea's finding. The writer's observation explains the discrepancy in the findings of the above investigators, since the pluglike protuberance is conspicuous in a shell not completely developed but is not recognizable in a well-developed shell (fig. 12, *A* and *B*). In a series of measurements involving about 50 eggs, length 41μ to 45μ, width 22μ to 26μ; according to Ciurea (*17*), length 39μ, width 17μ; according to Foster (*26*), length 34μ, width 15μ. Egg contains a well-developed embryo at time of oviposition.

EMBRYO

Embryos, obtained by crushing several eggs on a slide under a cover slip, 102μ to 107μ long by 6μ in maximum width. Embryo does not undergo further development until ingested by intermediate host. Morphology of embryo resembles that of young first-stage larva.

FIRST-STAGE LARVA

Shape and size.—First-stage larva similar in shape to that of *Ascarops strongylina* (fig. 12, *F*, *H*, and *I*); before molting sometimes attains a length of 448μ and a width of 38μ (table 9); according to Seurat (*117*), 420μ in length and 40μ in width.

Cuticle.—Cuticular structure and armature as in first-stage larva of *Ascarops strongylina*, but with the following differences: Posterior of two anterior hooks (fig. 12, *C* and *E*) about 3μ long, or about twice the length of corresponding hook of *A. strongylina*; rows of spines surrounding anterior portion of body about 15 in number and extending about 14μ from anterior end; spines of each row more widely spaced than corresponding spines of *A. strongylina*; each row of spines possessing one large spine dorsally (fig. 12, *C* and *D*), a feature lacking in *A. strongylina* (fig. 10, *C*).

Alimentary tract.—In general similar to that of first-stage larva of *Ascarops strongylina*. Esophagus 76μ to 144μ long, depending on degree of development in intermediate host.

Nervous system.—As in first-stage larva of *Ascarops strongylina*. Nerve ring, 42μ to 60μ from anterior end.

Excretory system.—Excretory pore 45μ to 68μ from anterior end, leading into a short dilated duct, this duct opening into a large excretory cell possessing a large nucleus.

Genital primordium.—In living specimens, hardly distinguishable from large muscle cells of body wall.

Table 9 shows the rate of development of first-stage larvae of *Physocephalus sexalatus* in the intermediate host (*Altaenius cognatus*), the measurements having been made on different days after experimental infection.

TABLE 9.—*Principal measurements of 5 first-stage larvae of Physocephalus sexalatus at various periods of development in dung beetles*

Item		Period of development and measurements of larva no.—				
		1	2	3	4	5
Period of development	days	2	2	10	10	[1] 16
Length of body	microns	121	136	152	155	448
Maximum width of body	do	8	6	11	11	38
Length of esophagus	do	76	76	82	80	144
Distance of nerve ring from anterior end	do	42	45	50	50	60
Distance of excretory pore from anterior end	do	45	48	55	55	68
Length of tail	do	20	24	28	28	34

[1] Larva undergoing first molt.

SECOND-STAGE LARVA

Shape and size.—Larva similar in shape to second-stage larva of *Ascarops strongylina*, but tip of tail more rounded in *Physocephalus sexalatus* (fig. 12, *G*). Larvae about 456μ to 1.3 mm long by 40μ to 60μ wide, the size depending on degree of development (table 10.)

Cuticle.—Cuticle without armature at anterior end; faint transverse striations present.

Alimentary tract.—In general similar to that of corresponding larvae of *Ascarops strongylina*; esophagus 146μ to 545μ long.

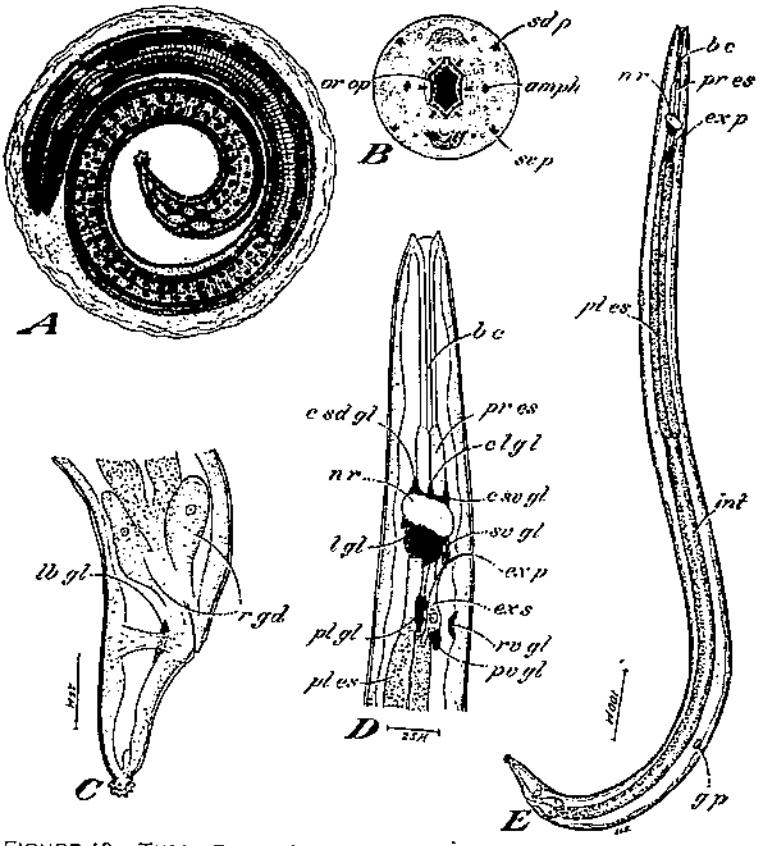

FIGURE 13.—THIRD-STAGE LARVAE OF PHYSOCEPHALUS SEXALATUS.

A, Encysted larva (from Hobmaier, 1925); *B*, anterior end of larva, en face view (from Chitwood, unpublished); *C*, posterior portion, lateral view (original); *D*, anterior portion, lateral view (original); *E*, lateral view of larva (original).

Nervous system.—Nerve ring, 60μ to 98μ from anterior end; details of this system similar in general to that of third-stage larva.

Excretory system.—As in previous stage. Excretory pore 68μ to 115μ from anterior end.

Genital primordium.—More easily recognized in older larvae of this stage, appearing as a small elliptical body; in a specimen 1.3 mm long, genital primordium 425μ from posterior end.

Table 10 shows the rate of development of second-stage larvae of *Physocephalus sexalatus* in the intermediate host (*Maenius cognatus*), the measurement having been made on different days after experimental infection.

TABLE 10.—*Principal measurements of 4 second-stage larvae of Physocephalus sexalatus at various periods of development in dung beetles*

Item	Period of development and measurements of larva no.—			
	1	2	3	4
Period of development.....days	20	20	26	[1] 34
Length of body.....microns	458	462	610	1,308
Maximum width of body.....do	40	40	48	60
Length of esophagus.....do	146	150	210	545
Distance of nerve ring from anterior end.....do	60	62	73	98
Distance of excretory pore from anterior end.....do	68	71	85	115
Distance of genital primordium from posterior end.....do				425
Length of tail.....do	30	38	48	70

[1] Larva undergoing second molt.

THIRD-STAGE LARVA

Shape and size.—General shape and structure (fig. 13, *A*-*E*) of larva similar to that of corresponding stage of *Ascarops strongylina*, with the exception of the position of cervical papilla and tip of tail. Cervical papilla (deirid) on right side of body opposite to excretory pore, 131μ to 170μ from anterior end; cervical papilla (deirid) on left side near region of base of buccal cavity, 68μ to 80μ from anterior end; tip of tail ending in a characteristic small knob, about 7μ to 8μ long, bearing about 20 to 23 small digitiform cuticular processes (fig. 13, *C*). Larvae 1.35 to 1.6 mm long by 60μ to 68μ wide; according to Seurat (*117*), 940μ to 1.81 mm. long by 75μ wide; according to Hobmaier (*52*), 1.3 to 1.35 mm long by 55μ wide.

Cuticle.—With prominent transverse striations.

Alimentary tract.—In general as in *Ascarops strongylina*, with the exception of the length of the buccal cavity. Buccal cavity comparatively long, 72μ to 106μ long (fig. 13, *D* and *E*); procesophagus 80μ to 102μ long; postesophagus 436μ to 585μ long, extending posteriorly almost to equator of body; rectum 34μ to 38μ long.

Nervous system.—General structure as in corresponding stage of *Ascarops strongylina* (fig. 13, *C*, *D*, and *E*). Nerve ring 110μ to 140μ from anterior end.

Excretory system.—Excretory pore 129μ to 167μ from anterior end; according to Seurat (*117*), 145μ from anterior end. Duct of excretory pore opening into a triangular excretory sinus; sinus walls containing a large nucleus.

Genital primordium.—As in *Gongylonema pulchrum* and *Ascarops strongylina*, sex can be differentiated at this stage. Male genital primordium elliptical in shape, about 15μ long and 9μ wide, located ventrally between body wall and intestine, 320μ to 340μ from posterior end; as in *A. strongylina*, composed of 2 large epithelial cells enclosing a group of germinal cells. Female genital primordium also somewhat elliptical, about 11μ long and 7μ wide, attached to body wall ventrally by means of a cell, 428μ to 460μ from tip of tail. Measurements given in table 11 indicate that the female genital primordium is closer to the posterior end of the larva than the genital primordium of the male.

Table 11 gives the measurements of third-stage larvae of *Physocephalus sexalatus* in an intermediate host (*Ataenius cognatus*).

TABLE 11.—*Principal measurements of 6 third-stage larvae of Physocephalus sexalatus at various periods of development in dung beetles*

Item	Period of development and measurements of larva no.					
	1[1]	2[1]	3[1]	4[2]	5[3]	6[1]
Period of development.....days	36	36	50	50	50	50
Length of body.....millimeters	1.35	1.40	1.42	1.45	1.59	1.60
Maximum width of body.....microns	60	60	62	60	66	68
Length of buccal cavity.....do	72	70	81	79	95	100
Length of procesophagus.....do	80	83	91	91	98	102
Length of postesophagus.....do	430	468	440	462	510	585
Distance of nerve ring from anterior end.....do	110	110	117	125	130	140
Distance of excretory pore from anterior end.....do	129	140	135	148	148	167
Distance of cervical papillae from anterior end:						
Right papilla.....do	131	140	138	118	150	170
Left papilla.....do	68	72	72	79	75	80
Distance of genital primordium from anterior end.....do	428	448	320	340	.....	460
Length of tail.....do	53	53	57	50	60	68

[1] Female larva. [2] Male larva. [3] Sex undetermined.

DIFFERENCES IN FIRST THREE STAGES

The outstanding differential features of the first three developmental stages of *Physocephalus sexalatus* are similar to those already noted for *Ascarops strongylina*, except that in the former the knob at the tip of the tail in the third-stage larva possesses several digitiform processes, whereas in the latter the knob is smooth.

DEVELOPMENT IN THE INTERMEDIATE HOST

Eggs of *Physocephalus sexalatus* were obtained and fed to dung beetles (*Ataenius cognatus*) as described for *Ascarops strongylina*. Beetles dissected 24 hours after experimental infection showed several newly hatched first-stage larvae in the body cavity. About 16 days after infection, first-stage larvae, about 448μ long, were found encysted in the Malpighian tubules of the beetles and were undergoing the first molt (fig. 12, *J*). The cyst wall at this time is very thin, and in addition to the larva, it encloses 2 or 3 small cells, probably fat cells. Second-stage larvae were noted 20 to 26 days after experimental infection; larvae undergoing the second molt were noted in beetles 34 days after infection; third-stage larvae were noted in beetles 36 days after infection. During the development of the larva, the cyst increased in size, being about 300μ to 650μ in greater diameter and 420μ to 700μ in lesser diameter at the time the cyst was fully developed (fig. 13, *A*). Completely formed cysts were found either attached to Malpighian tubules or free in the abdominal portion of the body cavity, frequently interlaced superficially by small tracheal tubules of the insect.

Hobmaier (*52*) in his study of the larval stages of *Physocephalus sexalatus*, shows in his figures that the larva molts four times in the intermediate host; Seurat (*115, 116, 117*) and the writer find that the larva undergoes only two molts in the intermediate host. The latter observations are in harmony with the known facts of nematode development.

OBSERVATIONS ON EFFECTS OF ENVIRONMENT

RESISTANCE OF EGGS TO LOW TEMPERATURES

On February 17, 1933, about 1,000 eggs of *Physocephalus sexalatus* were mixed with sterile sand, and the mixture was placed in two small glass tubes. These tubes were plugged with cotton and placed in a refrigerator. On March 9, 20 days later, the tubes were removed from the refrigerator. The eggs were separated from the sand, placed on pieces of blotting paper, and the blotting paper and eggs put in a tube containing several dung beetles (*Ataenius cognatus*). These beetles were examined at various intervals from 10 to 20 days after exposure to infection, and each was found to contain several young larvae of *Physocephalus sexalatus*.

The temperature range during the 20 days' exposure in the refrigerator was as follows: Minimum, —4° C; maximum, 2°. The total time during which the temperature was 2° was 48 hours; 1°, 24 hours; 0°, 48 hours; —1°, 24 hours; —2°, 216 hours; —3°, 24 hours; —4°, 76 hours. The eggs were thus kept at freezing or below-freezing temperatures for about 85 percent of the entire period of refrigerator exposure without being killed.

METASTRONGYLIDAE

METASTRONGYLUS SALMI GEDOELST, 1923

(Figs. 14–15)

Synonym.—*Metastrongylus elongatus* Salm, 1918, not Railliet and Henry, 1911.
Hosts.—Definitive: Swine. Intermediate: *Lumbricus terrestris* and *Helodrilus caliginosus* var. *trapezoides*.
Location.—Adults in trachea, bronchi, and bronchioles of definitive host; third-stage larvae in circulatory system and in walls of alimentary tract (usually esophagus) of intermediate host.
Distribution.—Africa (Belgian Congo), Asia (Java, Philippine Islands), Europe (Spain), and North America (United States).

Description of Egg, Embryo, and Larval Stages

EGG

Eggshell thick and elliptical in shape, possessing rough surface with the appearance of small mammillations (fig. 14, *A*); incompletely developed eggshell showing a thin covering, and within it an inner vitelline membrane enclosing embryo (fig. 14, *B*). Before the egg is deposited by the female worm the shell appears to undergo considerable hardening and contraction, this process possibly giving rise to the unevenness found on the surface of the fully developed eggshell. In a series of measurements involving about 50 fully developed eggs, length 43μ to 57μ, width 38μ to 41μ; according to Gedoelst (*32*), length 52.5μ to 55.5μ, width 33μ to 40μ. Egg contains a well-developed embryo at time of oviposition.

The eggs of *Metastrongylus salmi*, as well as those of *M. elongatus* and *Choerostrongylus pudendotectus* discussed in this bulletin, contrary to reports of some other investigators, usually pass out of the host unhatched. Hatching usually does not take place until the eggs are taken into the body of a susceptible intermediate host.

EMBRYO

Embryos, obtained by pressing several embryonated eggs under cover slip, 275μ to 295μ long by 12μ in maximum width; possess numerous and somewhat large granules; usually tightly coiled within eggshells as shown in figure 14, *A*. When embryo is mechanically removed from eggshell, posterior half of body is coiled ventrad, giving embryo appearance of open figure 6 (fig. 14, *D*). Embryo as found in egg in freshly passed feces of swine usually does not leave eggshell nor undergo additional development before being ingested by intermediate host.

FIRST- AND SECOND-STAGE LARVAE

First three larval stages of this parasite not distinctly separated from one another as are those of most strongyle larvae. Soon after cuticle of first molt becomes separated from anterior end of body, a second molt is evident (fig. 14, *E, G*). First cuticle usually shed before cuticle of second molt becomes completely detached from body of larva; second cuticle is, however, retained throughout life of larva in intermediate host; period of second stage is then possibly represented by short period from time that first and second molts are evident, that is, second stage is represented by a molt, but is otherwise more or less suppressed so far as a distinct existence for a definite period is concerned. Differentiation of various larval molts in *Metastrongylus salmi* involves essentially the same features as those pointed out by Schwartz and Alicata (*111*) for larvae of *M. elongatus* and *Choerostrongylus pudendotectus*.
Shape and size.—Larvae of the first and second stages slender, with tapering anterior and posterior portions. Anterior end (fig. 14, *C*) of young first-stage larva rounded and slightly set off by a small constriction, which is not evident in older larva of first stage. In first-stage larva, posterior end (fig. 14, *D*) has a broad rounded tip turned ventrad; after the first molt, tip of tail more pointed (fig. 14, *F*). First-stage larvae 1 day after infection, 275μ to 300μ long by 12μ wide; at time of first molt, larvae 500μ to 525μ long by 22μ to 26μ wide; undergoing second molt, 550μ to 610μ long by 26μ to 28μ wide (table 12).

Cuticle.—Very thin, transparent, with fine transverse striations; cuticle, in contrast with that of other heteroxenous nematodes discussed in this bulletin, with no armature.

Alimentary tract.—Oral opening leading into a short tripartite buccal cavity lined with three longitudinally arranged annules; these annules best distinguished in third-stage larva (fig. 15, *D*). Esophagus 110μ to 160μ long, slender, with a

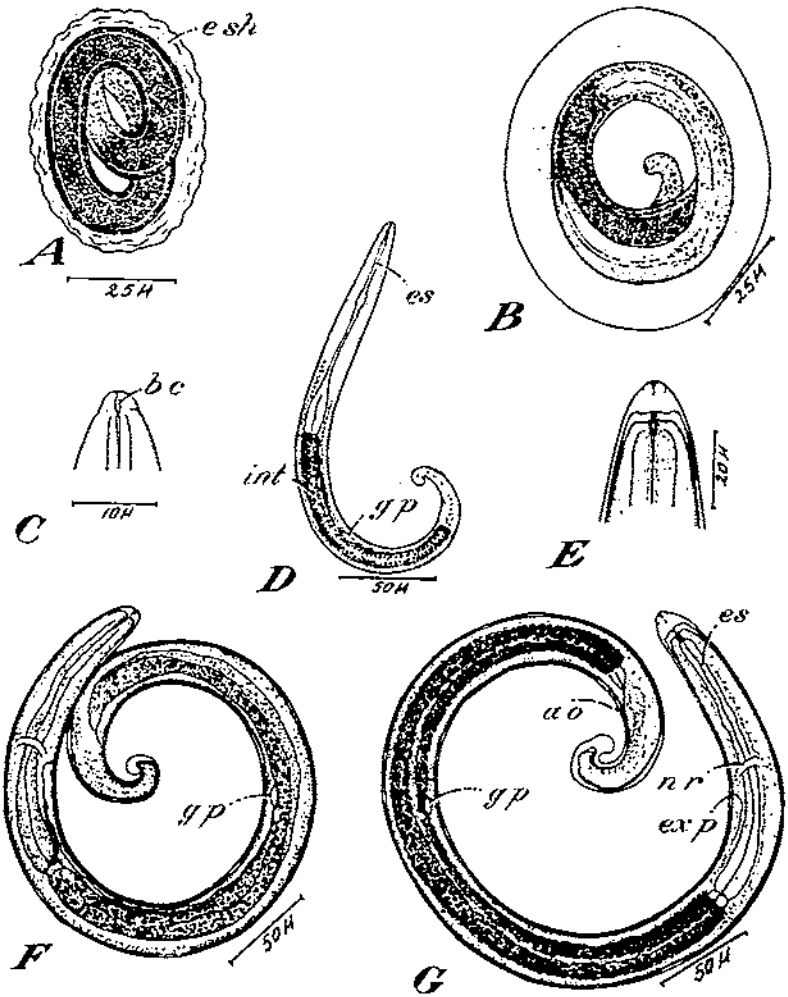

FIGURE 14.—VARIOUS STAGES IN THE DEVELOPMENT OF METASTRONGYLUS SALMI.

Egg: *A*, With fully developed embryo; *B*, showing incompletely formed shell.
First-stage larva: *C*, Anterior portion; *D*, newly hatched larva; *F*, larva undergoing first molt.
Second-stage larva: *E* and *G*, On verge of second molt while still enclosed within the cuticle of first molt.

distinct swelling at its anterior third (fig. 14, *D*). Intestine slender, its walls closely packed with large dark-brown granules; intestine connecting with a short and narrow rectum.

Nervous system.—Larva stained with aqueous methylene blue, showing nerve ring 50μ to 72μ from anterior end; nerve ring surrounded by several nuclei of nerve cells.

Excretory system.—Excretory pore 58μ to 80μ from anterior end; its duct connecting posteriorly with excretory glands.

Genital primordium.—Represented by small ellipsoidal group of cells, ventral in position, 160μ to 330μ from anterior end.

TABLE 12.—*Principal measurements of 10 first- and second-stage larvae of Metastrongylus salmi at various periods of development in earthworms*

Item	Period of development and measurements of larva no.—									
	1	2	3	4	5	6	7	8	9	10
Period of development ..... days..	1	1	8	[1] 13	[1] 13	[1] 13	[2] 15	[2] 15	[2] 15	[2] 15
Length of body ..... microns.	275	300	380	500	520	525	550	585	600	610
Maximum width of body ..... do.	12	12	18	22	26	26	26	26	28	28
Length of esophagus ..... do.	110	112	115	120	130	130	145	150	155	160
Distance of nerve ring from anterior end ..... microns	50	50	55	60	70	70	68	72	72	72
Distance of excretory pore from anterior end ..... microns	58	60	65	72	72	78	78	80	80	80
Distance of genital primordium from anterior end ..... microns.	160	164	200	280	285	285	290	318	325	330
Length of tail ..... do.	28	28	34	38	40	45	48	48	48	50

[1] Larva undergoing first molt.
[2] Larva undergoing second molt.

Table 12 shows the rate of development of *Metastrongylus salmi* in the intermediate host (*Helodrilus caliginosus* var. *trapezoides*), the measurements having been made on different days after experimental infection.

THIRD-STAGE LARVA

Shape and size.—Shape of larva, mostly as in previous stages (fig. 15, *E*). Each lateral half of head, in en face view, apparently surrounded by 3 small elevations (fig. 15, *C*), probably representing the beginning of the formation of the 2 lateral trilobed lips of the adult worm. Head with an outer circle of 2 subdorsal and 2 subventral papillae and 2 lateral amphids; an inner circle of 2 subdorsal and 2 subventral small papillae also apparently present. Tail tapering, terminating in a pointed tip; just above tip, lateral view, 2 dorsal notches somewhat indistinct (fig. 15, *B* and *F*). Larvae 550μ to 630μ long, enclosed in sheath of last molt.

Cuticle.—With prominent transverse striations.

Alimentary tract.—Oral opening leading into a tripartite buccal cavity about 5μ long, lined with three longitudinally arranged annules (fig. 15, *D*). Esophagus strongyliform, 150μ to 175μ long. Intestine slender, lying, for the most part, close to ventral side of body wall, and opening into a narrow rectum about 22μ long.

Nervous system.—Nerve ring 64μ to 72μ from anterior end of body. In stained specimens, 6 nerve strands anterior to nerve ring, forming papillary nerves, 2 subdorsal, 2 subventral, and 2 lateral. Posterior to nerve ring, 2 large lateral ganglia, each connected posteriorly to the posterolateral ganglion, the latter extending midway between nerve ring and end of esophagus. Dorsally and ventrally, groups of nuclei of nerve cells, possibly representing dorsal and ventral ganglia. Excretory duct surrounded by a group of nuclei of nerve cells; slightly posterior to it, retrovesicular ganglion represented by a group of nuclei of nerve cells.

Excretory system.—Excretory pore 72μ to 80μ from anterior end, leading into long excretory glands extending posterior to rectum (fig. 15, *E*).

Genital primordium.—In about same position as in previous stages, 300μ to 345μ from anterior end.

Table 13 shows the measurements of third-stage larvae of *Metastrongylus salmi* in an intermediate host (*Helodrilus caliginosus* var. *trapezoides*).

TABLE 13.—*Principal measurements of 5 third-stage larvae of Metastrongylus salmi at various periods of development in earthworms*

Item		Period of development and measurements of larva no.—				
		1	2	3	4	5
Period of development	days	18	18	30	30	30
Length of body	microns	550	550	550	575	630
Maximum width of body	do	26	26	26	26	26
Length of esophagus	do	170	170	150	160	175
Distance of nerve ring from anterior end	do	64	64	64	68	72
Distance of excretory pore from anterior end	do	72	78	76	78	80
Distance of genital primordium from anterior end	do	315	305	300	325	345
Length of tail	do	50	40	40	40	45

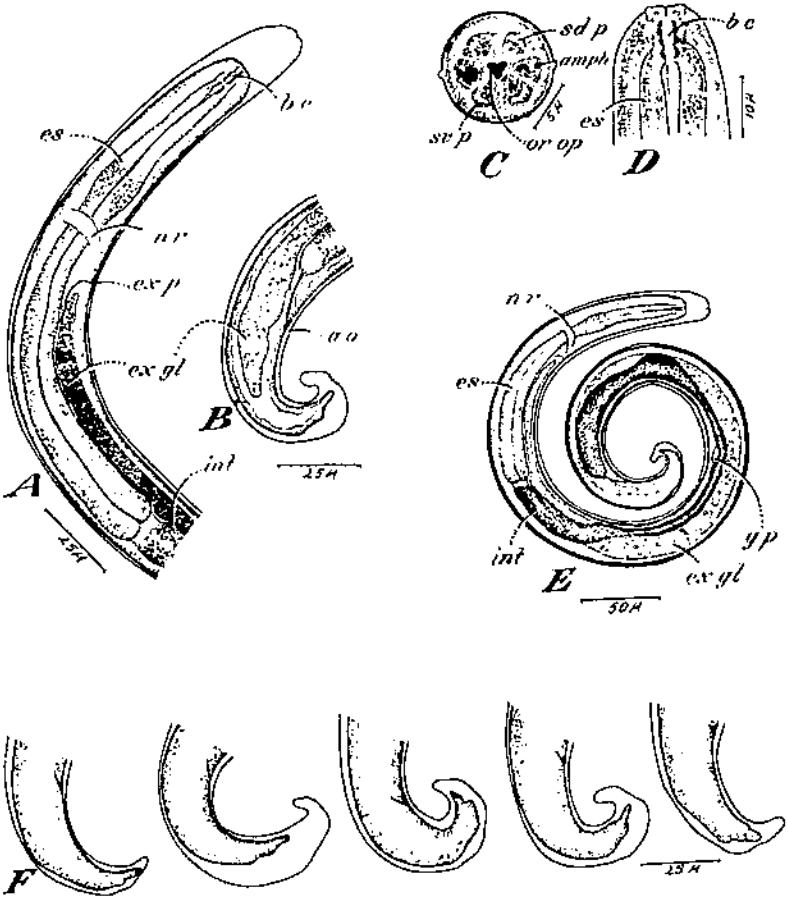

FIGURE 15.—THIRD-STAGE LARVAE OF METASTRONGYLUS SALMI

A, Anterior portion, lateral view; *B*, posterior portion, lateral view; *C*, anterior end, en face view; *D*, anterior end, lateral view; *E*, lateral view of larva; *F*, variations noted in tails

DIFFERENCES IN FIRST THREE STAGES

The outstanding differential features of the first three developmental stages of *Metastrongylus salmi* are as follows:

First-stage larva.—About 275μ to 380μ long by 12μ to 18μ wide; anterior end conical and somewhat set off by a small constriction; cuticle at anterior end conical when loosened in preparation for first molt.

Second-stage larva.—About 500μ to 525μ long by 22μ to 26μ wide; anterior end slightly flattened.

Third-stage larva.—About 550μ to 630μ long, enclosed in a sheath formed by cuticle of second stage; sheath flattened at anterior end (fig. 14, *E*); tail with 2 somewhat indistinct dorsal notches when viewed laterally.

Development in Intermediate Host

Eggs of *Metastrongylus salmi* were obtained by chopping up gravid female worms in a few drops of distilled water. This material was then transferred to a 250-cc beaker and mixed with a small quantity of soil; several live earthworms (*Helodrilus caliginosus* var. *trapezoides*) were put in this soil. In order that the earthworms used should be free from natural infestation with this parasite, they were collected from the yard of a city dwelling. Earthworms were allowed to remain overnight in the soil containing the lungworm eggs and were then transferred to a beaker containing soil free from lungworm eggs. These experiments were carried out at room temperatures (22° to 24° C.).

Earthworms dissected about 30 hours after being exposed to infection in soil contained several first-stage larvae in the wall of the esophagus, especially the posterior part of this organ. In earthworms dissected about 13 days after infection, several larvae were undergoing the first molt (fig. 14, *F*); earthworms dissected 2 days later contained larvae in the process of the second molt, while still enclosed in the cuticle of the first molt (fig. 14, *E* and G), the larvae having 2 sheaths at this time. Completely developed third-stage larvae were recovered from the esophageal wall and hearts of earthworms 18 days after infection.

Observations on Effects of Environment

Longevity of third-stage larvae in body of intermediate host

On March 24, 1933, 4 earthworms which were experimentally infected with *Metastrongylus salmi* were placed in a large, tightly nailed, wooden box containing soil. This box was placed outdoors, partially underground, and was covered to prevent rain from falling upon it. On July 24, 4 months later, only 1 earthworm was found in the box; apparently the others had died. This earthworm was found to harbor 4 third-stage larvae of *Metastrongylus salmi*, 1 larva in the esophageal wall, and 3 larvae in one of the hearts. These larvae, after being isolated from the earthworm and placed in water, showed considerable activity. The above experiment shows that larvae of *Metastrongylus salmi* may remain in the body of the intermediate host for at least 4 months.

METASTRONGYLUS ELONGATUS (DUJARDIN, 1845) RAILLIET AND HENRY, 1911

(Figs. 16-17)

Synonyms.—*Gordius pulmonalis apri* Ebel, 1777; *Ascaris apri* Gmelin, 1789; *A. filiformis* Schrank, 1788; *Fusaria apri* Zeder, 1803; *Strongylus suis* Rudolphi, 1809; *S. paradoxus* Mehlis, 1831; *S. elongatus* Dujardin, 1845; *S. longivaginatus* Diesing, 1851; *Metastrongylus paradoxus* (Mehlis, 1831) Molin, 1860; *Filaria trachealis* Cobbold, 1864; *S. apri* (Gmelin, 1789) R. Blanchard, 1895; *Cloacina octodactyla* Linstow, 1906; *M. apri* (Gmelin, 1789) Railliet and Henry, 1907.

Hosts.—Definitive: Swine, cattle, sheep, goat, deer, roe deer, man, and, by experimental feeding, dog, as reported by the writer (*6*). Intermediate: *Helodrilus foetidus, H. caliginosus* var. *trapezoides, Lumbricus terrestris, L. rubellus, Bimastus tenuis.*

Location.—Adults in trachea, bronchi, and bronchioles of definitive host; third-stage larvae in circulatory system or wall of intestine or, usually, esophagus of intermediate host.

Distribution.—Africa (Belgian Congo), Asia (Annam, China, Japan), Australia, Europe, North America (British West Indies, Puerto Rico, Mexico, United States), South America (Argentina).

Description of Egg, Embryo, and Larval Stages

EGG

As in *Metastrongylus salmi*, egg, fully developed, with thick shell, elliptical in shape, and with a corrugated surface (fig. 16, *A*). In a series of measurements involving about 50 eggs, length 45μ to 57μ, width 38μ to 41μ; according to Gedoelst (*83*), length 51μ to 54μ, width 33μ to 36μ; according to Zebrowski (*140*), length 50μ to 80μ. Egg contains a well-developed embryo at time of oviposition.

EMBRYO

Embryos similar to those of *Metastrongylus salmi* (fig. 16, *B*), 275μ to 305μ long by 12μ wide.

FIRST- AND SECOND-STAGE LARVAE

Shape and size.—Shape of these larvae same as in corresponding stages of *Metastrongylus salmi*. Newly hatched larvae 275μ to 305μ long by 12μ wide; at time of first molt, 520μ to 540μ long by 22μ wide; undergoing second molt, 560μ to 650μ long by 26μ to 28μ wide (table 14).

Cuticle.—Thin, with fine transverse striations.

Alimentary tract.—Morphologically, as in *Metastrongylus salmi;* esophagus 114μ to 160μ long.

Nervous system.—As in *Metastrongylus salmi;* nerve ring 52μ to 76μ from anterior end.

Excretory system.—Excretory pore 58μ to 86μ from anterior end, its duct connecting posteriorly with excretory glands.

Genital primordium.—Small, ellipsoidal, and ventral in position, 160μ to 365μ from anterior end.

Table 14 shows the rate of development of first- and second-stage larvae of *Metastrongylus elongatus* in an intermediate host (*Helodrilus caliginosus* var. *trapezoides*), the measurements having been made at different days after experimental infection.

TABLE 14.—*Principal measurements of 9 first- and second-stage larvae of Metastrongylus elongatus at various periods of development in earthworms*

Item	Period of development and measurements of larva no.—								
	1	2	3	4	5	6	7	8	9
Period of development.....days.	1	1	9	[1] 15	[1] 15	[2] 19	[2] 19	[2] 19	[2] 19
Length of body.....microns.	275	305	392	520	540	560	600	645	650
Maximum width of body.....do.	12	12	18	22	22	26	28	28	28
Length of esophagus.....do.	114	117	120	130	138	145	148	158	160
Distance of nerve ring from anterior end microns.	52	52	56	68	68	68	72	76	76
Distance of excretory pore from anterior end microns.	58	64	68	72	78	80	82	86	86
Distance of genital primordium from anterior end.....microns.	160	164	210	285	280	298	328	368	365
Length of tail.....do.	28	28	34	38	38	38	40	48	50

[1] Larva undergoing first molt.
[2] Larva undergoing second molt.

THIRD-STAGE LARVA

Shape and size.—Shape of larva resembles that of corresponding stage of *Metastrongylus salmi* (fig. 16, *D–G*); in lateral view of posterior portion, notches

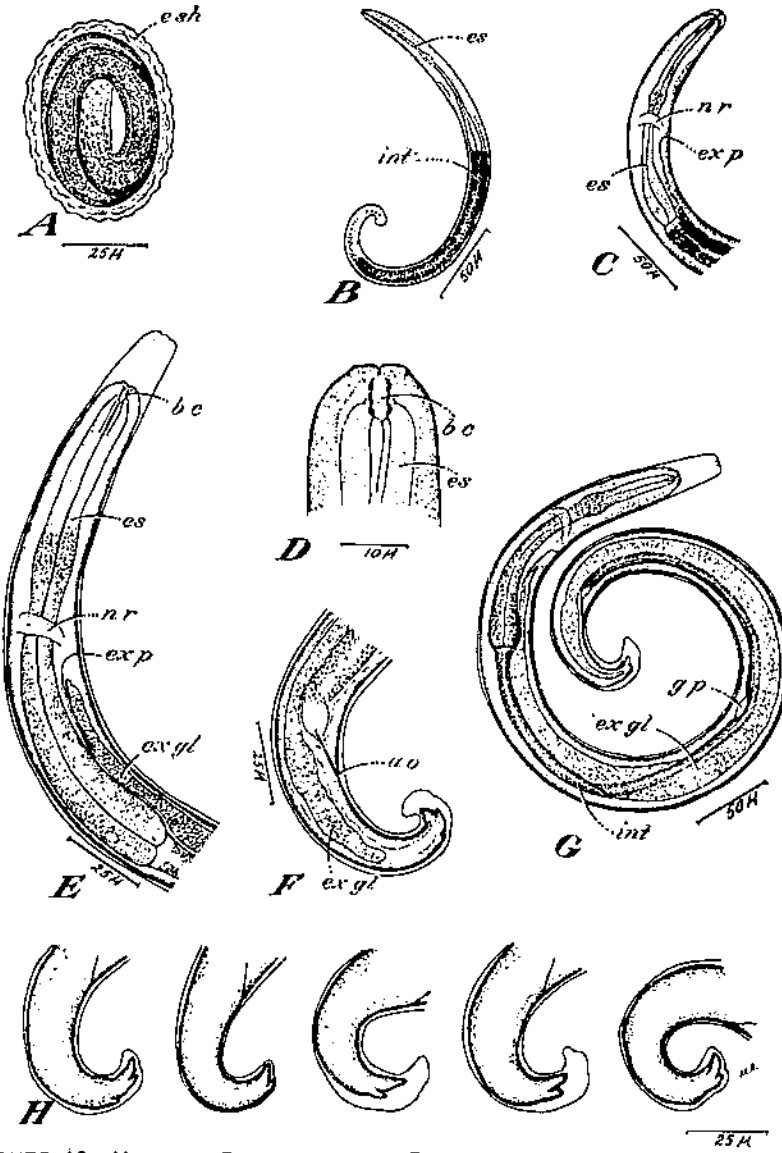

FIGURE 16.—VARIOUS STAGES IN THE DEVELOPMENT OF METASTRONGYLUS ELONGATUS.

A, Egg with fully developed embryo.
First-stage larva: *B*, Newly hatched; *C*, anterior portion of larva undergoing first molt.
Third-stage larva: *D*, Anterior end, lateral view; *E*, anterior portion, lateral view; *F*, posterior portion, lateral view; *G*, lateral view of larva; *H*, variations noted in tails.

near tip of tail usually more prominent in *M. elongatus* (fig. 16, *H*) than in *M. salmi*. Larvae 625μ to 665μ long by 26μ wide, enclosed in sheath of last molt.

Cuticle.—With prominent transverse striations.

Alimentary tract.—Morphologically, as in *Metastrongylus salmi;* esophagus 155μ to 177μ long.

Nervous system.—In general, as in *Metastrongylus salmi;* nerve ring 72μ to 80μ from anterior end.

Excretory system.—Excretory pore 80μ to 87μ from anterior end, leading into long excretory glands extending posterior to rectum (fig. 16, *G*).

Genital primordium.—Position resembles that of previous stages; 340μ to 390μ from anterior end.

Table 15 gives the measurements of third-stage larvae of *Metastrongylus elongatus* in an intermediate host (*Helodrilus caliginosus* var. *trapezoides*).

TABLE 15.—*Principal measurements of 5 third-stage larvae of Metastrongylus elongatus at various periods of development in earthworms*

Item	Period of development and measurements of larva no.—				
	1	2	3	4	5
Period of development.....days...	20	20	28	28	28
Length of body.....microns...	625	630	650	650	665
Maximum width of body.....do....	26	26	26	26	26
Length of esophagus.....do....	155	160	160	160	177
Distance of nerve ring from anterior end.....do....	72	72	75	75	80
Distance of excretory pore from anterior end.....do....	80	87	87	87	84
Distance of genital primordium from anterior end.....do....	340	360	390	390	390
Length of tail.....do....	60	60	62	60	60

DIFFERENCES IN FIRST THREE STAGES

The outstanding differential features of the three developmental stages of *Metastrongylus elongatus* are as given for *M. salmi.*

DEVELOPMENT IN INTERMEDIATE HOST

Earthworms (*Helodrilus caliginosus* var. *trapezoides*) were exposed to soil containing eggs of *Metastrongylus elongatus,* in the same manner as described for *M. salmi.* Earthworms dissected about 16 hours after being exposed to infected soil contained several first-stage larvae in the wall of the esophagus. In earthworms dissected 15 days after infection, several larvae were undergoing the first molt (fig. 16, *C*). Earthworms dissected 4 days later contained larvae in the process of shedding the second cuticle, while still enclosed in the cuticle of the first molt. Completely developed third-stage larvae were recovered from the esophagus and hearts of earthworms 20 days after infection (fig. 17, *A* and *B*).

Occasionally, larvae which had not yet undergone the first molt were found in the circulatory system, especially in the hearts of the earthworm. In this connection Schwartz and Alicata (*109*) pointed out that the migration of the larvae of *Metastrongylus elongatus* and *Choerostrongylus pudendotectus* in the blood vessels of the intermediate host was not an essential feature in the development of the larvae, since complete larval development may take place in the walls of the digestive tract.

The time of occurrence of the various molts seems variable; Hobmaier and Hobmaier (*49*) in referring to the development of *Metastongylus elongatus* in earthworms, state that a molt took place 10 days after infection. Schwartz and Alicata (*109*) reported that evidence of first and second molts was found on the eighth and ninth days, respectively, after infection.

OBSERVATIONS ON EFFECTS OF ENVIRONMENT

On December 20, 1932, 15 earthworms (*Helodrilus caliginosus* var. *trapezoides*) which had been experimentally infected with *Metastrongylus elongatus* were placed in a box outdoors, as described for *M. salmi*. On September 20, 1933, 9 months later, only 2 earthworms were recovered from the box; apparently the others had died. One of these earthworms harbored 2 larvae in the esophageal wall and 3 larvae coiled in one of the hearts; the other earthworm harbored 2

FIGURE 17.—*A*, Esophagus of earthworm containing larvae of *Metastrongylus elongatus* (indicated by arrows) in hearts and esophageal wall 30 days after experimental infection; *B*, heart of earthworm enclosing larvae of *M. elongatus* (indicated by arrows).

larvae in the hearts. These larvae showed considerable activity when isolated and placed in water. These observations show that third-stage larvae of *M. elongatus* were able to survive in the tissues of the intermediate host for at least 9 months, including the winter and summer seasons.

CHOEROSTRONGYLUS PUDENDOTECTUS (WOSTOKOW, 1905) SKRJABIN, 1924

(Fig. 18)

Synonyms.—*Metastrongylus pudendotectus* Wostokow, 1905; *M. brevivaginatus*, Railliet and Henry, 1907; *Choerostrongylus brevivaginatus* (Railliet and Henry, 1907) Gedoelst, 1923.

Hosts.—Definitive: Swine. Intermediate: *Helodrilus foetidus*, *H. caliginosus* var. *trapezoides*, *Lumbricus terrestris*, *L. rubellus*.

Location.—Adults in trachea, bronchi, and bronchioles of definitive host; third-stage larvae in circulatory system and in walls of alimentary tract (usually esophagus) of intermediate host.

Distribution.—Africa (Belgian Congo), Asia (Annam), Europe, North America (British West Indies, United States), and South America (Argentina).

Description of Egg, Embryo, and Larval Stages

EGG

As in *Metastrongylus salmi*, egg fully developed with thick shell, elliptical in shape, and with a corrugated surface (fig. 18, *B*). In a series of measurements involving about 50 eggs, length 60μ to 64μ, width 43μ to 45μ; according to Gedoelst (*82*), length 57μ to 63μ, width 39μ to 42μ; according to Zebrowski (*140*), length 70μ to 100μ, width 50μ to 80μ. Zebrowski's measurements indicate that he was measuring eggs with incompletely developed shells. Egg contains a well-developed embryo at time of oviposition.

EMBRYO

Embryos similar to those of *Metastrongylus salmi* (fig. 18, *D*), 300μ to 315μ long by 12μ to 14μ wide.

FIRST- AND SECOND-STAGE LARVAE

Shape and size.—Shape of these larvae similar to that of corresponding stage of *Metastrongylus salmi* (fig. 18, *D*). Larvae newly hatched, 300μ to 315μ long by 12μ to 14μ wide; at time of first molt, 525μ to 548μ long by 22μ wide; undergoing second molt, 580μ to 630μ long by 26μ to 28μ wide (table 16).

Cuticle.—With fine transverse striations.

Alimentary tract.—Morphologically, as in *Metastrongylus salmi;* esophagus, 117μ to 160μ long.

Nervous system.—Nervous system as in *Metastrongylus salmi;* nerve ring, 52μ to 76μ from anterior end.

Excretory system.—Excretory pore 64μ to 86μ from anterior end, its duct connecting posteriorly with excretory glands.

Genital primordium.—Small and ellipsoidal, 164μ to 355μ from anterior end.

Table 16 shows the rate of development of first- and second-stage larvae of *Choerostrongylus pudendotectus* in an intermediate host (*Helodrilus caliginosus* var. *trapezoides*), the measurements having been made on different days after experimental infection.

TABLE 16.—*Principal measurements of 8 first- and second-stage larvae of Choerostrongylus pudendotectus at various periods of development in earthworms*

Item	Period of development and measurements of larva no.							
	1	2	3	4	5	6	7	8
Period of development.....days	1	1	9	[1] 14	[1] 15	[2] 18	[2] 18	[2] 18
Length of body.....microns	300	315	385	525	548	580	590	630
Maximum width of body.....do	12	14	18	22	22	26	28	28
Length of esophagus.....do	117	120	122	125	130	140	140	160
Distance of nerve ring from anterior end...do	52	54	50	60	70	70	72	76
Distance of excretory pore from anterior end...do	64	70	72	72	76	80	82	86
Distance of genital primordium from anterior end.....microns	164	170	215	200	296	315	330	355
Length of tail.....do	28	30	34	38	40	40	45	50

[1] Larva undergoing first molt.
[2] Larva undergoing second molt.

THIRD-STAGE LARVA

Shape and size.—Larvae resembling in shape those of corresponding stages of *Metastrongylus salmi* (fig. 18, *C* and *E*). In lateral view, notches on tip of tail usually as in *M. salmi*, but not so prominent as those of *M. elongatus* (fig. 18, *G*), the findings of the writer being contrary to those of Hobmaier and Hobmaier (*51*), who state that the notches at the tip of the tail of *Choerostrongylus pudendotectus*

are deeper than those of *Metastrongylus elongatus*. Larvae 600μ to 655μ long by 26μ wide, enclosed in sheath of last molt.

Cuticle.—With prominent transverse striations.

Alimentary tract.—Morphologically similar to that of corresponding stage of *Metastrongylus salmi;* esophagus, 160μ to 180μ long.

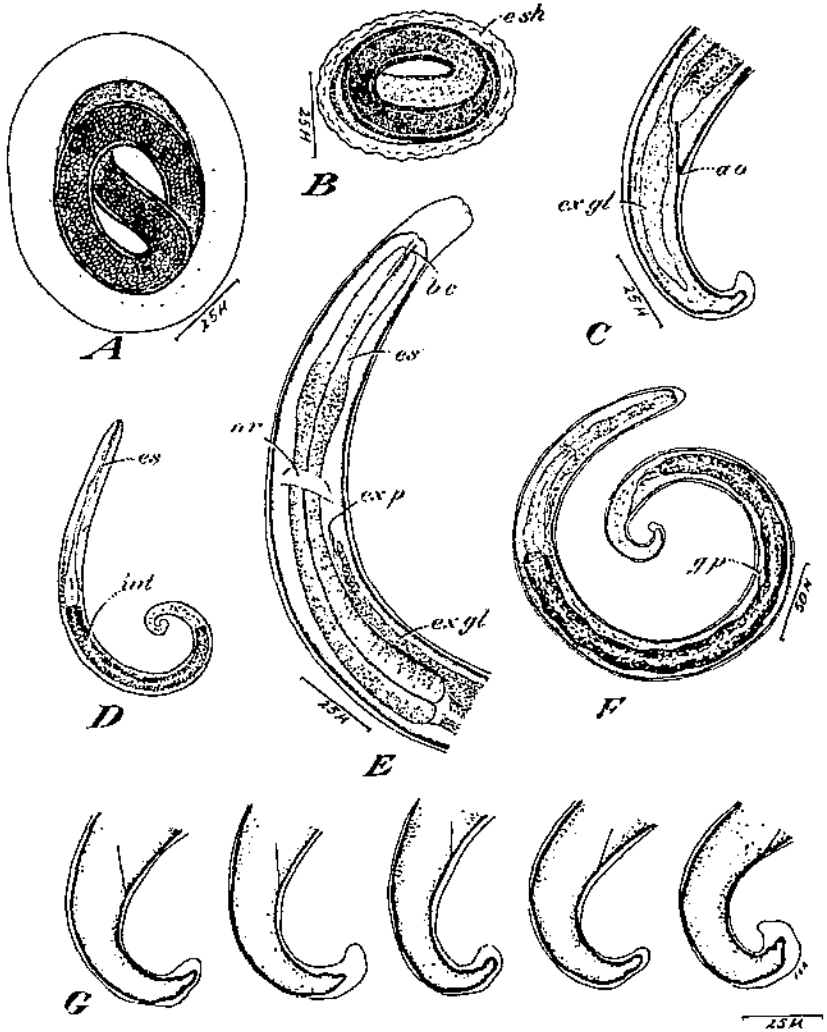

FIGURE 18.—VARIOUS STAGES IN THE DEVELOPMENT OF CHOEROSTRONGYLUS PUDENDOTECTUS.

Egg: *A*, Showing incompletely formed shell; *B*, with fully developed shell and embryo.
First-stage larva: *D*, Newly hatched; *F*, undergoing first molt.
Third-stage larva: *C*, Posterior portion, lateral view; *E*, anterior portion, lateral view; *G*, variations noted in tails.

Nervous system.—In general, as in *Metastrongylus salmi;* nerve ring 72μ to 82μ from anterior end.

Excretory system.—Excretory pore 84μ to 87μ from anterior end, leading into long excretory glands extending posterior to rectum (fig. 18, *C* and *E*).

Genital primordium.—In approximately the same position as in previous stage; 342μ to 390μ from anterior end.

Table 17 shows the measurements of third-stage larvae of *Choerostrongylus pudendotectus* in an intermediate host (*Helodrilus caliginosus* var. *trapezoides*).

TABLE 17.—*Principal measurements of 5 third-stage larvae of Choerostrongylus pudendotectus at various periods of development in earthworms*

Item	Period of development and measurements of larva no.—				
	1	2	3	4	5
Period of development.....days..	19	19	19	25	25
Length of body.....microns..	600	625	640	650	655
Maximum width of body.....do....	26	26	26	26	26
Length of esophagus.....do....	160	160	170	175	180
Distance of nerve ring from anterior end.....do....	72	75	75	80	82
Distance of excretory pore from anterior end.....do....	84	84	87	87	87
Distance of genital primordium from anterior end.....do....	342	360	380	390	390
Length of tail.....do....	55	60	55	60	62

The outstanding differential features of the three developmental stages of *Choerostrongylus pudendotectus* are as given for *Metastrongylus salmi*.

DEVELOPMENT IN INTERMEDIATE HOST

Earthworms (*Helodrilus caliginosus* var. *trapezoides*) were exposed to soil containing eggs of *Choerostrongylus pudendotectus*, in the same manner as described for *Metastrongylus salmi*. Earthworms dissected about 30 hours after exposure to infected soil contained several first-stage larvae in the wall of the esophagus. In earthworms dissected 14 days after infection, several larvae were undergoing the first molt (fig. 18, *F*). Earthworms dissected 4 days later contained larvae in the process of shedding the second cuticle, while still enclosed in the cuticle of the first molt. Earthworms dissected 19 days after infection contained fully developed third-stage larvae. As in *M. elongatus*, larvae which had not yet undergone the first molt were found in the circulatory system, especially in the hearts of the earthworms.

OBSERVATIONS ON EFFECTS OF ENVIRONMENT

On December 20, 1932, 15 earthworms (*Helodrilus caliginosus* var. *trapezoides*) which had been experimentally infected with *Choerostrongylus pudendotectus* were placed in a box outdoors, as described for *Metastrongylus salmi*. Nine months later, only 1 earthworm was recovered from the box; apparently the others had died. In this earthworm there were found 4 third-stage larvae, 2 larvae in the esophageal wall and 2 larvae in one of the hearts. These larvae showed considerable activity when isolated and placed in water. These observations show that third-stage larvae of *Choerostrongylus pudendotectus* were able to survive in the tissues of the intermediate host for at least 9 months, including the winter and summer seasons.

ASCARIDAE

ASCARIS SUUM GOEZE, 1782

(Fig. 19)

Synonyms.—*Ascaris lumbricoides* Linnaeus, 1758, of authors; *A. suilla* Dujardin, 1845.

Hosts.—Definitive: Swine, sheep, cattle, orang-utan, squirrel. Since *Ascaris suum* is morphologically identical with *A. lumbricoides*, so far as the literature indicates, the reports of *Ascaris* for the above-mentioned hosts other than swine, the

type host of *A. suum*, are possibly reports of *A. suum* and possibly of *A. lumbricoides*. Larval forms of *A. suum* in goat, guinea pig, mouse, rabbit, rat, and probably many other mammals.

Location.—Adults in small intestine usually, but sometimes as wandering parasites in esophagus, stomach, cecum, respiratory passages, liver, gall bladder, pancreas, kidneys, and possibly other parts of the body.

Distribution.—Cosmopolitan.

Description of Egg and Embryo

Egg

Egg usually rounded or elliptical; shell thick, covered with an albuminous coat irregularly mammillated (fig. 19, *A*), and usually stained yellowish brown by intestinal contents. It has been pointed out by many authors, namely, Miura and Nishiuchi (*75*), Foster (*27*), Wharton (*136*), Martin (*73*), Otto (*83*), and Keller (*53*), that unfertilized *Ascaris* eggs are occasionally found in host feces; such eggs are usually thin-shelled, elongated ovoid, and frequently asymmetrical, the albuminous covering present or absent. Otto (*83*), in examining 59,329 eggs of *A. lumbricoides*, noted that 15.9 percent were infertile.

In a series of measurements involving 50 eggs, length 68μ to 84μ, width 50μ to 76μ; according to Foster (*27*), length 56μ to 73μ, width 46μ to 56μ. Egg usually unsegmented when oviposited and on appearance in feces; when embryo within shell is fully formed and molted, development apparently ceases until egg is swallowed by suitable host.

Embryo

Fully developed embryos (fig. 19, *G*), obtained by crushing several embryonated eggs, 235μ to 266μ long by 11μ wide; according to Ransom and Foster (*97*), 200μ to 300μ long by 13μ wide; body nearly uniform in diameter, anterior end with small knob composed of 1 dorsal and 2 subventral lips (fig. 19, *B*) a feature first pointed out by Stiles (*130*), lips surrounded by outer circle of 2 subdorsal and 2 subventral double papillae and 1 pair of lateral single papillae; an inner circle of papillae also present, composed of 1 pair of large papillae on each lip (fig. 19, *B*), 1 amphid present laterally on each subventral lip. Esophagus 95μ to 102μ long, occupying about one-third of entire length of worm. Intestine very granular, connecting posteriorly with a slender rectum. Genital primordium not visible in living specimens. Tail conical, usually pointing dorsad, 21μ to 25μ long; according to Ransom and Foster (*97*), 40μ long. Fully developed embryos are enclosed within a sheath (fig. 19, *G*) indicating that they have molted once while within the shell.

Development and Infectivity of Embryo

Although voluminous data are available in the literature on the development of *Ascaris* eggs, there are certain peculiarities in connection with the infectivity which are not yet understood. The references to "embryonated eggs" commonly found in the literature presumably refer to infective eggs, but observations of the writer indicate that the so-called "embryonated eggs" are not always infective. Apparently an egg is not infective until the embryo within the shell has undergone a first molt. The time required for this molt depends on the temperature at which the egg is incubated.

That larvae of *Ascaris* undergo a molt while in the egg has already been noted by Ransom and Foster (*97*); these writers do not specify, however, that the molt is essential before the egg is infective. They report that some eggs cultured by them at from 33° to 34° C. contained "fully developed embryos in 10 days", but they give no information to show that these eggs were actually infective. To obtain information on the development and infectivity of embryos of *A. suum*, the author made the following experiment:

A large number of *Ascaris* eggs obtained from the uteri of gravid females were cultured in Petri dishes in 1-percent formalin solution at various temperatures, namely, 22° to 24°, 30° to 33° C. When the

eggs contained young embryos, such as is shown in figure 19, *F*, about 4,000 of the eggs were fed to 1 or 2 young guinea pigs weighing about 140 g each; feedings were continued thereafter daily until the eggs

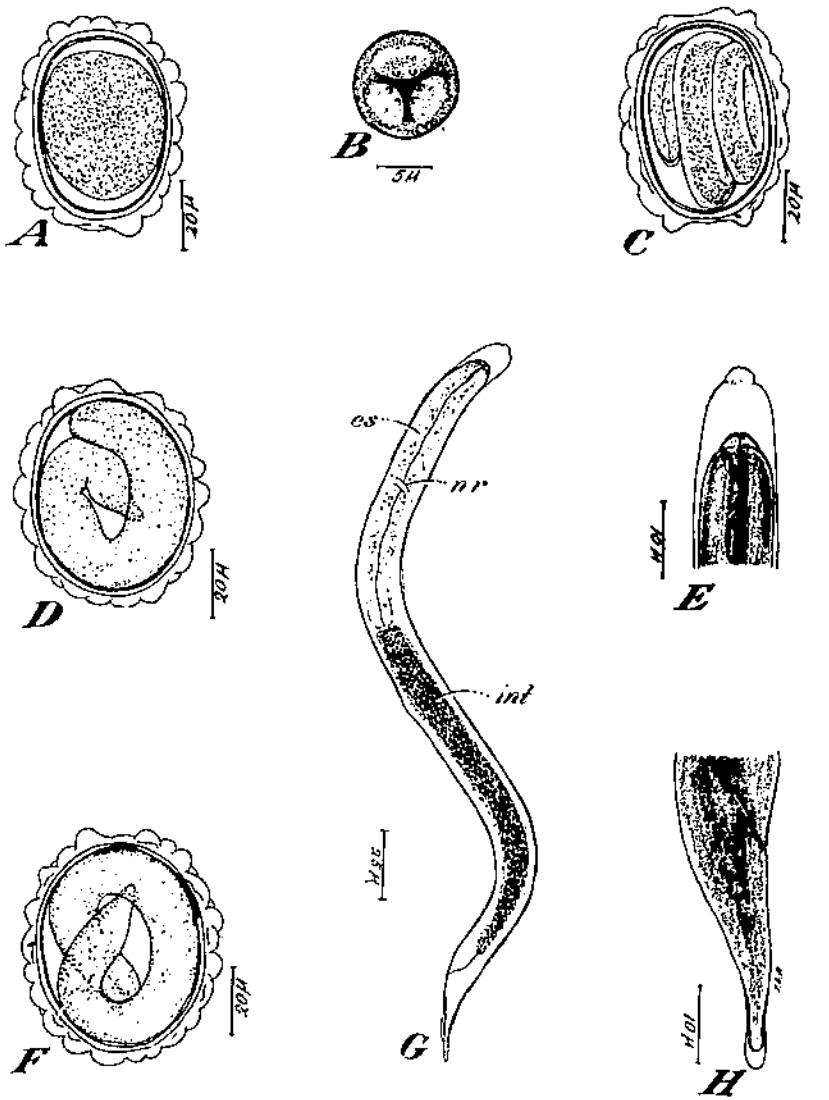

FIGURE 19.—EGGS AND EMBRYOS OF ASCARIS SUUM.

Egg: *A*, As found in freshly deposited feces of swine; *D*, containing young embryo; *F*, containing young embryo later in development; *C*, with an infective embryo.
Embryo: *B*, Fully developed, anterior end, en face view; *G*, infective embryo, obtained by crushing the eggshell, showing cuticle of first molt; *E*, anterior portion of infective embryo, side view; *H*, tail of embryo on verge of first molt.

fed contained embryos which had molted. Molting of the embryos was best observed by placing the eggs under a cover slip and then tapping the latter sharply with the handle of a dissecting needle until

most of the embryos became liberated from their shells. The guinea pigs were killed 5 days after feeding; the lungs were examined for gross lesions and were also chopped in small pieces and placed in a small Baermann apparatus. The results of the observation of the larvae cultured at 33° and of the feeding experiments are shown in table 18.

TABLE 18.—*Development and infectivity of eggs of Ascaris suum after incubation at 33° C.*

[Date of incubation, June 14, 1933]

Days after incubation (number)	Stage of egg development	Results of lung observations of guinea pigs 5 days after being fed *Ascaris* eggs[1]	Days after incubation (number)	Stage of egg development	Results of lung observations of guinea pigs 5 days after being fed *Ascaris* eggs[1]
0	1 cell		16	10 percent of embryos in first molt	Several petechial hemorrhages; 5 *Ascaris* larvae recovered.
5	Morula				
10	Late tadpole				
12	Young embryo, as shown in figure 19, *F*.	No lesions or larvae.	17	50 percent of embryos in first molt	Lungs moderately congested; 16 *Ascaris* larvae recovered.
13	do	Do.			
14	Apparently fully developed embryo.	Do.	18	90 percent of embryos in first molt	Lungs heavily congested; 29 *Ascaris* larvae recovered.
15	do	Do.			

[1] Eggs not fed 0, 5, and 10 days after incubation

Table 18 shows that eggs of *Ascaris suum* incubated at 33° C. contained fairly well-developed embryos in from 12 to 15 days after incubation (fig. 19, *F*), and that the eggs reached the infective stage at the time the embryos had molted (fig. 19, *G*), which was 16 days after incubation.

Eggs which were incubated at 30° and at 22° to 24° C. (room temperature) contained some molted embryos 18 and 28 days, respectively, after the cultures were made. Previous to the molting of the embryos, these eggs failed to produce lesions when fed to guinea pigs, and no larvae were recovered from the lungs; lesions and *Ascaris* larvae were noted, however, in the lungs of guinea pigs which were fed the eggs at the time the embryo began to molt.

TRICHURIDAE

TRICHURIS SUIS (SCHRANK, 1788) A. J. SMITH, 1908

(Fig. 20)

Synonyms.—*Trichocephalus suis* Schrank, 1788; *T. apri*, Gmelin, 1790; *T. crenatus* Rudolphi, 1809.

Hosts.—Swine, wild boar, and wild pig (*Sus bengalensis*).

Location.—Adults in cecum and colon.

Distribution.—Cosmopolitan.

DESCRIPTION OF EGG AND EMBRYO

EGG

Eggshell usually barrel shaped, thick, dark brown, and provided with a clear knob at each pole (fig. 20, *A*). In a series of measurements involving about 50 eggs, length 60μ to 68μ, width 28μ to 31μ; according to Hall (*A*), length 52μ to 56μ. Egg unsegmented when passed in feces. Development of embryo proceeds outside the host until embryo is fully formed; subsequently development apparently ceases until egg is ingested by suitable host.

EMBRYO

Fully formed embryos, obtained by crushing several embryonated eggs, 136μ to 163μ long by 11μ wide (fig. 20, *I–K*). Body tapering slightly at anterior and posterior portions; anterior end rounded, bearing a small lancet-shaped spear, the latter sometimes protruding from mouth cavity and sometimes retracted within cavity; spear connecting posteriorly with a small, slender, dark base. Fülleborn (*30*) has reported a similar spear and dark base in embryos of *Trichuris trichiura* and stated that this base, called by him "Lanzen-Schaft", is fixed to the digestive tract; when the embryo of *T. suis* is viewed laterally, the spear appears to lie slightly oblique to the longitudinal axis of body. Esophagus 60μ long, poorly defined, extending slightly less than one-half entire length of embryo; posterior to esophagus an undifferentiated mass of granules extending to posterior end of body, most of this representing the intestinal tract. Tip of tail ending in a characteristic rounded knob (fig. 20, *K*).

Development of the Embryo

The development of the embryo of *Trichuris suis* within the egg-shell is apparently dependent upon the environment. Considerable information is available with reference to the time necessary and other factors essential for development of the eggs of several species of *Trichuris*. Davaine (*23*) reported that *Trichuris* eggs isolated from feces of man and cultured in water required 8½ months to become embryonated. Railliet (*91*) noted that eggs of *Trichocephalus depressiusculus* (=*Trichuris vulpis*) required about 6 months to embryonate when cultured in water. According to Fülleborn (*29*), eggs of *Trichuris trichiura* cultured at 26° C. were embryonated in about 3½ weeks; Hasegawa (*45*) also found that some eggs of *T. trichiura* were embryonated in 28 days at from 28° to 30°. Cort and collaborators (*19, 20, 21*), in a field survey of helminthic infestation in southwestern Virginia, Panama, and China, noted that infestation with *T. trichiura* was high in regions where shade, warmth, and abundance of moisture prevailed; in some cases, however, where the incidence of *Trichuris* infestation was high, these investigators found that climatic and soil conditions were not the primary determining factors in the distribution of this parasite. Spindler (*122*) suggested that a considerable quantity of moisture was probably necessary for the development of the whipworm of man. The same writer (*123*), in a study of temperature and moisture requirements in the development of *T. vulpis*, found that eggs cultured in water at 30° became embryonated in 16 days, whereas those cultured at 37° became embryonated in from 12 to 15 days; eggs on wet soil and those in a saturated atmosphere at 22° and 30° developed normally, whereas on dry soil at 30°, 98 percent of the eggs failed to become embryonated and were no longer viable after 29 days. In field studies of the trichurid of man in Louisiana, Otto (*82*) concluded that heavy rains in addition to long warm seasons and shade proved ideal for optimum culture conditions of *Trichuris* eggs. Nolf (*80*) has also noted that the eggs of whipworms of man require highly saturated atmosphere for development of the embryo.

The writer obtained eggs of *Trichuris suis* from feces of a heavily infested pig and cultured them in water in an incubator at 37.5° and 33° C., and in charcoal and feces at room temperature (22 to 24°), and outdoors underground. The mean temperature in Washington, D. C., during the period of the outdoor experiment from March 16 to October 12, 1933, was as follows: March, 6.1° C.; April, 7.3°; May, 19.6°; June, 23.7°; July, 24.5°; August, 24°; September, 21.6°; October, 16°.

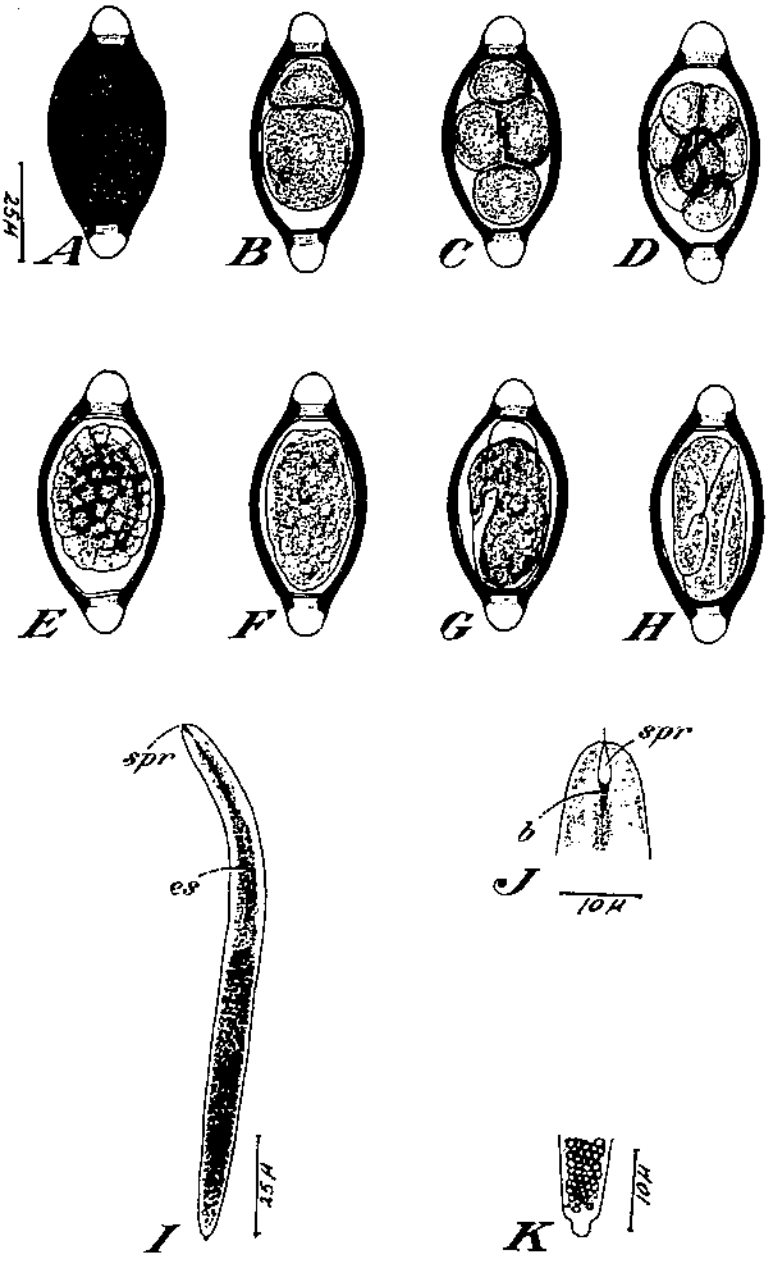

FIGURE 20.—EGGS AND EMBRYOS OF TRICHURIS SUIS.

Egg: *A*, As found in freshly deposited feces of swine; *B*, egg 1 day after incubation at 37.5° C.; *C*, 2 days after incubation at 37.5°; *D*, 5 days after incubation at 37.5°; *E*, 7 days after incubation at 37.5°; *F*, 12 days after incubation at 37.5°; *G*, 16 days after incubation at 37.5°; *H*, 18 days after incubation at 37.5°, with a fully developed embryo.

Embryo: *I*, Obtained by crushing the eggshell; *J*, anterior end of embryo; *K*, posterior end.

Eggs cultured in water were first isolated from pig feces by the method outlined by McCoy (*70*). The eggs were then placed in a small glass receptacle containing water to a depth of about 3 mm. In order to avoid excessive evaporation the receptacle was placed within a 100-cc beaker containing moistened cotton at the bottom. The beaker was then covered with a thick layer of cotton and placed in the incubator. The small glass receptacle containing eggs was removed from the incubator from time to time and the development of the eggs recorded.

For observations on the development of eggs at room temperature and outdoors, the hog feces containing trichurid eggs were mixed with animal charcoal up to one-third of the mass of feces. This mixture was slightly moistened with water and transferred to large specimen bottles about 12 cm high and 5 cm in diameter; the bottles were covered with a paper cap through which many minute openings were made. Specimen bottles containing the feces-charcoal mixture were kept at room temperature for observation, the bottles being placed under bell jars. Within each bell jar was placed a large culture dish containing water, the purpose of which was to keep the moisture content of the jar as high as possible. The bottom of the bell jar was raised above the surface of the table to a distance of about 2 mm in order to allow free air circulation. For observations on the development of eggs under outdoor conditions, the specimen bottles referred to were covered with aluminum caps in which several small openings were made; these bottles were then taken outdoors and placed upside down in sheltered areas about 2 inches underground. After the desired lapse of time, each bottle was removed, and eggs were recovered for examination by the salt-flotation method.

The results of the observations on the development of eggs and embryos of *Trichuris suis* are given in table 19.

TABLE 19.—*Stage of development of eggs of Trichuris suis in water and in feces-and-charcoal media*

[Date of incubation Mar. 16, 1933]

Days after incubation (number)	Stage of development of eggs, at indicated temperature, in			
	Water		Charcoal-feces culture	
	37.5° C.	33° C.	22°–24° C.	6.1°–24.5° C.
0	1 cell	1 cell	1 cell	1 cell.
1	2 cells	do	do	
2	4 cells	do		
3		2 cells		
5	8 cells	4 cells		
7	Early morula	16 cells	1 cell	
9	Advanced morula	Early morula		
12	Early gastrula	Advanced morula		
16	Late tadpole	do	2 cells	1 cell.
18	Fully embryonated	Early tadpole		
20		Late tadpole		
22		20 percent fully embryonated.		
26		All fully embryonated	Early morula	
40			Advanced morula	
51			Late tadpole	1 to 2 cells.
54			30 percent fully embryonated.	
60			Majority fully embryonated.	1 to 8 cells.
128				Some in late morula.
183				Some in late tadpole.
210				10 percent fully embryonated.

Table 19 shows that temperature is one of the important factors in the development of the embryo. Eggs cultured at 37.5° C. were embryonated in 18 days, whereas some eggs cultured at 33° were embryonated in 22 days. At room temperature (22° to 24°) some eggs became embryonated in about 54 days, and of those kept outdoors underground at temperatures of from 6.1° to 24.5° some eggs became embryonated in 210 days. It was also observed that whereas practically 100 percent of the eggs incubated at 33° and 37.5° became embryonated, about 30 percent of the eggs at lower temperatures appeared to be in the process of degeneration. The observation on the development of the eggs outdoors may give some clue as to what takes place under field conditions.

TRICHOSTRONGYLIDAE

HYOSTRONGYLUS RUBIDUS (HASSALL AND STILES, 1892) HALL, 1921

(Figs. 21–26)

Synonyms.—*Strongylus rubidus* Hassall and Stiles, 1892; *Haemonchus rubidus* (Hassall and Stiles, 1892) Sluiter and Swellengrebel, 1912; *Ostertagia rubida* (Hassall and Stiles, 1892) Travassos, 1918; *Trichostrongylus rubidus* (Hassall and Stiles, 1892) Fiebiger, 1923.

Hosts.—Swine and, experimentally, guinea pig.

Location.—Adults in stomach of host.

Distribution.—Asia (Philippine Islands), Europe (England, Germany, and Hungary), Central America (Panama), North America (United States).

Description of Egg, Embryo, and Preparasitic Larval Stages

EGG

Eggshell thin, transparent, and oval in shape, with poles usually unequal, one being less convex than the other (fig. 21, *A*). In a series of measurements involving about 50 eggs, length 60μ to 76μ, width 31μ to 38μ; according to Skrjabin and Bekensky (*121*), length 71μ to 78μ, width 35μ to 42μ. Egg containing an early tadpole-stage embryo when deposited with the feces of the host.

EMBRYO

Embryo, when ready to hatch, resembling first-stage larva; about 280μ to 300μ long by 17μ wide.

FIRST-STAGE LARVA

Shape and size.—Larva resembling corresponding stage of related strongyles; body slender and cylindrical for most of its length, tapering slightly anteriorly and more so posteriorly (fig. 21, *F*); anterior end with 6 minute elevations, possibly representing 2 subdorsal and 2 subventral papillae, and 2 lateral amphids (fig. 21, *C*); posterior portion terminating in a long, slender, pointed tail. Newly hatched larvae 290μ to 315μ long by 17μ wide; before molting, first-stage larvae attain a length of about 540μ to 554μ and a width of 22μ (table 20).

Cuticle.—Thin, transparent, and with very fine transverse striations.

Alimentary tract.—Oral opening leading into a cylindrical buccal cavity, 11μ to 15μ long. Esophagus rhabditoid, the anterior part, or corpus, separated from posterior bulb by a constriction, the isthmus; bulb possessing the usual Y-shaped valve; base of esophagus with cells representing primordium of esophageal intestinal valve. Intestine somewhat granular, composed of 8 dorsal and 8 ventral cells; nuclei of these cells alternating with one another, causing cells to bulge out into lumen of intestine, giving the latter a zigzag or serpentine appearance when viewed laterally; intestine connecting posteriorly with a fine slitlike canal about 15μ long, lined with a thin cuticularized membrane.

Nervous system.—Nerve ring appearing as a band encircling esophagus 75μ to 91μ from its anterior end; ring surrounded by several nuclei of nerve cells.

Excretory system.—Excretory pore opening ventrally 80μ to 95μ from anterior end.

Genital primordium.—Represented by a small elliptical body, composed of 2 epithelial cells enclosing 2 germinal cells, 165μ to 275μ from anterior end, ventral in position, and near junction of fourth and fifth intestinal cells. As is pointed

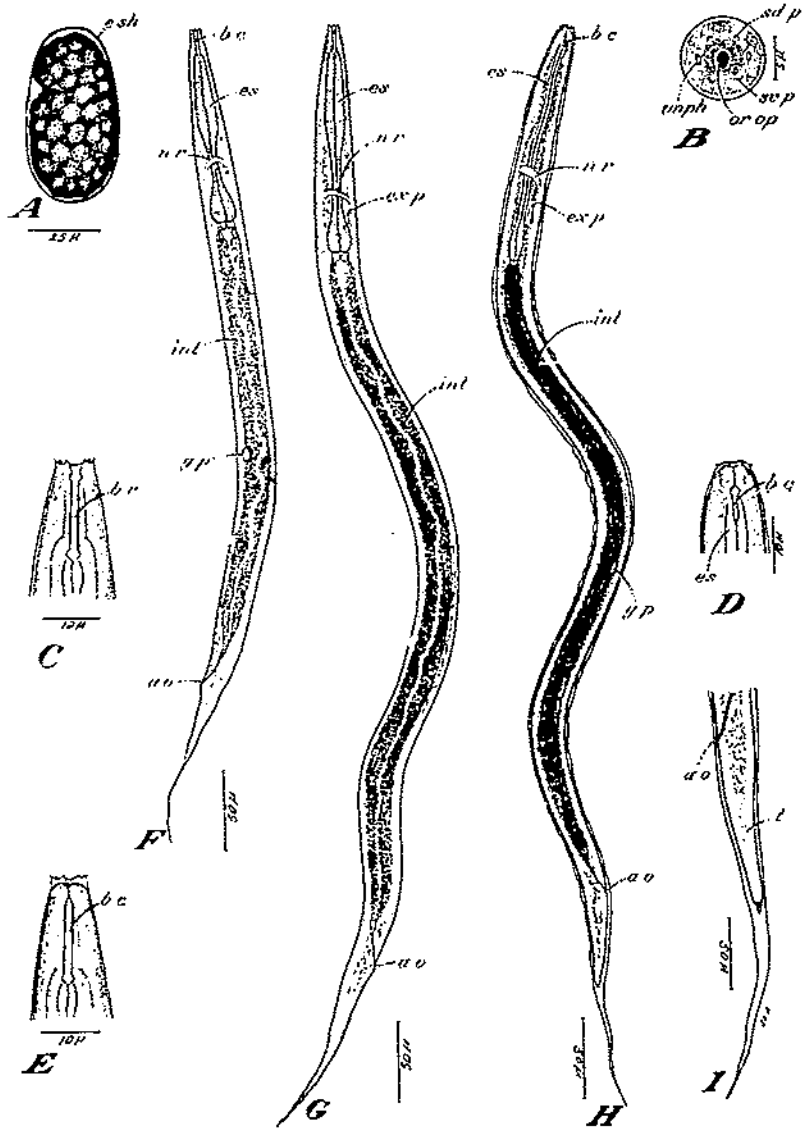

FIGURE 21.—VARIOUS STAGES IN THE DEVELOPMENT OF HYOSTRONGYLUS RUBIDUS.

First-stage larva: *C*, Anterior end; *F*, lateral view.
Second-stage larva: *E*, Anterior end, undergoing second molt, lateral view; *G*, lateral view of larva.
Third-stage larva: *B*, Anterior end, en face view; *D*, anterior portion showing shape of buccal cavity; *H*, lateral view of larva; *I*, tail.

out later, the sex of some specimens of *Hyostrongylus rubidus* can be determined in this stage.

Table 20 shows the rate of development of first-stage larvae of *Hyostrongylus rubidus* in water-charcoal feces media at room tem-

perature (22° to 24° C.), the measurements having been made at different periods after the preparation of the cultures.

TABLE 20.—*Principal measurements of 5 first-stage larvae of Hyostrongylus rubidus at various periods of development*

Item	Period of development and measurements of larva no.—				
	1	2	3	4	5
Period of development ..hours..	2	2	50	174	[1] 74
Length of body ..microns..	290	315	436	546	554
Maximum width of body ..do...	17	17	19	22	22
Length of buccal cavity ..do...	11	11	15	15	15
Length of esophagus ..do...	87	87	95	117	110
Distance of nerve ring from anterior end ..do..	75	75	-------	91	87
Distance of excretory pore from anterior end ..do..	80	82	-------	95	-------
Distance of genital primordium from anterior end ..do..	170	165	255	205	275
Length of tail ..do..	75	80	105	121	125

[1] Larva undergoing first molt.

SECOND-STAGE LARVA

Shape and size.—Second-stage larva similar in shape to larva of first stage (fig. 21, *G*). In this stage the larvae grow considerably, and before the second molt attain a length of about 702μ to 748μ and a width of 26μ (table 21).

Cuticle.—With fine transverse striations.

Alimentary tract.—Buccal cavity as in first-stage larva, but in the transition to the next stage, anterior portion of lumen narrowing gradually (fig. 21, *E*); ultimately one-half of original lumen left open posteriorly, lumen of buccal cavity then being shaped like a spearhead. Esophagus rhabditiform, 117μ to 133μ long; intestine as in first-stage larva.

Nervous system.—In general, as in first-stage larva; nerve ring 97μ to 106μ from anterior end.

Excretory system.—Excretory pore 102μ to 117μ from anterior end.

Genital primordium.—During most of second larval stage, composed of group of 4 cells as in first stage; during transition to third stage, epithelial cells increasing to about 10 or 11 in number.

Table 21 shows the rate of development of second-stage larvae of *Hyostrongylus rubidus* in water-charcoal-feces media at room temperature (22° to 24° C.), the measurements having been made at different periods after preparation of the culture.

TABLE 21.—*Principal measurements of 5 second-stage larvae of Hyostrongylus rubidus at various periods of development*

Item	Period of development and measurements of larva no.—				
	1	2	3	4	5
Period of development ..hours	85	98	98	[1] 122	[1] 122
Length of body ..microns..	577	624	670	702	748
Maximum width of body ..do...	24	24	26	26	26
Length of buccal cavity ..do...	15	15	15	15	15
Length of esophagus ..do...	117	125	129	133	133
Distance of nerve ring from anterior end ..do..	-------	106	102	97	102
Distance of excretory pore from anterior end ..do..	102	-------	-------	106	117
Distance of genital primordium from anterior end ..do...	275	-------	285	357	368
Length of tail ..do...	129	114	129	127	136

[1] Larva undergoing second molt.

THIRD-STAGE LARVA

Shape and size.—Body similar in shape to that of previous stage, but more slender (fig. 21, *H*), head surrounded by outer circle of 2 subdorsal and 2 subventral papillae, and 2 lateral amphids (fig. 21, *B*); tail conical and shorter than that of previous stages, ending in a characteristic short digitiform process (fig. 21,

I). Due to reduction in size of tail, third-stage larva shorter than some larvae of previous stage, those of third stage being 715μ to 735μ long by 22μ wide (table 22).

Cuticle.—With prominent transverse striations.

Alimentary tract.—In en face view, oral opening oval, being slightly elongated dorsoventrally (fig. 21, *B*). Mouth aperture leading into a narrow lumen connecting posteriorly with a characteristic spearhead-shaped cavity about 8μ long

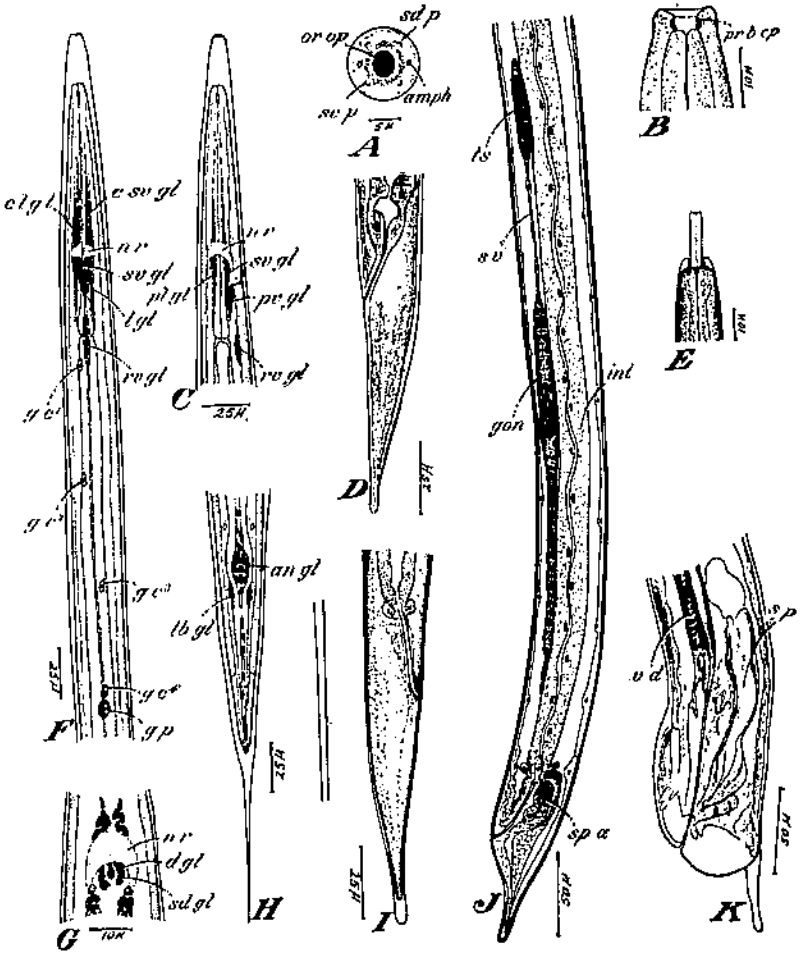

FIGURE 22.—THIRD- AND FOURTH-STAGE LARVAE OF HYOSTRONGYLUS RUBIDUS.

Third-stage larva: *C*, Anterior portion, lateral view; *F*, anterior half showing general features of nervous system and position of giant cells, ventral view; *G*, region of nerve ring, dorsal view; *H*, posterior portion of larva, ventral view.

Fourth-stage larva: *A*, Anterior end, en face view; *B*, anterior portion showing provisional buccal capsule; *E*, anterior portion of larva undergoing fourth molt; *D*, tail of female; *I*, tail of female undergoing fourth molt; *J*, posterior portion of male showing development of genitalia; *K*, posterior portion of male undergoing fourth molt.

representing remains of buccal cavity of previous stage (fig. 21, *D*). Esophagus strongyliform, more slender than in that of previous larval stages, and 130μ to 148μ long. Intestine composed of 8 dorsal and 8 ventral cells, and less granular than that of previous larval stages; posteriorly, intestine connecting with a slender rectal canal.

Nervous system.—Closely related to that of other strongyles, such as *Ancylostoma duodenale*, *Haemonchus contortus*, *Trichostrongylus instabilis*, and *T. douglasi*, described by Looss (*67*), Veglia (*135*), Mönnig (*76*), and Theiler and Robertson

(*134*), respectively. In stained specimens, nerve ring appearing as a light band surrounding the esophagus, 97μ to 106μ from anterior end. Anterior to nerve ring, 6 papillary nerves, of which 2 subventral, 2 subdorsal, and 2 lateral (fig. 22, *C, F, G*). Posterior to nerve ring, 2 lateral ganglia extending almost to base of esophagus; a group of ventral nuclei, posterior to nerve ring, probably corresponding to cells of subventral ganglion; posterior to nerve ring, a dorsal and a subdorsal ganglion. The posteroventral ganglion apparently represented by the group of nuclei of cells surrounding excretory canal; retrovesicular ganglion slightly posterior to posteroventral ganglion and at level of base of esophagus; the 2 lumbar ganglia located near region of anus (fig. 22, *H*).

Excretory system.—Excretory pore, 117μ to 125μ from anterior end; excretory pore connected with a canal leading backward and becoming indistinguishable in passing between cells of nervous system.

Genital primordium.—Location as in previous stages; 368μ to 395μ from anterior end; primordium composed of a group of about 12 or 13 cells, 2 of these germinal cells and the others epithelial cells. A further discussion of the genital primordia of this and other larval stages is given on page 59.

Table 22 gives the measurements of third-stage larvae of *Hyostrongylus rubidus*.

TABLE 22.—*Measurements of 5 third-stage larvae of Hyostrongylus rubidus* [1]

Item	Period of development and measurements of larva no.—				
	1	2	3	4	5
Period of development.....days	7	7	7	7	7
Length of body.....microns	715	717	717	720	735
Maximum width of body.....do	22	22	22	22	22
Length of esophagus.....do	135	135	148	135	130
Distance of nerve ring from anterior end.....do	106	102	102	97	106
Distance of excretory pore from anterior end.....do	123	117	121	120	125
Distance of genital primordium from anterior end.....do	368	370	382	370	395
Length of tail.....do	68	64	60	64	68

[1] Measurements do not include sheath.

DIFFERENCES IN FIRST THREE STAGES

The outstanding differential features of the first three larval stages of *Hyostrongylus rubidus* are as follows:

First-stage larva.—Buccal cavity long, with parallel rod-shaped walls, opening directly to the exterior; esophagus rhabditiform; tail long and pointed. Larva 290μ to 554μ long by 17μ to 22μ wide.

Second-stage larva.—Buccal cavity, esophagus, and tail as in first-stage larva. Larva 577μ to 748μ long by 24μ to 26μ wide.

Third-stage larva.—Buccal cavity short, spearhead-shaped, and opening anteriorly by a narrow lumen; esophagus strongyliform; tail short and conical, terminating in a small digitiform process. Larva 715μ to 735μ long by 22μ wide.

DEVELOPMENT OF PREPARASITIC LARVAL STAGES

The method of obtaining eggs and studying the various developmental stages was in some respects similar to that described by Schwartz (*108*) in connection with a study of the preparasitic development of *Monodontus phlebotomus* (=*Bustomum phlebotomum*). The eggs were obtained by cutting up gravid females of *Hyostrongylus rubidus*, which liberated most of the eggs from the uteri. In order to separate the eggs from the fragments of tissue, the chopped-up worm material was put in a sieve of fine mesh placed over a small porcelain dish, and a gentle stream of water was played on it. The

water passing through the filter carried most of the eggs into the porcelain dish. This water was then centrifuged, and the eggs recovered from this sediment were placed in two small Petri dishes. In one dish was put just enough water to cover the bottom, and in the other dish was put a small quantity of large granules of washed animal charcoal to which were added about 3 drops of filtrate from boiled hog feces. The dish containing only water served for noting the development of the eggs and to obtain first-stage larvae for morphological study; the other dish containing charcoal-and-feces medium served for noting the development of first-, second-, and third-stage larvae and to enable the writer to study the morphology and process of development of these larvae. The reason for using small quantities of water in these cultures was that in a large quantity of water larvae would disintegrate before reaching the second or third stage.

At the time of their removal from the uteri of female worms, eggs were in most cases in a well-advanced morula stage, though the range in development was from one cell to the early tadpole stage. At room temperature (22° to 24° C.), the majority of eggs contained coiled and moving embryos after a period of 24 hours. Hatching was observed 39 hours after incubation. Newly hatched larvae moved very slowly, but as they began to feed they became active and moved in a series of wriggling jerks. The period of activity and growth was followed by the first lethargus, during which the larvae were inactive and began to shed their first cuticle. The first lethargus was observed 103 hours after incubation, and complete shedding of the cuticle occurred 10 hours later. Second-stage larvae moved even more actively than those of the previous stage. This period of activity was again checked by the second lethargus, which occurred 161 hours after eggs were placed in culture; 7 hours later ensheathed or third-stage larvae were seen moving actively in the dish.

Third-stage larvae of *H. rubidus* wriggled very actively in serpentine movements. Another peculiarity noted was the attempt of these larvae to rise when in water; if a small Petri dish half full of water and containing larvae was slightly agitated, the larvae would be quickly carried up by the water currents and would be seen swimming upward for a considerable period, the anterior end of a larva during this time being very close to the surface of the water. The writer has found this peculiarity very helpful in differentiating these larvae from those of other strongyle larvae encountered in hog feces.

The following tabulation shows the time required for the development of larvae to the third stage in a moist charcoal-and-feces medium at room temperature (22° to 24° C.):

Hours after incubation	*Degree of development*
0	1-cell to early tadpole stage.
24	Most eggs embryonated.
39	Eggs hatching.
65	Few larvae in first stage.
89	Majority of larvae in first stage.
103	First lethargus in progress.
113	Few larvae in first molt.
124	Few larvae in second stage.
137	Majority of larvae in second stage.
161	Second lethargus in progress.
168	Larvae in second molt (= third stage).

Development of Parasitic Stages in Final Host

As already reported by the writer (7), infective larvae of *Hyostrongylus rubidus* develop to maturity in the stomachs of guinea pigs. These animals were used throughout the investigation on the parasitic stages of *H. rubidus*. Young guinea pigs weighing between 130 and 150 g were used; these animals were kept without feed for about 24 hours before they were fed third-stage larvae of *Hyostrongylus*.

In guinea pigs fed ensheathed third-stage larvae and killed 15 minutes after infection, the larvae had exsheathed and were adherent to the mucosa of the stomach; this observation was made by first washing the contents of the stomach and then examining the scrapings of its inner lining. These larvae were found to remain in the stomach

FIGURE 23.—Cross section of stomach of guinea pig, showing *Hyostrongylus rubidus* worms (indicated by arrows) and erosion of the gastric epithelium.

and to develop to maturity there without any extensive migration in the tissues of the host, as in the case of the hookworm and some other strongyle larvae. In the process of development the larvae penetrated the epithelial folds of the mucosa and frequently caused ulceration of gastric glands and blood vessels (fig. 23); large masses of coagulated blood have been found in the stomach contents of such guinea pigs.

As shown in table 23, the rate of development of male and female third- and fourth-stage larvae in the final host is approximately the same. Some larvae of both sexes were found in the third molt 5 days after experimental infection, and larvae which had completely shed the third cuticle were noted 3 and 4 days later. Fourth-stage larvae of both sexes were noted undergoing the fourth or final molt 13 days after experimental infection (fig. 22, *E*, *I*, and *K*). Completely formed adult males and females were noted 17 and 19 days, respectively, after experimental infection. The outstanding morphological features during the development of third- and fourth-stage larvae in the final host are as follows:

Third-stage larvae.—Larvae grow in size, and at the time of the third molt male larvae attain a length of 925μ to 1.12 mm (table 23); the genital primordia have undergone considerable differentiation, the primordia of the future testis and ducts being recognizable (fig. 24, *K*). At this stage female larvae have also increased in size, and at the time of the third molt are about 889μ long (table 23); the female genital primordia have also undergone considerable differentiation, the primordia of the ovary and ducts being recognizable (fig. 26, *I*).

Fourth-stage larvae.—These larvae have a provisional buccal capsule (fig. 22, *B*). Male larvae are 1.38 to 3.1 mm long by 30μ to 60μ wide; the tail, which will form the future bursa, becomes distended (fig. 22, *K*); the primordium of the vas deferens is connected with the rectum. Female larvae are 1.01 to 3 mm long by 26μ to 45μ wide; the genital primordium is definitely attached to the body wall ventrally and the future vulva is recognizable. Further discussion of the development of the genital primordium of the larval stages is given on page 59. Table 23 shows the development of *Hyostrongylus rubidus* in a final host (guinea pig), the measurements having been made on different days after experimental infection.

TABLE 23.—*Principal measurements of third- and fourth-stage larvae and adult (fifth-stage) Hyostrongylus rubidus in various periods of development in the guinea pig*

Item	MALES									
	Period of development and measurements of									
	Third-stage larva no.				Fourth-stage larva no.				Adult (fifth stage) no	
	1	2	3	4	1	2	3	4	1	2
Period of development.....days	2	5	5	6	9	9	13	[2] 13	17	19
Length of body.....microns	740	820	925	1,123	1,388	1,440	2,100	3,100	3,800	4,900
Maximum width of body.....do	22	22	26	30	30	38	46	60	60	65
Length of esophagus.....do	155	197	228	220	285	284	326	539	524	539
Distance of nerve ring from anterior end.....microns	106	110	110	121	141	148	160	174	197	197
Distance of excretory pore from anterior end.....microns	125	125	136	144	172	180	178	258	258	281
Distance of cervical papillae from anterior end.....microns									273	281
Distance of genital primordium from posterior end.....microns	335	350	315	310						
Length of spicules.....do								114	121	114
Length of gubernaculum.....do								55	57	57
Length of tail.....do	72	76	72	83	72	72	72			

Item	FEMALES									
Period of development.....days	2	3	5	[1] 5	8	8	9	[2] 13	19	21
Length of body.....microns	742	795	811	889	1,014	1,404	1,528	3,000	4,800	8,000
Maximum width of body.....do	22	22	22	22	20	30	30	45	68	100
Length of esophagus.....do	158	200	212	220	258	288	288	540	530	608
Distance of nerve ring from anterior end.....microns	106	110	106	114	110	121	125	170	190	220
Distance of excretory pore from anterior end.....microns	123	125	120	135	140	148	163	205	205	234
Distance of cervical papillae from anterior end.....microns									290	315
Distance of genital primordium from posterior end.....microns	330	250	200	150						
Distance of genital opening from posterior end.....microns					220	273	286	532	920	1,372
Length of tail.....do	68	72	78	76	76	83	89	106	120	152

[1] Larva undergoing third molt. [2] Larva undergoing fourth molt.

Sex Differentiation of Preparasitic Larvae and General Development of Primary Reproductive Organs

Although sexual dimorphism in the preparasitic larval stages of strongyles has not been reported previously, so far as the writer can ascertain, such differentiation has been noted in early stages of free-living nematodes and in spirurid larvae. Maupas (*74*) found that in third-stage larvae of *Rhabditis causaneli* the genital primordium was composed of a long cylindrical body in the center of which was a group of small cells which gave rise to the uterus and the oviduct in the fourth stage. Pai (*86*), in studying the life cycle of *Anguillula aceti*, noted sex differentiation, based apparently on differences in size of the early larval forms. Pai does not specify sex in the larval stages, but states that in newly hatched larvae the genital primordium of the 2 sexes appeared identical; in larvae 2 or 3 days old the morphological features of the male and female sex primordia appeared different. Pai also mentions that the female worms of *A. aceti* reached sexual maturity in 6 or 7 days, whereas the males reached this condition in 9 days. Seurat (*118*, *119*) pointed out that third-stage larvae of *Gongylonema scutatum* (=*G. pulchrum*) which later developed into females already showed the genital primordium attached to the body wall in the third stage. The writer has confirmed Seurat's observation and has also noted a similar attachment of the genital primordium in third-stage larvae of two other spirurids, *Physocephalus sexalatus* and *Ascarops strongylina*. The male genital primordium in these cases is not attached to the body wall but lies in the ventral region between the body wall and the intestine. Yokogawa (*137*), in his observation on larval development of *Heligmosomum muris* (=*Nippostrongylus muris*), could distinguish sex in third-stage larvae soon after they entered the host. Yokogawa's bases for sexual differentiation were: (1) The posterior migration of the genital primordium in larvae that develop to females; (2) structural differences in the caudal region in the 2 sexes; and (3) differences in the shape of the genital primordia in the 2 sexes. As is shown later, all these changes have been noted by the writer in the parasitic third-stage larvae of *Hyostrongylus rubidus*, but sexual dimorphism has also been noted in the preparasitic stages of these larvae.

According to observations by the writer, sex in preparasitic larval stages of *H. rubidus* may be determined by the position of a large nucleated cell, referred to later as the genital giant cell, close to the genital primordium. A cell apparently similar to this one was mentioned by Looss (*67*) as occurring in larvae of *Ancylostoma duodenale*, and by Mönnig (*76*) in larvae of *Trichostrongylus instabilis* and *T. rugatus*, and was also figured by the writer (*5*) in larvae of *Obeliscoides cuniculi*.

On several occasions, while studying stained specimens of third-stage larvae of *H. rubidus*, the writer observed that the genital giant cell was sometimes anterior to the genital primordium and sometimes lateral or posterior to it. Studies of the parasitic stages of the third and fourth stages of this parasite indicated that larvae having the genital giant cell anterior to the genital primordium developed to males, and those having the genital giant cell lateral or posterior to the genital primordium developed to females. The general development of the male and female genital systems and the probable significance of the genital giant cell are discussed later.

In a recent abstract, the writer (*8*) pointed out that the genital giant cell has been found to be the posterior cell of a group of 4 giant cells situated in the body cavity approximately equidistant from one another and for the most part ventral to the intestine (fig. 22, *F*). Each of these giant cells is about 5μ to 8μ long by 3μ to 6μ wide and is the primordium of each of 4 giant cells found in the body cavity of the adult worms. Four similar giant cells have been reported by Looss (*67*) in third-stage larvae of *Ancylostoma duodenale* and are referred to by him as "cells of unknown significance."

In the study of the detailed organization of the genital primordium the writer used Looss's (*67*) method, which consisted of staining the larvae with acid carmine. This stain brings out the structural details which are described in the following paragraphs:

MALES

First-stage larva.—Genital primordium of *Hyostrongylus rubidus*, like that described for several nematode larvae of first stage, composed of 4 cells, of which 2 are epithelial cells enclosing the other 2, which are germinal cells; in newly hatched larvae, the group of 4 cells is arranged at right angle to main axis of body (fig. 24, *A*). As larva becomes older during this stage, genital primordium rotates almost 90° to lie parallel with body wall (fig. 24, *B* and *C*); at the time genital primordium has rotated about 90°, genital giant cell lies anterior and close to genital primordium. Position of this giant cell usually differentiates male from female; in what are considered female first-stage larvae, genital giant cell lateral to genital primordium; in male larvae giant cell anterior to genital primordium. In early first-stage larvae giant cell, in some cases, slightly lateral and slightly anterior to genital primordium; in these transitional cases, impossible at present to differentiate sex. In late first-stage larvae, location of giant cell appears to be more nearly constant, either anterior to genital primordium in male larvae, or lateral to genital primordium in female larvae.

Second-stage larva.—Genital primordium during most of this stage composed of 2 germinal cells and 2 epithelial cells, corresponding to that of late first-stage larva. At time of transition to third stage, epithelial cells of genital primordium increase by cell division to about 11, whereas the 2 germinal cells do not divide. Genital giant cell still remains anterior and usually close to genital primordium (fig. 24, *D* and *E*).

Third-stage larva (preparasitic).—Genital primordium composed of about 11 epithelial cells surrounding 2 germinal cells (fig. 24, *F*), located 325μ to 343μ from posterior end; genital giant cell still anterior to genital primordium.

Third-stage larva (parasitic).—In larvae 48 hours after infection, most epithelial cells of genital primordium rearranged so that the two germinal cells lie in posterior portion of epithelial-cell group (fig. 24, *G*). Genital giant cell still anterior and close to genital primordium, which is 355μ to 360μ from posterior end of larva. Four days after experimental infection, epithelial cells of genital primordium increasing in size, apparently accompanied by movement of entire genital primordium directed toward changing ends and reversing original position of structure (fig. 24, *I* and *J*). In 5 or 6 days after infection, in late phase of third stage, genital primordium has completely reversed former position (fig. 24, *K* and *L*); during this rotation no division of either epithelial or germinal cells observed. At this time genital primordium shows somewhat definite differentiation; anterior portion containing germinal cells represents primordium of testis; "neck" region, composed usually of three epithelial cells, destined to form seminal vesicle; posterior portion, also epithelial in nature, is primordium of male gonoduct. Genital primordium 310μ to 315μ from posterior end. Reversal of position of male genital primordium in *H. rubidus* resembles similar rotation of male genital primordium of developing larvae of *Anguillula aceti*, a free living nematode, as determined by Pai (*88*). In *H. rubidus*, genital giant cell up to third molt is still anterior to genital primordium (fig. 24, *L*).

Fourth-stage larva and adult.—In 9 to 11 days after infection larva shows further differentiation of various genital structures (fig. 24, *M* and *N*), and further cell division, involving both epithelial and germinal cells (fig. 22, *J*). Genital giant cell far removed from anterior portion of genital primordium and slightly more anterior to latter (fig. 24, *O*) than in third-stage larva. Entire genital primordium

during early part of this stage grows considerably in length, and by the thirteenth day after infection its long and slender posterior portion becomes united with the rectum; when the vas deferens shows definite connection with the rectum,

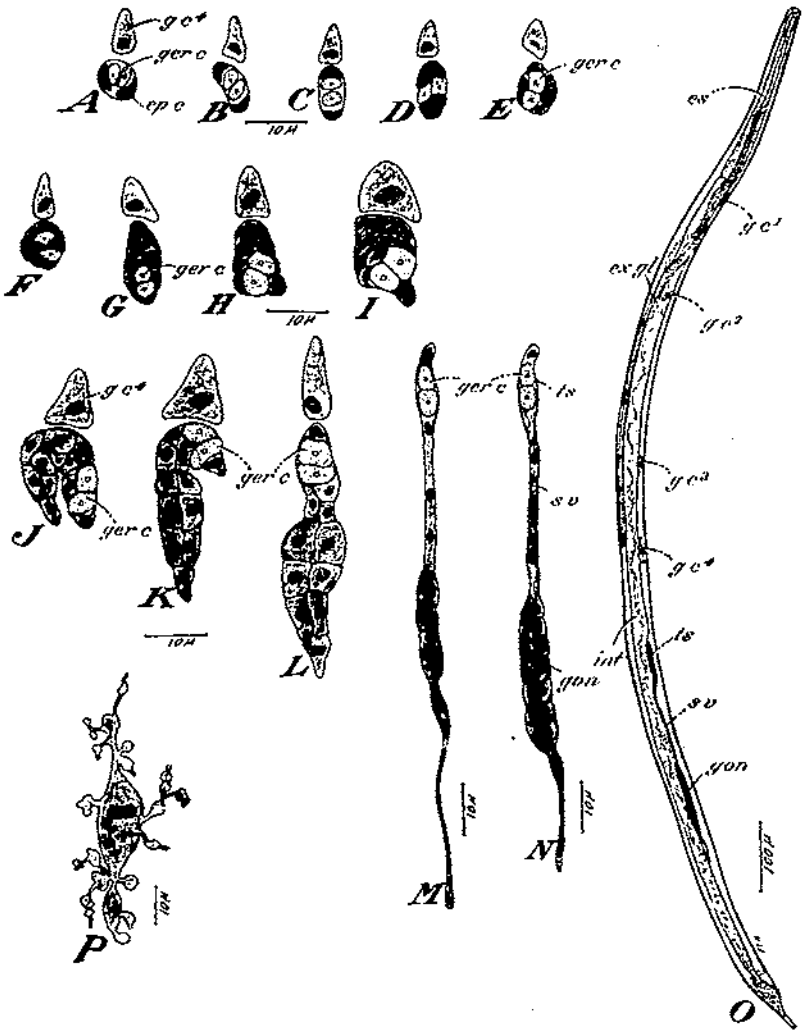

FIGURE 24.—PHASES IN THE DEVELOPMENT OF THE MALE GENITALIA AND POSITION OF THE GENITAL GIANT CELL IN HYOSTRONGYLUS RUBIDUS.

Genital primordium of first-stage larva: *A*, Of a newly hatched larva; *B*, of a larva a few hours after hatching; *C*, of a larva several hours after hatching.

D and *E*, Genital primordium of second-stage larva at time of second molt.

Genital primordium of third-stage larva: *F*, Of a preparasitic larva; *G*, of a larva recovered from a guinea pig 2 days after experimental infection; *H*, *I*, and *J*, of larvæ recovered from a guinea pig 4 days after experimental infection; *K*, of a larva recovered from a guinea pig 5 days after experimental infection; *L*, of a larva recovered from a guinea pig 6 days after experimental infection (larva undergoing third molt).

M and *N*, Differentiation of testis and gonoduct of fourth-stage larvæ 9 and 11 days, respectively, after experimental infection (genital giant cell not shown).

O, Fourth-stage male larva 11 days after experimental infection showing position of the four giant cells and genitalia.

P, Giant cell and its proliferations in body cavity of a young fifth-stage female worm.

larva begins to discard fourth or last larval cuticle (fig. 22, *K*); various portions of genitalia, corresponding to those of adult (fig. 25, *E*), now easily differentiated. Adult genital system (fig. 25, *E*) similar to that of related strongyles, consists

of an anterior portion, the testis, followed by the seminal vesicle, a thin- and transparent-walled tube containing three nuclei, followed in turn by the gonoduct which connects with the rectum. At center axis of testis there appears to be a

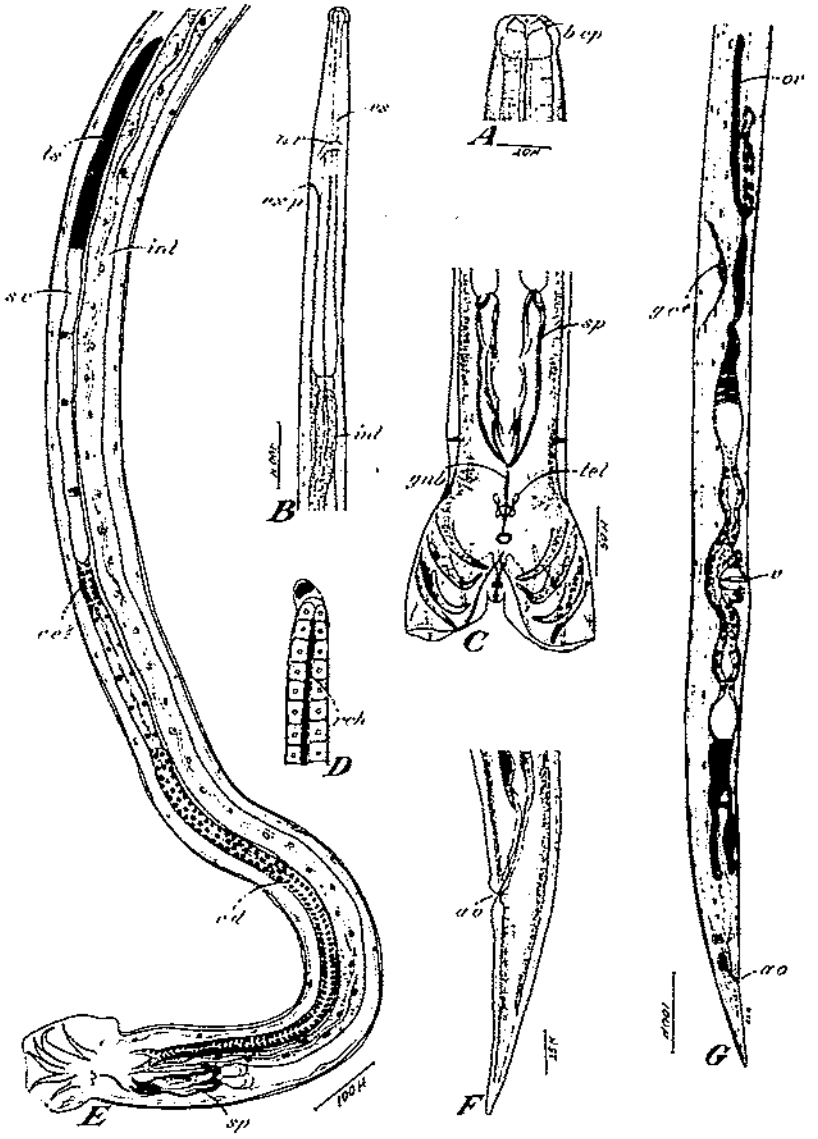

FIGURE 25.—YOUNG ADULT WORMS (FIFTH STAGE) OF HYOSTRONGYLUS RUBIDUS.

A, Anterior end of male; *B*, anterior portion of female; *C*, posterior portion of male showing spicules and bursa; *D*, anterior portion of testis, showing rachis; *E*, posterior portion of male showing genitalia; *F*, tail of female, lateral view; *G*, posterior portion of female, showing genitalia.

slender and solid longitudinal core from which radiate the germinal cells; this core probably corresponds to the rachis (fig. 25, *D*), a structure which has been mentioned by several writers as occurring in *Ascaris lumbricoides*, *Strongyloides* from sheep according to Rovelli (*105*), *Pseudalius inflexus* according to List (*64*), and other nematodes.

The fate of the giant cells in the adult *Hyostrongylus rubidus* is discussed below.

FEMALES

First-stage larva.—Genital primordium of first-stage larva composed, as in male, of 2 epithelial cells and 2 germinal cells (fig. 25, *A*); in position and arrangement these cells similar to those of corresponding male first-stage larva. In somewhat late female first-stage larva, genital giant cell slightly lateral to genital primordium; in some early first-stage larva, as already mentioned, giant cell slightly anterior to genital primordium; in such cases sex of larva cannot be ascertained.

Second-stage larva.—Cells of genital primordium similar in number and position to those in second-stage male larva; giant cell usually lateral to genital primordium.

Third-stage larva (preparasitic).—Genital primordium composed of about 10 epithelial cells enclosing 2 germinal cells (fig. 26, *B*); in position, this developing organ corresponds to that of third-stage larva of male, being located 320μ to 338μ from posterior end. Genital giant cell usually lateral (fig. 26, *B*), and sometimes slightly posterior to genital primordium (fig. 26, *C*).

Third-stage larva (parasitic).—In 48 hours after infection, epithelial and germinal cells have become rearranged, so that germinal cells are one at each end of genital primordium (fig. 26, *D*), which has migrated slightly and is more posterior than in corresponding male larva; in female, genital primordium 330μ to 340μ from posterior end. Genital giant cell usually lateral to genital primordium; unlike that of male, female genital primordium does not reverse position but simply elongates anteriorly and posteriorly, carrying along at each end one germinal cell. In 4 days after infection, slight constriction at middle portion of genital primordium (fig. 26, *H*); in 5 days after infection, genital primordium has migrated farther posteriorly than that of corresponding male larva, primordium in female being 200μ from posterior end. At time of third molt genital primordium 159μ from posterior end and on verge of attachment to ventral side of body wall (fig. 26, *I*).

Fourth-stage larva and adult.—Genital primordium in general same as in previous stage, except that there is a multiplication of epithelial and germinal cells (fig. 26, *K*); it also becomes definitely attached to body wall as a result of proliferation of cells of body wall which connect with those of genital primordium (fig. 26, *J*). Genital giant cell has been found during fourth stage near end of anterior ovarian primordium (fig. 26, *L*); in young adult this cell not far from primordium of anterior uterine duct (fig. 25, *G*); soon becomes remote from ovary because latter grows anteriorly and extends as far forward as posterior portion of esophagus.

The four giant cells in the adult specimens of *Hyostrongylus rubidus* are not regularly arranged but are placed more or less equidistant from one another throughout the anterior half of the worm. Each of these giant cells in the adult worm is somewhat elongated dorsoventrally and sends off several branches from its periphery (fig. 24, *P*). In shape these giant cells resemble cells figured by Nassonov (*79*) in the body cavity of *Strongylus paradoxus* (=*Metastrongylus elongatus*) and referred to by him as the "phagocytic organ." Other stellate or branching structures have been reported in the body cavity of nematodes, notably by Bojanus, cited by Schneider (*105*), Bastian (*10*), Shipley (*120*), Hamann (*44*), and Looss (*65*).

The close association of one of these giant cells with the genital primordium indicates that it might have some relation to the genital system. Chitwood and Chitwood (*16*), in studying the anatomy of the adult *Cephalobellus papilliger*, have found in its body cavity two-celled structures referred to by them as "x-bodies", which are associated with the gonads. They expressed the opinion that these x-bodies might function as endocrine glands. Since little is known about such cells in the body cavity of nematodes, the writer is not

certain whether the giant cells in *Hyostrongylus rubidus* represent phagocytic cells or x-bodies, and whether or not these cells have any connection with the genital system.

In connection with the study of sex differentiation in stained specimens of *H. rubidus*, the male third-stage larvae appeared to be more numerous than the females. An actual count of 100 third-stage

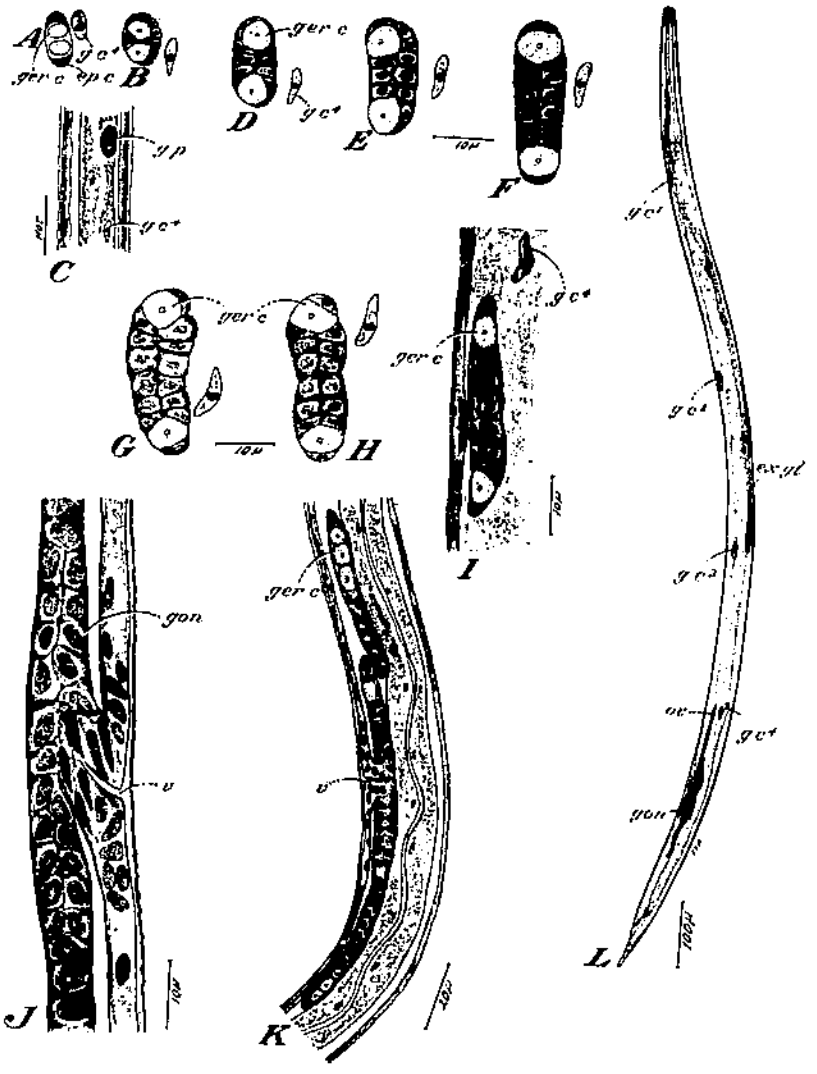

FIGURE 26.—PHASES IN THE DEVELOPMENT OF FEMALE GENITALIA AND POSITION OF GENITAL GIANT CELLS OF HYOSTRONGYLUS RUBIDUS.

A, Genital primordium of first-stage larva.

Genital primordium of third-stage larva: *B*, Of a preparasitic larva; *C*, of a preparasitic larva (position of one of the giant cells also shown); *D*, of a larva recovered from guinea pig 2 days after experimental infection; *E*, *F*, *G*, and *H*, of larvae recovered from guinea pig 4 days after experimental infection; *I*, of a larva recovered from guinea pig 5 days after experimental infection (larva on verge of third molt).

Fourth-stage larva: *J*, Region of vulva showing proliferation of cells from body wall into that of gonoduct 7 days after experimental infection; *K*, region of vulva showing differentiation of ovary and gonoduct 9 days after experimental infection; *L*, female larva 9 days after experimental infection, showing position of the four giant cells and genitalia.

larvae taken at random showed that the number of males was slightly more than twice that of females, males constituting 68 percent and females, 32 percent. However, in a count of 150 fourth-stage larvae recovered from a guinea pig 10 days after an experimental infection, 46.6 percent were males and 53.4 percent were females.

Observations on Effects of Environment

ATTEMPTS TO INDUCE SKIN PENETRATION

This experiment was conducted in accordance with the technic described by Goodey (*35*). The skin of a 2-day-old rat was stretched, hair upward, on a cork ring, floated in a beaker containing warm physiological salt solution, and kept in an incubator at a temperature of 37° C. A small drop of water containing about 150 larvae was placed on the piece of skin and then allowed to evaporate in an incubator. One hour after the drop containing the larvae had evaporated, a drop of water was placed on the rat skin and removed to a slide by means of a pipette. Microscopic examination revealed many ensheathed larvae. No larvae were found in the salt solution. The rat skin was then fixed in 70-percent alcohol and superficial layers were mechanically separated from the deeper layers. These layers were then cleared in an alcohol-phenol mixture. Several ensheathed larvae were found on the surface of the skin, but there were no larvae in the subcutaneous layers. These findings agree with those of Goodey (*37*), who tested eight *Hyostrongylus rubidus* larvae by the cork-ring method previously described and noted that these larvae failed to penetrate the skin.

In another experiment about 200 infective larvae were placed on small areas on the skin of two young guinea pigs, the hair having been clipped from these areas. The guinea pigs were kept under restraint until the water evaporated. An hour later, a few drops of water were placed on the skin area of one guinea pig where the larvae had been placed, and after the water had remained on the skin for a short time it was transferred to a glass slide for microscopic examination. Practically all the larvae that were originally placed on the skin were recovered. The skin of the other guinea pig was thoroughly washed with water and the animal was put in a clean cage. Ten days later this animal was killed, and no larvae were recovered from the skin, lungs, or stomach.

These observations indicate that infection with these larvae does not take place through the intact skin. In their failure to penetrate the skin these larvae resemble those of other trichostrongyles, such as *Haemonchus contortus* according to Veglia (*135*) and *Obeliscoides cuniculi* according to the writer (*5*). However, some trichostrongyles, namely, *Trichostrongylus calcaratus* according to Stoll (*132*) and *Nippostrongylus muris* according to Yokogawa (*137*), have been found to be skin penetrators.

REACTION TO COLD

The ability of nematode larvae to withstand low temperatures is variable. According to Cameron (*15*), third-stage larvae of *Monodontus trigonocephalus* do not revive after being frozen for a few minutes. Ransom (*92*) noted that third-stage larvae of *Haemonchus*

contortus remained alive in sheep feces after an exposure outdoors to temperatures ranging from 21.6° to —13.8° C. for 85 days. Schwartz (*108*) reported that infective larvae of *Bustomum phlebotomum* (=*Monodontus phlebotomus*) frozen solid for about 15 hours, became active when thawed. Third-stage larvae of *Stephanurus dentatus*, according to Schwartz and Price (*113*), can withstand a temperature of —19° for 6 hours, but are killed when exposed to this temperature for 9 hours. Third-stage larvae of *Trichostrongylus* spp., according to Mönnig (*77*), were still alive after an exposure to 0° for 14 days. Ortlepp (*81*) noted that infective larvae of *Triodontophorus tenuicollis* could withstand freezing in an ice chest overnight. De Blieck and Baudet (*12*) noted that infective larvae of *Strongylus vulgaris*, *S. edentatus*, and *Cylicostomum* spp. in a culture of water and feces could withstand a temperature of 0° for 15 days. Raffensperger (*90*) exposed horse manure containing infective strongyle larvae of various species to Montana weather conditions for 20 months; some larvae did not succumb despite the fact that in the course of the experiment the temperature ranged from —18.3° to —38° for a period of 26 days in January and February 1929.

The effects of various low temperatures on the infective larvae of *Hyostrongylus rubidus* are shown in table 24. Each record is based on observations involving about 50 infective larvae. The larvae were placed in small glass tubes containing moist animal charcoal, and the tubes were placed in a refrigerator and removed from time to time for examination. The tubes remained at room temperature for about 4 hours before each examination. In case the larvae showed no motility they were kept under observation for 4 more consecutive days before being declared dead.

TABLE 24.— *Effects of low temperatures on third-stage larvae of Hyostrongylus rubidus, each culture involving about 50 larvae*

Culture no.	Period of refrigeration	Temperature of refrigerator	Condition of larvae after exposure to refrigeration	Culture no.	Period of refrigeration	Temperature of refrigerator	Condition of larvae after exposure to refrigeration
	Hours	°C.			*Hours*	°C.	
1	33	5	All active.	4	3	—20	About 20 percent active; all others dead.
2	144	3 to 5	Majority active; several showed only slight movement.	5	5	—20	About 10 percent active; all others dead.
3	720	—5 to 1	All dead.	6	9	—20	All dead.

In this experiment the *Hyostrongylus* larvae were resistant to a temperature of from 3° to 5° C. for 144 hours, but not to a temperature of from —5° to 1° for 720 hours. Temperatures during this second period of exposure lasted as follows: 1°, 24 hours; 0°, 408 hours; —1°, 24 hours; —2°, 216 hours; —5°, about 42 hours. A temperature of —20° for 9 hours destroyed the vitality of the larvae.

RESISTANCE TO DRYING

Infective larvae of strongyles vary considerably in their ability to resist desiccation. Looss (*67*) reported that infective larvae of *Strongylus* spp. and *Cylicostomum* spp. can resist desiccation in a

Petri dish for 14 days: Raffensperger (*90*) reported that 10 percent of infective larvae of *Strongylus* spp. withstood desiccation in an incubator at 26° C. for 4 months. According to Ransom (*92*), infective larvae of *Haemonchus contortus* which had been dried in feces for 35 days, revived after being moistened. Ortlepp (*81*) found that infective larvae of *Triodontophorus tenuicollis* revived after they had been dried in an incubator overnight at 26°. In contrast to these observations, Looss (*67*) pointed out that larvae of *Ancylostoma duodenale* perish as soon as their surroundings become dry. Goodey (*35*) also found that larvae of *Necator americanus* fail to revive after a few minutes of desiccation. The following experiments were conducted to determine the resistance of *Hyostrongylus* larvae to desiccation:

A small drop of water containing a number of third-stage larvae was placed on each of several glass slides. At the moment the water evaporated, the time was noted, and the slide remained exposed at room temperature for various periods, as shown in table 25. At the expiration of the various periods of time, a few drops of water were added to the dried larvae and the preparations were examined at intervals for about 24 hours. The results of these observations are given in table 25. The table shows that *Hyostrongylus* larvae are not very resistant to drying, since a 240-minute exposure proved fatal.

TABLE 25.—*Results of 5 observations on the resistance of third-stage larvae of Hyostrongylus rubidus to air drying at room temperature (24° C.)*

Larvae used in experiment (number)	Duration of exposure	Condition of larvae after the addition of water	Larvae used in experiment (number)	Duration of exposure	Condition of larvae after the addition of water
	Minutes			*Minutes*	
5	15	All active.	12	180	2 moving slowly; others dead.
5	45	2 active; 3 dead.	10	240	All dead.
5	150	1 active; 4 dead.			

LONGEVITY OF LARVAE IN WATER AT ROOM TEMPERATURE

On November 26, 1932, a number of third-stage larvae of *Hyostrongylus rubidus* were placed in a small glass receptacle containing tap water to a depth of 2 mm and also containing several large granules of washed animal charcoal. The glass receptacle was then covered with a glass slide and was placed in a large Syracuse dish which contained moistened cotton. A cover was placed on the Syracuse dish and sealed with petrolatum. The experiment was conducted at room temperature (22° to 24° C.). Larvae in this condition were found to be somewhat active on February 10, 1933. When examined on March 15, the larvae were dead and somewhat disintegrated. Apparently third-stage larvae will survive in water, with some charcoal added, for a period of 2½ months but not for 3½ months, at room temperature.

STRONGYLIDAE

OESOPHAGOSTOMUM DENTATUM (RUDOLPHI, 1803) MOLIN, 1861

(Fig. 27)

Synonyms.—*Strongylus dentatus* Rudolphi, 1803; *Sclerostoma dentatum* (Rudolphi, 1803) Rudolphi, 1809; *Oesophagostomum subulatum* Molin, 1861; *Strongylus follicularis* (?) Ostertag, in Olt, 1898.

Hosts.—Swine and wild boar.

Location.—Adults in large intestine.

Distribution.—Africa (Zanzibar), Asia (China, Philippines, India), Europe, North America (British West Indies, Puerto Rico, United States), South America, and Oceania (Tonga Island).

Description of Egg and Preparasitic Larval Stages

EGG

Eggshell thin, transparent, and usually elliptical (fig. 27, *A*). In a series of measurements involving about 50 eggs, length 61μ to 83.5μ, width 38μ to 53μ. Eggs in an early cleavage stage, containing from about 8 to 16 cells, when passed in feces.

EMBRYO

Embryo, when ready to hatch, resembling first-stage larva.

FIRST-STAGE LARVA

Shape and size.—In shape (fig. 27, *C* and *E*) first-stage larva similar to corresponding stage of *Hyostrongylus rubidus*. Larvae, soon after hatching, 304μ to 311μ long by 15μ wide, and before first molt 425μ to 433μ long by 19μ wide (table 26).

Cuticle.—Thin with very fine transverse striations.

Alimentary tract.—In general, same as in first-stage larva of *Hyostrongylus rubidus*. Buccal cavity 11μ to 15μ long; esophagus rhabditiform, 83μ to 97μ long; intestine slightly granular, with a sinous lumen.

Nervous system.—Nerve ring appearing as a band encircling esophagus 76μ to 85μ from anterior end.

Excretory system.—Excretory pore inconspicuous in young larva of this stage, about 90μ from anterior end.

Genital primordium.—Small elliptical body, 155μ to 225μ from anterior end.

Table 26 shows the rate of development of first-stage larvae of *Oesophagostomum dentatum* in moist charcoal-feces media at room temperature (22° to 24° C.), the measurements having been made at different periods after preparation of the culture.

TABLE 26.—*Principal measurements of 7 first-stage larvae of Oesophagostomum dentatum at various periods of development*

Item	Period of development and measurements of larva no.—						
	1	2	3	4	5	6	7
Period of development ..... hours	1	1	18	18	18	[1] 27	[1] 27
Length of body ..... microns	304	311	400	410	421	425	433
Maximum width of body ..... do	15	15	19	19	19	19	19
Length of buccal cavity ..... do	11	11	15	15	15	15	15
Length of esophagus ..... do	83	83	95	97	97	97	.....
Distance of nerve ring from anterior end ..... do	76	.....	83	85	85	80	80
Distance of excretory pore from anterior end ..... do	.....	.....	90	90	90	90	.....
Distance of genital primordium from anterior end ..... do	155	162	212	218	220	215	225
Length of tail ..... do	83	83	114	114	117	117	117

[1] Larva undergoing first molt.

SECOND-STAGE LARVA

Shape and size.—In shape (fig. 27, *D*) similar to second-stage larva of *Hyostrongylus rubidus*. Larvae 440μ to 655μ long by 21μ to 32μ wide (table 27).

Cuticle.—With very fine transverse striations.

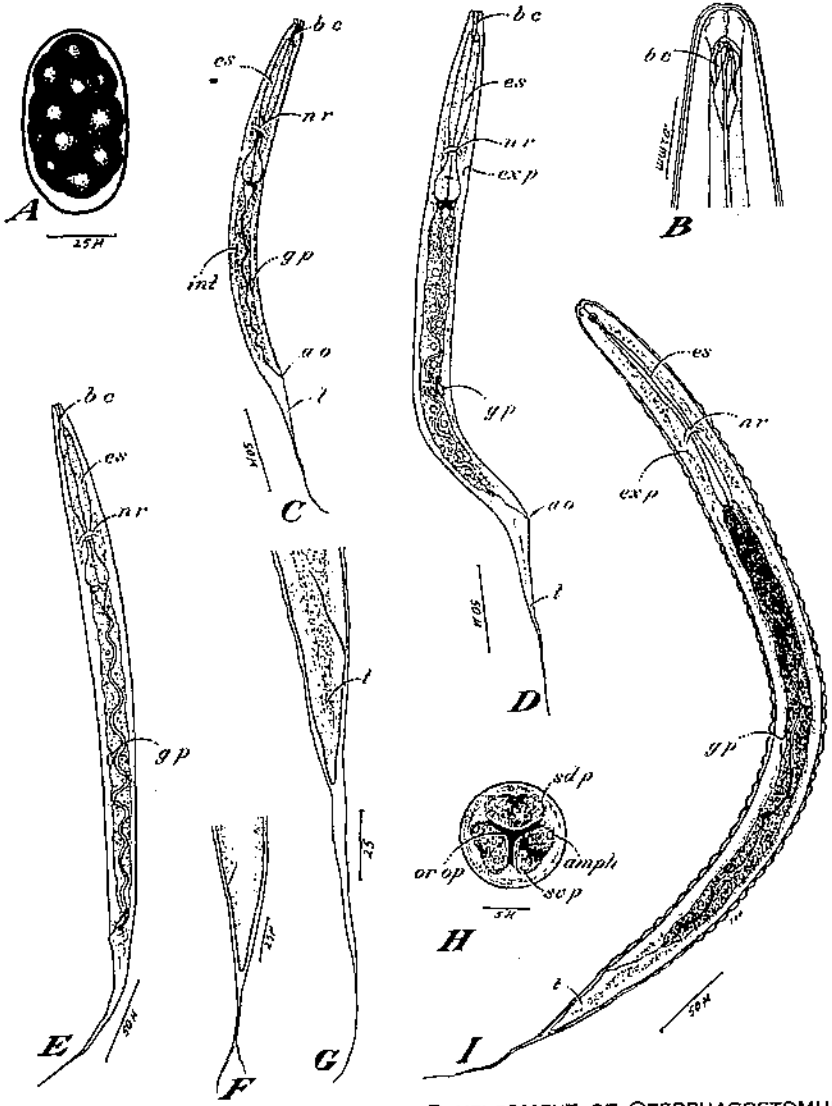

FIGURE 27.—VARIOUS STAGES IN THE DEVELOPMENT OF OESOPHAGOSTOMUM DENTATUM.

A, Egg.
First-stage larva: *C*, Newly hatched, lateral view; *E*, lateral view of fully grown larva.
D, Second-stage larva, lateral view.
Third-stage larva: *B*, Anterior end, showing features of buccal cavity (from Goodey (1924) slightly modified); *H*, anterior end of larva, en face view; *F*, malformation of tip of tail in shed cuticle; *G*, posterior portion of larva; *I*, lateral view of larva.

Alimentary tract.—In general, as in second-stage larva of *Hyostrongylus rubidus*. Buccal cavity 15μ long; esophagus rhabditiform, 102μ to 130μ long; intestine slightly granular and similar to that in first-stage larva.

Nervous system.—Nerve ring 85μ to 95μ from anterior end.

Excretory system.—Excretory pore 95μ to 121μ from anterior end.
Genital primordium.—As in first-stage larva; 225μ to 330μ from anterior end.

Table 27 shows the rate of development of second-stage larvae of *Oesophagostomum dentatum* in moist charcoal-feces media at room temperature (22° to 24° C.), the measurements having been made at different periods after preparation of the culture.

TABLE 27.—*Principal measurements of 7 second-stage larvae of Oesophagostomum dentatum at various periods of development*

Item	Period of development and measurements of larva no.—						
	1	2	3	4	5	6	7
Period of development.....hours	42	42	66	66	66	[1] 100	[1] 100
Length of body.....microns	440	497	509	521	624	639	655
Maximum width of body.....do	21	24	24	26	30	30	32
Length of buccal cavity.....do	15	15	15	15	15	15	15
Length of esophagus.....do	102	110	115	112	121	121	130
Distance of nerve ring from anterior end.....do	85	90	90	90	95	95	95
Distance of excretory pore from anterior end.....do	95	102	110	105	114	117	121
Distance of genital primordium from anterior end...do	225	255	260	265	310	322	330
Length of tail.....do	125	122	125	125	132	152	152

[1] Larva undergoing second molt.

THIRD-STAGE LARVA

Shape and size.—In general, body similar in shape to that of previous stage (fig. 27, *I*). In en face view, head with 3 inconspicuous lips, 1 dorsal and 2 subventral; dorsal lip with 2 subdorsal papillae; each subventral lip with 1 subventral papilla and 1 lateral amphid; an inner circle of 2 minute papillae apparently present on each of the lips (fig. 27, *H*). Tail of larva somewhat conical, ending in a characteristic, somewhat pointed tip (fig. 27, *G*). Characteristic sheath surrounding third-stage larva, showing many evenly arranged folds throughout most of its length (fig. 27, *I*). Larva, excluding sheath, 500μ to 532μ long by 26μ wide; according to Goodey (*36*), larva (including sheath) 660μ to 720μ long by 30μ wide.

Cuticle.—With fine transverse striations.

Alimentary tract.—Oral opening leading into short narrow lumen connecting posteriorly with modified portion of buccal cavity; cuticular lining surrounding buccal cavity, as observed by Goodey (*36*); lining apparently drawn out into thin strands of fibers, the fibers spreading out and interlacing, forming a complex network (fig. 27, *B*). Esophagus strongyliform, 144μ to 152μ long. Intestine composed of 8 dorsal and 8 ventral cells, connecting posteriorly with a slender rectal canal.

Nervous system.—In general, corresponding to that of *Hyostrongylus rubidus;* nerve ring 91μ to 95μ from anterior end.

Excretory system.—Excretory pore 98μ to 110μ from anterior end; pore connecting with a canal leading backward and becoming indistinguishable in passing between cells of nervous system.

Genital primordium.—Location as in previous stages; 273μ to 318μ from anterior end.

Table 28 shows the measurements of third-stage larvae of *Oesophagostomum dentatum.*

TABLE 28.—*Principal measurements of 6 third-stage larvae of Oesophagostomum dentatum* [1]

Item	Period of development and measurements of larva no.—					
	1	2	3	4	5	6
Period of development.....days	6	6	6	6	6	6
Length of body.....microns	500	509	516	520	530	532
Maximum width of body.....do	26	26	26	26	26	26
Length of esophagus.....do	144	148	144	152	152	152
Distance of nerve ring from anterior end.....do	91	91	95	95	91	95
Distance of excretory pore from anterior end...do	98	102	102	102	106	110
Distance of genital primordium from anterior end.....microns	273	300	296	298	311	318
Length of tail.....do	45	45	49	49	51	53

[1] Measurements do not include sheath.

DIFFERENCES IN FIRST THREE STAGES

The outstanding differential features of the first three larval stages of *Oesophagostomum dentatum* are as follows:

First-stage larva.—Buccal cavity long, with parallel rod-shaped walls, opening directly to the outside; esophagus rhabditiform; tail long and pointed; larvae 304μ to 433μ long by 15μ to 19μ wide.

Second-stage larva.—Buccal cavity, esophagus, and tail as in first-stage larva; larvae 440μ to 655μ long by 21μ to 32μ wide.

Third-stage larva.—Buccal cavity short, with cuticular walls drawn out into thin strands of fibers spreading out and interlacing into a complex network; buccal cavity opening to outside by narrow lumen; esophagus strongyliform; tail short, conical, terminating in a rounded tip. Larvae 500μ to 532μ long by 26μ wide, and surrounded by sheath of second molt, sheath possessing numerous evenly arranged folds throughout most of its length.

DEVELOPMENT OF PREPARASITIC LARVAL STAGES

Little information is available in the literature as to the time required for the development of the various preparasitic stages of *Oesophagostomum dentatum*. Goodey (*36*) noted that from 18 to 20 hours after being passed with the feces of pigs, eggs of this parasite contained an embryo which was apparently approaching the tadpole stage.

The writer has cultured eggs of *O. dentatum* in a moist charcoal-feces medium as described for *Hyostrongylus rubidus*. The results obtained at room temperature (22° to 24° C.) are shown in the following tabulation. It is noted that the movements of third-stage larvae are somewhat slow in contrast to those of *H. rubidus*.

Hours after incubation	*Degree of development*
0	1-cell to about 16-cell stage.
18	Majority of eggs containing fully developed embryos.
23	Eggs hatching.
41	Majority of larvae in first stage.
50	First lethargus in progress.
65	Few larvae in second stage.
89	Majority of larvae in second stage.
123	Second lethargus in progress.
137	Few larvae in second molt (=third stage).

OBSERVATIONS ON EFFECTS OF ENVIRONMENT

ATTEMPTS TO INDUCE SKIN PENETRATION

The writer has confirmed the findings of Goodey (*36*) that third-stage larvae of *Oesophagostomum dentatum* fail to penetrate the skin of young rats when the skin is stretched on a cork ring and floated on a warm physiologic saline solution.

REACTION TO COLD

There is no information in the available literature with reference to the reaction of third-stage larvae to cold. The writer made such a study, and the results of the observations are given in table 29. Each record is based on observations involving about 300 third-stage larvae. These larvae were placed in small glass tubes containing moist animal charcoal; larvae were removed from the culture from time to time for examination. Before the larvae were examined microscopically, the tubes were kept at room temperature for 6 hours, and the larvae were examined on the same day and for the next 4 consecutive days. The data in table 29 show that some third-stage

larvae of *O. dentatum* were somewhat resistant to low temperature. Some of the larvae still showed signs of life when exposed to —19° to —29° C. for 10 days, but their vitality was destroyed when kept at temperatures of —15° to —29° for 31 days.

TABLE 29.—*Effects of low temperatures on the infective larvae of Oesophagostomum dentatum, each culture involving about 800 larvae*

Culture no.	Period of refrigeration		Temperature of refrigerator	Condition of larvae after exposure to refrigeration
	Days	*Hours*	*° C.*	
1	1	9	5	All active.
2	0		3 to 5	Majority active; few slightly active.
3		0	—20	15 moved slowly, others responded to heat only.
4	2		—20 to —21	About 10 moved slowly and about 50 percent responded to heat; others dead.
5	5		—17 to —21	About 10 moved slowly, and about 25 percent responded to heat; others dead.
6	10		—17 to —20	About 8 responded to heat; others dead.
7	10		—19 to —20	2 responded to heat; others dead.
8	31		—15 to —29	All dead.

REACTION TO DESICCATION

Goodey (*36*) reports that third-stage larvae of *O. dentatum*, when dried in glass capsules for 1 or 2 days, revived on the addition of water. The writer, however, could not confirm Goodey's observation. Larvae in a small drop of water were placed on each of several glass slides, and the water was allowed to evaporate. At the moment the water evaporated the time was noted, and the glass slide then remained exposed at room temperature for various periods, as shown in table 30. At the expiration of the desired lapse of time, a few drops of water were added to the dried larvae, and the preparation was examined microscopically at various intervals during about 24 hours. The results of the observations are given in the table which shows that some larvae died after being desiccated for 30 minutes, but that one larva showed signs of life even after 20 hours of drying. All the larvae were dead after 22 hours of drying.

TABLE 30.—*Results of observations on the resistance of third-stage larvae of Oesophagostomum dentatum to air drying at room temperature (22° to 24° C.)*

Larvae used (number)	Duration of exposure		Condition of larvae after the addition of water	Larvae used (number)	Duration of exposure		Condition of larvae after the addition of water
	Hours	*Minutes*			*Hours*	*Minutes*	
15		10	All active.	22	18		1 moved anterior end in response to heat; all others dead.
17		30	2 dead; all others active.				
19	1		5 dead; all others moved or responded to heat.	50	20		Do.
16	2		1 active; 3 responded to heat; all others dead.	30	22		All dead.
22	5		3 moved slowly; all others dead.				

LONGEVITY OF LARVAE IN WATER AT ROOM TEMPERATURE

On November 26, 1932, third-stage larvae of *Oesophagostomum dentatum* were placed in a small glass receptacle containing water and animal charcoal, as described for *Hyostrongylus rubidus*, kept at room temperature (22° to 24° C.), and examined at least once every month. The last examination was made on September 15, 1933, at which time the larvae were still active and appeared normal.

STEPHANURUS DENTATUS DIESING, 1839

(Fig. 28)

Synonyms.—*Sclerostoma dentatum* (Diesing, 1839) Leidy, 1856, not Rudolphi, 1803; *S. pinguicola* Verrill, 1870; *Strongylus dentatus* (Diesing, 1839) Dean, 1874, not Rudolphi, 1803; *Stephanurus nattereri* Cobbold, 1879; *Strongylus pinguicola* (Verrill, 1870) Malgalhães, 1894; *Sclerostomum renium* Drabble, 1922; *Stephanurus morai* Almeida, 1928.

Hosts.—Swine, cattle, and, experimentally, guinea pig.

Location.—Adults in kidney fat, kidneys, ureters, urinary bladder, lungs, pleural cavity, lumbar muscles, spleen, and spinal canal.

Distribution.—Africa (Belgian Congo, Dahomey, French Congo), Asia (Indo-China, Java, Philippines, Sumatra), Australia, Europe (Spain), Central America (Nicaragua, Panama), North America (British West Indies, Cuba, Mexico, United States), South America (Argentina, Brazil, Uruguay), and Oceania (Cook Islands, Guam).

DESCRIPTION OF EGG AND PREPARASITIC LARVAL STAGES

EGG

Egg with thin, transparent, oval shell; poles usually unequal, one usually more convex than other (fig. 28, *A*); abnormalities in shape of shell occasionally present, one or both extremities being somewhat flat instead of rounded. In a series of measurements involving 50 eggs, length 91μ to 114μ, width 53μ to 65μ; segmenting eggs 208μ long by 72μ wide have been found, but such extreme size possibly represents an abnormality; according to Bernard and Bauche (*11*) eggs 100μ to 120μ long by 55μ wide, according to Ross and Kauzal (*101*) most typical eggs 104μ to 136μ long by 56μ to 64μ wide. When deposited in urine of host, egg advanced in cleavage and composed of from 32 to 64 cells.

EMBRYO

Embryo, just before hatching, resembling first-stage larva.

FIRST-STAGE LARVA

Shape and size.—In shape (fig. 28, *E*) first-stage larva similar to corresponding stage of *Hyostrongylus rubidus*; anterior end somewhat rounded in lateral view; head papillae not very distinct. Three hours after hatching, larvae 410μ to 421μ long by 24μ wide, attaining a length of about 530μ and a width of 26μ before molting (table 31).

Cuticle.—Thin and apparently without transverse striations.

Alimentary tract.—In general, as in first-stage larva of *Hyostrongylus rubidus*. Buccal cavity 11μ to 15μ long; esophagus rhabditiform, 95μ to 114μ long. Intestine more granular than that of any other first-stage larva of a swine nematode; walls of intestinal cells inconspicuous; lumen of intestine sinous in outline.

Nervous system.—Nerve ring appearing as a band encircling esophagus, 66μ to 91μ from anterior end.

Excretory system.—Excretory pore inconspicuous in young larva of this stage, 85μ to 98μ from anterior end.

Genital primordium.—Represented by a small elliptical body ventral in position, 215μ to 273μ from anterior end.

Table 31 shows the rate of development of first-stage larvae of *Stephanurus dentatus* in moist charcoal-feces media at room temperature (22° to 24° C.), the measurements having been made at different periods after the preparation of the culture.

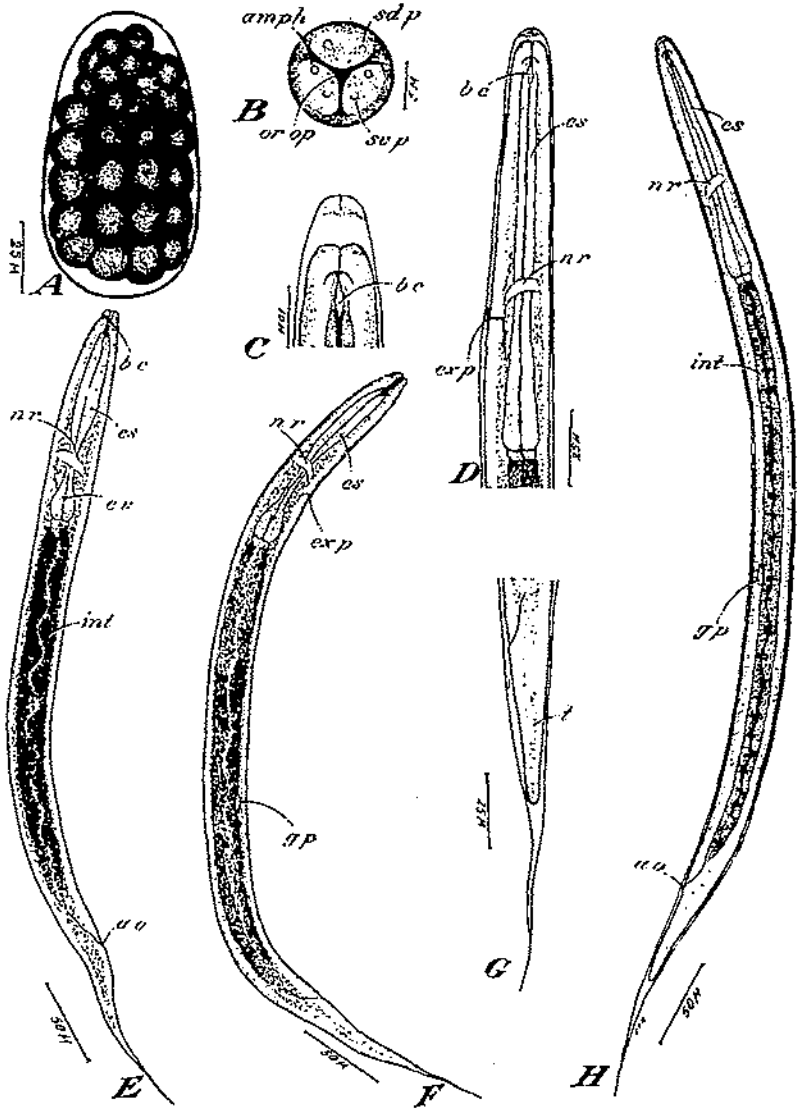

FIGURE 28.—VARIOUS STAGES IN THE DEVELOPMENT OF STEPHANURUS DENTATUS.
A, Egg.
E, First-stage larva, lateral view.
F, Second-stage larva, lateral view.
Third-stage larva: *B*, Anterior end, en face view; *C*, anterior end, showing shape of buccal cavity; *D*, anterior portion, lateral view; *G*, posterior portion, lateral view; *H*, lateral view of larva.

TABLE 31.—*Principal measurements of 5 first-stage larvae of Stephanurus dentatus at various periods of development*

Item	Period of development and measurements of larva no.—				
	1	2	3	4	5
Period of development.....hours	3	3	8	8	[1] 20
Length of body.....microns	410	421	452	468	530
Maximum width of body.....do	24	24	24	24	26
Length of buccal cavity.....do	11	11	11	15	15
Length of esophagus.....do	95	108	110	108	114
Distance of nerve ring from anterior end.....do	66	70	78	78	91
Distance of excretory pore from anterior end.....do				85	98
Distance of genital primordium from anterior end.....do	215	220	230	240	273
Length of tail.....do	95	102	102	106	106

[1] Larva undergoing first molt.

SECOND-STAGE LARVA

Shape and size.—In shape (fig. 28, *F*) similar to second-stage larva of *Hyostrongylus rubidus*. Papillae of anterior end not very distinct. Larvae 530μ to 580μ long by 26μ to 28μ wide (table 32); according to Ross and Kauzal (*101*), 540μ long by 27μ to 32μ wide.

Cuticle.—Transverse striations not visible at this stage.

Alimentary tract.—In general as in second-stage larvae of *Hyostrongylus rubidus*. Buccal cavity 15μ long; esophagus rhabditiform, 110μ to 136μ long; intestine somewhat granular.

Nervous system.—Nerve ring 83μ to 91μ from anterior end.

Excretory system.—Excretory pore inconspicuous, 91μ to 102μ from anterior end.

Genital primordium.—As in first-stage larva; 250μ to 304μ from anterior end.

Table 32 shows the rate of development of second-stage larvae of *Stephanurus dentatus* in moist charcoal-feces media at room temperature (22° to 24° C.), the measurements having been made at different periods after preparation of the culture.

TABLE 32.—*Principal measurements of 5 second-stage larvae of Stephanurus dentatus at various periods of development*

Item	Period of development and measurements of larva no.—				
	1	2	3	4	5
Period of development.....hours	41	41	41	65	[1] 70
Length of body.....microns	530	535	535	540	580
Maximum width of body.....do	26	26	26	26	28
Length of buccal cavity.....do	15	15	15	15	15
Length of esophagus.....do	110	114	125	114	136
Distance of nerve ring from anterior end.....do	91	83	91	91	91
Distance of excretory pore from anterior end.....do		91	98	98	102
Distance of genital primordium from anterior end.....do	250	256	256	281	304
Length of tail.....do	102	106	117	122	117

[1] Larva undergoing second molt.

THIRD-STAGE LARVA

Shape and size.—In general, body similar in shape to that of previous stage, but more slender (fig. 28, *H*). In en face view, head with 3 inconspicuous lips, 1 dorsal and 2 subventral; dorsal lip with 2 subdorsal papillae; each subventral lip with 1 subventral papilla and 1 lateral amphid; an inner circle of 2 minute

papillae apparently present on each of the lips (fig. 28, *B*). Number of lips as noted by the writer is at variance with that reported by Ross and Kauzal (*101*) who found 4 inconspicuous lips, 2 dorsal and 2 subventral. Tail of larva somewhat conical, ending in a characteristic rounded tip (fig. 28, *G*). Owing to reduced length of tail, larvae 518μ to 610μ long by 24μ to 26μ wide; according to Ross and Kauzal (*101*), 607μ long by 28μ wide.

Cuticle.—With fine transverse striations.

Alimentary tract.—Oral opening leading into short narrow lumen connecting posteriorly with characteristic spindle-shaped buccal cavity (fig. 28, *C*); in optical section cuticular lining of cavity apparently sending out a pair of short, laterally directed fibers at anterior portion of cavity. Esophagus strongyliform, its base characteristically broader than that of corresponding stage of other larvae of swine strongyles. Intestine less granular than in previous larval stages and with 16 dorsal and 16 ventral cells; intestine connecting posteriorly with a slender rectal canal.

Nervous system.—In general, corresponding to that of *Hyostrongylus rubidus;* nerve ring 85μ to 92μ from anterior end.

Excretory system.—Excretory pore 91μ to 105μ from anterior end; pore connecting with a canal extending backward and becoming indistinguishable in passing between cells of nervous system.

Genital primordium.—Location as in previous stages; 288μ to 342μ from anterior end.

Table 33 gives the measurements of third-stage larvae of *Stephanurus dentatus.*

TABLE 33.—*Principal measurements of 5 third-stage larvae of Stephanurus dentatus* [1]

Item		Period of development and measurements of larva no.—				
		1	2	3	4	5
Period of development	days	7	7	7	7	7
Length of body	microns	518	518	520	537	610
Maximum width of body	do	24	24	24	24	26
Length of esophagus	do	125	128	132	134	136
Distance of nerve ring from anterior end	do	85	87	85	85	92
Distance of excretory pore from anterior end	do	96	102	91	91	105
Distance of genital primordium from anterior end	do	288	290	290	294	342
Length of tail	do	49	53	50	49	57

[1] Measurements do not include sheath.

DIFFERENCES IN FIRST THREE STAGES

The outstanding differential features of the first three larval stages of *Stephanurus dentatus* are as follows:

First-stage larva.—Buccal cavity long with parallel rod-shaped walls, opening directly to exterior; esophagus rhabditiform; intestine very dark and granular; tail long and pointed; larvae about 410μ to 530μ long by 24μ to 26μ wide.

Second-stage larva.—Buccal cavity, esophagus, intestine, and tail as in first stage; larvae about 530μ to 580μ long by 26μ to 28μ wide.

Third-stage larva.—Buccal cavity short, spindle-shaped, opening anteriorly by a narrow lumen; esophagus strongyliform; intestine less dark and granular than in previous stages; tail short, conical, terminating in a rounded tip; larvae 518μ to 610μ long by 24μ to 26μ wide.

DEVELOPMENT OF PREPARASITIC LARVAL STAGES

Considerable information is already available concerning the development of the preparasitic stages of *Stephanurus dentatus.* Bernard and Bauche (*11*) noted that eggs cultured in animal charcoal and urine hatched in 24 hours and that the third stage was reached 5 days later. Schwartz and Price (*113*) reported that at a tempera-

ture of about 26° to 27° C., *Stephanurus* eggs hatched in 24 to 48 hours, and third-stage larvae were present in 5 to 6 days after the culture was prepared. They found that low temperatures retarded the development of the eggs and the larvae; thus, at a temperature of about 10°, the eggs not only failed to develop, but their vitality was destroyed in 10 days. Ross and Kauzal (*101*) noted that when eggs were cultured at 25° to 27.5° in a medium containing water with a few drops of filtered decoction of pig feces, the larvae hatched in 24 to 36 hours; the first molt occurred 16 to 30 hours after hatching and the second molt 36 to 48 hours after the commencement of the second stage; the third stage was usually reached 95 to 120 hours after culturing of the eggs, but in exceptional cases, at 30° the third stage was reached in 85 hours.

The writer cultured eggs of *S. dentatus* in a moist charcoal-feces medium, as described for *Hyostrongylus rubidus*. The results at room temperatures (22° to 24° C.) are given in the following tabulation. It was noted that the movement of third-stage larvae in moist charcoal medium was more active than that of the sluggish third-stage *Oesophagostomum* larvae, but slightly less active than that of third-stage larvae of *Hyostrongylus rubidus*.

Hours after incubation	*Degree of development*
0	1-cell to advanced morula stage.
23	Eggs hatching.
40	Majority of larvae in first stage.
43	First lethargus in progress.
64	Few larvae in second stage.
88	Majority of larvae in second stage.
93	Second lethargus in progress.
112	Few larvae in second molt (=third stage).

Observations on Effects of Environment

Attempts to Induce Skin Penetration

Information concerning the ability of third-stage larvae of *Stephanurus* to penetrate the skin of the final host has aroused considerable discussion among some parasitologists. Bernard and Bauche (*11*) stated that they infected pigs experimentally with *Stephanurus dentatus* as a result of skin penetration by third-stage larvae. Schwartz and Price (*112, 113, 114*) on the other hand, in an extensive investigation regarding skin penetration, concluded that when *Stephanurus* larvae are placed on the intact skin of pigs they are incapable of penetrating it; these writers showed, however, that if the skin of the pig were scarified, the larvae would penetrate and develop in their usual location. Ross and Kauzal (*101*), however, stated that they produced an experimental infection of kidney worms in pigs by placing larvae in feces and mud on the intact skin of live pigs or guinea pigs. Lately, Spindler (*125*) cleared up the difference of opinion with reference to skin penetration of *Stephanurus* larvae. He confirmed the findings of Schwartz and Price to the effect that third-stage larvae in water when placed on the intact skin of pigs are unable to infect these animals, and confirmed the findings of Ross and Kauzal to the effect that infection occurs readily when charcoal and feces or soil and feces cultures containing third-stage larvae are spread on the intact skin of pigs. Spindler expressed the opinion that traction

probably plays a part in aiding the larvae to penetrate the intact skin.

In two cases the writer, using Goodey's (*35*) cork-ring skin-penetration method, failed in attempts to bring about the penetration of *Stephanurus* larvae through the skin of 2-day-old rats, results which are in harmony with the findings of Schwartz and Price (*113*) and Ross and Kauzal (*101*).

REACTION TO COLD

Infective *Stephanurus* larvae are not very resistant to low temperature. Schwartz and Price (*113*) showed that at a temperature of —19° C. the vitality of the larvae was destroyed in 9 hours, but that 6 hours' exposure to this temperature was not fatal. Experiments conducted by the writer on the resistance of larvae to a temperature of —19° confirmed the finding of Schwartz and Price (*113*).

RESISTANCE TO DESICCATION

Infective larvae of *Stephanurus dentatus* are not very resistant to desiccation. Schwartz and Price (*113*) noted that when larvae were air-dried at room temperature on a slide, their vitality was destroyed in about 30 minutes, but shorter periods of exposure did not prove fatal to all larvae. Ross and Kauzal (*101*) ascertained that some larvae exposed in bright sunshine (43.3° C.) resisted 5 to 10 minutes of desiccation, but all were found dead in 15 minutes. Practically the same results were obtained by De Jesus[4] who found that *Stephanurus* larvae in dust exposed to bright sunlight for 5 or 10 minutes remained alive, whereas an exposure of 15 or more minutes proved fatal.

The writer has dried larvae on glass slides at room temperature and has noted that only a few larvae survived an exposure of 30 minutes. Drying for 20 minutes proved fatal to some larvae, but all of them succumbed to an exposure of 1 hour or longer (table 34). These findings are in agreement with those of Ross and Kauzal (*101*) who found that a few larvae survived an exposure of 30 minutes, but that an exposure of 1 hour was fatal.

TABLE 34.—*Results of observations on the resistance of third-stage larvae of Stephanurus dentatus to air drying at room temperature (24° C.)*

Larvae used (number)	Length of exposure	Condition of larvae after addition of water	Larvae used (number)	Length of exposure	Condition of larvae after addition of water
	Minutes			*Minutes*	
25	5	All active.	30	30	4 active; 26 dead.
20	10	Do.	28	60	All dead.
20	15	Do.	25	180	Do.
25	20	12 active; 13 dead.			

LONGEVITY OF INFECTIVE LARVAE

According to Ross and Kauzal (*101*), the longevity of infective larvae varies considerably in the different media used. In soil and feces media they recovered most of the larvae alive up to the one hundred and eighth day. Beyond this period the number of live

[4] JESUS, Z. DE. THE RESISTANCE OF THE EGGS AND LARVAE OF SWINE KIDNEY WORM, STEPHANURUS DENTATUS DIESING, WITH SPECIAL REFERENCE TO THE CONTROL OF STEPHANURIASIS. Philippine Islands Bur. Anim. Indus. Circ. 3(2): 98–108. 1933. [Mimeographed.]

larvae which they recovered diminished progressively; on the one hundred and fifty-fourth day only a few larvae were alive. These authors also noted that in liquid medium few larvae survived more than 50 days, and in agar all larvae degenerated in 28 days. De Jesus [6] reported that when third-stage larvae were placed in a layer of dust about 3 mm deep, about half of them died after 47 days, and by the fifty-fifth day all the larvae were disintegrated.

The writer has kept infective larvae of *Stephanurus* in a moist charcoal-and-feces mixture for 40 days at room temperature, and after that period several active larvae were recovered with the aid of the Baermann apparatus.

STRONGYLOIDIDAE

STRONGYLOIDES RANSOMI SCHWARTZ AND ALICATA, 1930

(Figs. 29–30)

Host.—Swine.
Location.—Adults in small intestine.
Distribution.—North America (United States).

Larval Stages and Development

Eggs of *Strongyloides ransomi* derived from parasitic female worms give rise to embryos which after hatching pursue one of two cycles of development, direct or indirect. In the direct cycle, the larvae grow and after two molts, according to Lucker (*69*), transform into third-stage (strongyliform) larvae capable of infecting the host. In the indirect cycle the larvae grow and after four molts, according to Lucker, develop into adult free-living males and females; the females give rise to embryonated eggs which hatch, the larval worms molting twice and developing to third-stage strongyliform larvae capable of infecting a suitable host.

The number of molts observed by Lucker in *Strongyloides ransomi* is at variance with the number reported for other species of *Strongyloides*. Various writers, namely, Grassi and Parona (*39*), Perroncito (*88*), Leuckart (*61*), Grassi and Segré (*40*), Golgi and Monti (*33*), Zinn (*148*), Leichtenstern (*57*), Gonder (*34*), and Kreis (*55*), mention only one molt in the direct cycle of development of *Strongyloides stercoralis* and other species of *Strongyloides*.

Bavay (cited by Schuurmans Stekhoven (*107*)) and Oudendal (*84*) state that young first-stage larvae of *S. stercoralis* molt in the intestine of the host before passing out with the feces. Unfortunately there is no clue as to whether larvae seen molting in the intestine are of the direct or indirect cycle of development; however, such a molt may explain why many investigators have noted only one molt in larvae which followed the direct cycle of development, the explanation being that one molt had already occurred during the passage of the larvae through the intestine to the outside world.

According to Looss (*67*), larvae of *S. stercoralis* pursuing the indirect cycle molted only once before reaching sexual maturity. On the other hand, Lucker reports four molts in the corresponding cycle of *S. ransomi*. The progeny of the free-living forms of *S. stercoralis* and *S. ransomi*, according to Looss (*66, 67*) and Lucker, respectively, molt twice before reaching the strongyliform stage (=third stage). The writer also has noted two molts in the course of development of eggs from free-living females to strongyliform larvae.

[6] See footnote on p. 78.

Description of Egg, Embryo, and Larval Stages Undergoing Direct Development

EGG (FROM PARASITIC FEMALE)

Eggshell thin, transparent, and usually elliptical (fig. 29, *A*). In a series of measurements of 50 eggs, length 53μ to 57μ, width 30μ to 34μ; usually in late tadpole stage when passed with feces of host.

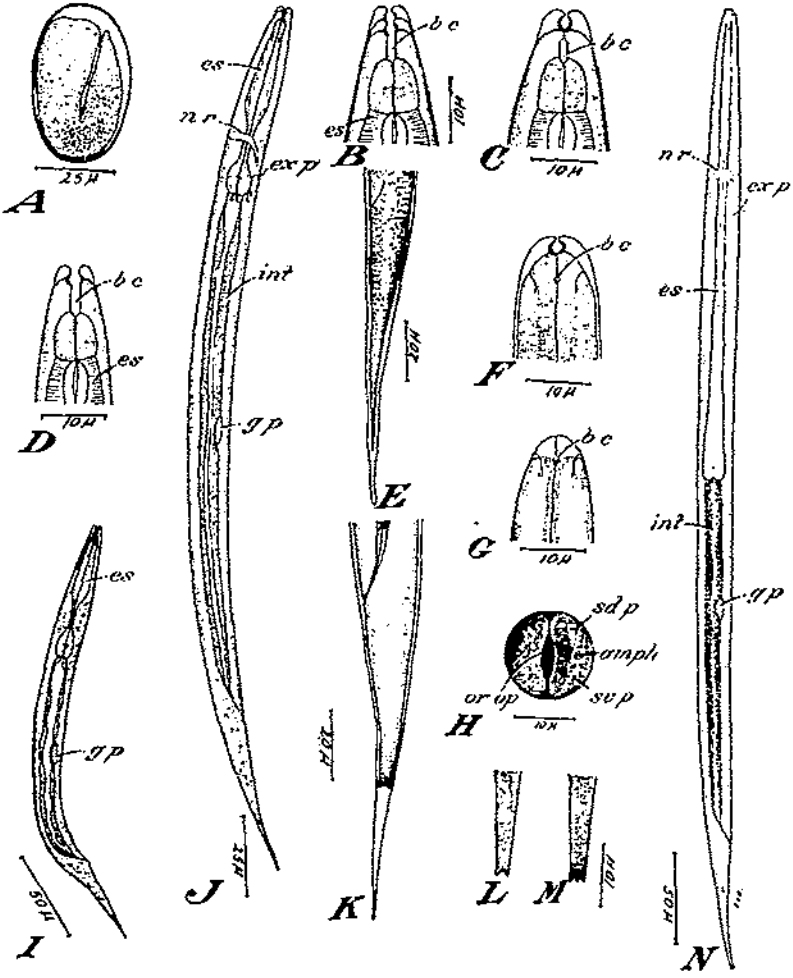

FIGURE 29.—VARIOUS STAGES IN THE DEVELOPMENT OF STRONGYLOIDES RANSOMI.

A, Egg, as recovered from freshly deposited feces.

First-stage larva: *D*, Anterior portion; *I*, newly hatched larva; *J*, larva of direct cycle of development; *B*, anterior end of larva of indirect cycle (?) of development undergoing first molt; *E*, posterior end of larva of indirect cycle (?) of development undergoing first molt.

Second-stage larva (direct cycle): *C* and *F*, Anterior end of larvae undergoing second molt; *K*, posterior end of larva undergoing second molt.

Third-stage (strongyliform) larva: *G*, Anterior end, lateral view; *H*, anterior end, en face view; *L*, showing processes at tip of tail, lateral view; *M*, showing processes at tip of tail, ventral view; *N*, lateral view of larva.

EMBRYO

Just before oviposition, embryo fairly well developed, resembling first-stage larva; just before hatching embryos 228μ to 235μ long by 15μ wide; morphology of fully developed embryo as in first-stage larva.

FIRST-STAGE LARVA

Shape and size.—Body cylindrical for most of its length, tapering slightly anteriorly and more so posteriorly (fig. 29, *I* and *J*); anterior end with 6 minute elevations possibly representing 2 subdorsal and 2 subventral papillae and 2 lateral amphids. About 2 hours after hatching, larvae 228μ to 265μ long and 13μ to 15μ in maximum width; posterior portion terminating in a long, slender, pointed tail, 41μ to 57μ long. According to Lucker, larvae, as a rule, still in first stage when about 325μ long.

Cuticle.—Very thin, transparent, and without transverse striations; apparently set off from body at anterior end by a small constriction (fig. 29, *D*).

Alimentary tract.—Mouth aperture leading into a cylindrical buccal cavity; its walls, in optical section, appearing as two sharp refringent rods (fig. 29, *D*); a short and fibrous structure (fig. 29, *D*) interpolated posterior to buccal cavity and anterior to esophagus. Esophagus rhabditiform and very muscular, 64μ to 95μ long in larvae about 3 hours after hatching. Intestine somewhat granular, its individual cells not easily differentiated; lumen of intestine in lateral view usually straight (fig. 29, *J*), differing in this respect from corresponding larvae of strongyles, which usually have a lumen with a sinuous outline; intestine connected posteriorly with a short rectum.

Nervous system.—Nerve ring encircling narrow portion of esophagus; 41μ to 60μ from anterior end in larvae about 3 hours after hatching.

Excretory system.—Excretory pore 64μ to 68μ from anterior end and leading into a short, narrow duct.

Genital primordium.—Represented by a small elliptical body ventral in position, near equator of body; 117μ to 140μ from anterior end in larvae about 3 hours after hatching. According to Lucker, genital primordium 10μ to 15μ long at time of first molt.

SECOND-STAGE LARVA

General morphology of second-stage larvae presumably similar to that of first-stage larvae. Lucker states that size attained by these larvae is variable, depending to some extent upon environmental condition during the period of feeding and growth; ordinarily maximum length is about 450μ to 550μ during process of second molt. Genital primordium 10μ to 17μ long.

THIRD-STAGE LARVA (STRONGYLIFORM)

Shape and size.—Body very slender, tapering slightly anteriorly and more so posteriorly (fig. 29, *K*); head end appearing very light in color, the color setting it off from the more granular posterior portion of the body (fig. 29, *G*). The extremely small size of the larva has led several investigators to speculate on the structure of the head. Perroncito (*87*) and L. laurmans Stekhoven (*107*) were of the opinion that the strongyliform larva possesses 3 lips. Kreis (*55*) states that the strongyliform larva of *S. similae* possesses 4 indistinctly developed lips. According to the observations of the writer, the en face view of the head of *S. ransomi* shows 2 lateral slightly raised elevations, surrounded by 2 subdorsal and 2 subventral papillae and 2 lateral amphids (fig. 29, *H*). Tail shorter than in previous stage, 68μ to 76μ long, and terminating in the characteristic 3 small processes, 2 dorsal and 1 ventral (fig. 29, *L* and *M*); the latter observation is in opposition to that of previous reports in which the tip of the tail of third-stage *Strongyloides* larvae is described as notched, with only 2 processes

Size of third-stage larvae is apparently dependent on the nutritive properties of the medium in which they grow. Schwartz and Alicata (*110*) reported that when these larvae were grown in a feces culture, they were 504μ to 635μ long by 15μ to 19μ wide; in a culture containing a small quantity of water and very little fecal decoction, the writer found that they were 405μ to 420μ long by 13μ wide.

Cuticle.—Thin, transparent, with very fine transverse striations.

Alimentary tract.—Oral opening elongated dorsoventrally (fig. 29, *H*), opening into a narrow lumen connecting posteriorly with a small and distinct buccal cavity (fig. 29, *G*). Esophagus slender and strongyliform, 250μ to 258μ long, occupying about one-half the entire length of body; inconspicuous sphincter at union of esophagus and intestine. Intestinal cells slightly granular, cell walls inconspicuous; intestine connecting posteriorly with a short rectum.

Nervous system.—Nerve ring very inconspicuous, encircling esophagus at union of anterior and middle thirds, 87μ to 90μ from anterior end.

Excretory system.—Excretory pore very inconspicuous, 102μ to 114μ from anterior end.

Genital primordium.—Somewhat elliptical in shape, about 17μ long, and 310μ to 330μ from anterior end.

Description of Larval Stages Undergoing Indirect Development

FIRST-STAGE LARVA

First-stage larva presumably rhabditiform like corresponding larva of direct cycle. According to Lucker, larvae distinguishable from first-stage larvae of direct cycle by possession of a large genital primordium, 15μ to 26μ long in larvae 230μ to 350μ long. Contrast in size of genital primordium in first-stage larvae of the two cycles of development previously noted by Schuurmans Stekhoven (*107*) in larvae of *Strongyloides stercoralis*.

SECOND-STAGE LARVA

Second-stage larva presumably rhabditiform and similar in shape to first-stage larva. According to Lucker, larvae, tentatively determined by him as second-stage larvae, 300μ to 450μ long by 20μ to 25μ wide. Genital primordium 25μ to 70μ long; tail 60μ to 78μ long.

THIRD-STAGE LARVA

Third-stage larva presumably rhabditiform and similar in shape to those of previous stages. According to Lucker, larvae, tentatively determined by him as third-stage larvae, 420μ to 550μ long by 25μ to 31μ wide. Genital primordium 60μ to 160μ long; tail 70μ to 80μ long.

FOURTH-STAGE LARVA

Fourth-stage larva presumably rhabditiform and similar in shape to previous stages; according to Lucker, sex can be differentiated at this stage. Males about 750μ long, possessing a completely formed spicular apparatus; females 750μ to 875μ long just preceding last molt; uterus and ovaries at this stage more or less completely formed.

FIFTH OR ADULT STAGE

Male.—According to Schwartz and Alicata (*110*), males (fig. 30, *D*) 868μ to 899μ long; smaller specimens 748μ long by 38μ wide also seen by writer. Body nearly equal in diameter for most of its length, tapering toward anterior and posterior ends. In en face view, head with 2 lateral liplike elevations, each possessing 1 subdorsal and 1 subventral papilla and a lateral amphid (fig. 30, *A*). According to Schuurmans Stekhoven (*107*), free-living adults of *Strongyloides stercoralis* with 3 lips; this investigator did not study head in en face view. Head of *S. ransomi*, in lateral view, distinctly set off from rest of body by shallow constriction (fig. 30, *B*). Oral opening leading into wide buccal cavity lined with thick walls (fig. 30, *B* and *C*). A short fibrous structure 9μ long interpolated anterior to esophagus and set off from it by a constriction. Esophagus very muscular, rhabditiform; in a male specimen 748μ long, esophagus 121μ long. Two spicules present; according to Schwartz and Alicata, each spicule 26μ to 29μ long, shaped like a curved blade (fig. 30, *F* and *G*). Gubernaculum 18μ to 22μ long by 9.4μ wide. Several papillae present at posterior portion of body, namely, 2 pairs preanal and 3 pairs postanal; also 1 papillalike elevation present above cloacal opening (fig. 30, *F*). Tail somewhat slender and pointed, 83μ to 90μ long.

Female.—According to Schwartz and Alicata (*110*), females (fig. 30, *G*) 1 to 1.1 mm long by 62μ in maximum width; specimens 0.8 to 1.3 mm long also observed by the writer. Shape of body and structure of head and digestive tract as in free-living adult males. Vulva with salient lips, located near equator of body. Gravid females containing several shelled eggs in various degrees of segmentation. Tail somewhat slender and pointed, 150μ to 158μ long.

Description of Egg, Embryo, and Larval Stages from Progeny of Free-Living Generation

EGG

Shape of eggs similar to those of parasitic females. In a series of measurements involving 25 eggs, length 45μ to 60μ, width 26μ to 34μ; usually embryonated

when deposited by gravid females; frequently old females fail to oviposit and their eggs hatch in the uterus.

EMBRYO

Embryo morphologically similar to embryo derived from egg of parasitic female.

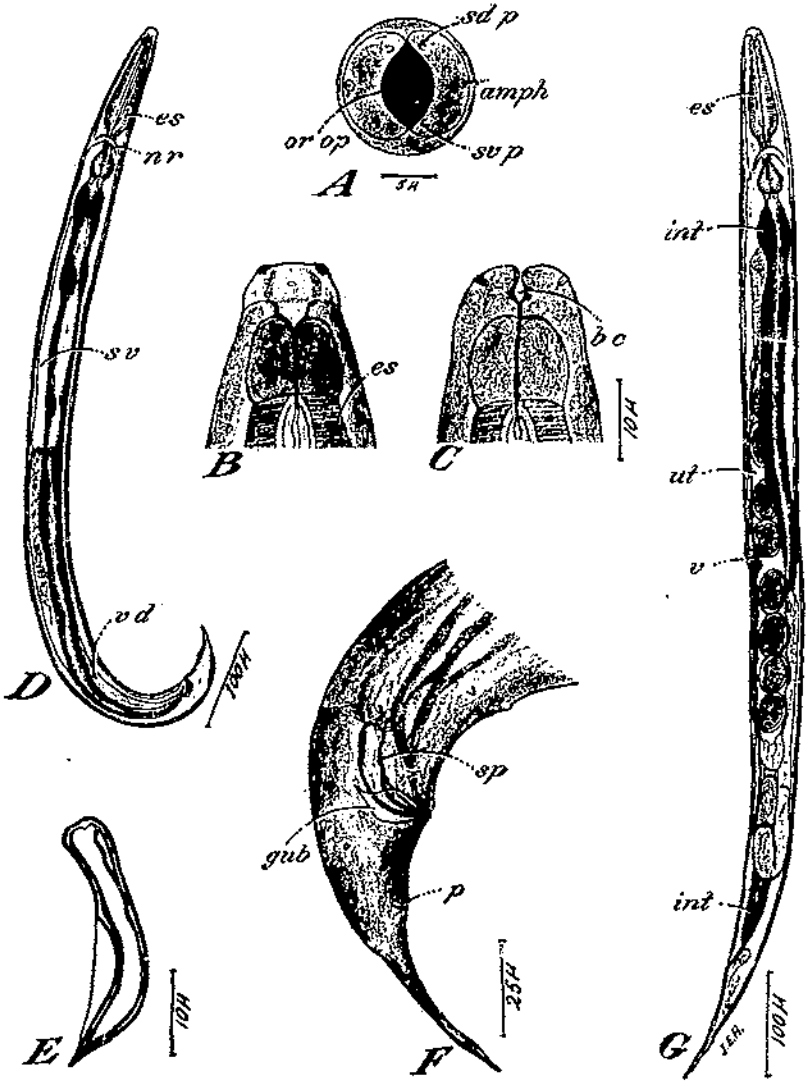

FIGURE 30.—ADULT FREE-LIVING MALES AND FEMALES OF STRONGYLOIDES RANSOMI.

Male: *D*, Lateral view; *E*, view of spicule; *F*, posterior portion of male, lateral view.
Female: *A*, Anterior end, en face view; *B*, anterior end, lateral view; *C*, anterior end, dorsal view; *G*, lateral view of female.

FIRST-, SECOND-, AND THIRD-STAGE LARVAE

These three larval stages, according to observations by Lucker, morphologically similar to corresponding stages in direct cycle.

Development of Strongyloides Ransomi Outside the Host

The development of the various stages of *Strongyloides ransomi* outside the host, in accordance with information obtained by Lucker, is as follows:

DIRECT DEVELOPMENT OF THIRD-STAGE LARVAE FROM EGGS OF PARASITIC FEMALES

At room temperature (22° to 24° C.), eggs of parasitic females hatch in from 4 to 18 hours and the larvae undergo the first molt from 12 to 18 hours later. Second-stage larvae, after a period of growth, molt a second time in less than 48 hours after incubation (fig. 29, *K*); after the second molt the larvae are in the third stage.

INDIRECT DEVELOPMENT OF FREE-LIVING ADULT MALES AND FEMALES FROM EGGS OF PARASITIC FEMALES

At room temperature (22° to 24° C.), eggs of parasitic females hatch in from 4 to 18 hours and larvae undergo the first molt about 10 hours after hatching (fig. 29, *B* and *E*). Second-stage larvae, after a period of growth, undergo a second molt from 12 to 14 hours after the first molt. The time required for the third and fourth molt is not stated; however, Lucker noted adult free-living males and females from 36 to 48 hours after incubation.

DEVELOPMENT OF THIRD-STAGE LARVAE FROM EGGS OF FREE-LIVING FEMALES

Eggs deposited by free-living females usually hatch in 12 hours. The first molt in a water medium is apparently variable, and may require about 48 hours, especially when food is scarce. Second-stage larvae apparently molt from 48 to 60 hours after incubation, as that was the period when third-stage larvae were noted by Lucker in water cultures.

Observations on Effects of Environment

SKIN PENETRATION

Lucker has shown that third-stage larvae of *Strongyloides ransomi* are capable of penetrating the skin of live pigs and rabbits, this resulting in an intestinal infestation.

RESISTANCE TO DESICCATION

A small drop of water containing a number of third-stage larvae was placed on each of several glass slides. At the moment the water evaporated, the time was noted, and the slides remained exposed at room temperature for various periods as shown in table 35. At the expiration of the desired lapse of time, a few drops of water were added to the dried larvae, and the preparations were examined at various intervals for about 24 hours.

Table 35 shows that exposure of 5 to 10 minutes to air drying destroyed the vitality of most larvae, and a 20-minutes' exposure proved fatal to all larvae.

TABLE 35.—*Results of observations on the resistance of third-stage larvae of Strongyloides ransomi to air drying at room temperature (24° C.)*

Number of larvae used	Duration of exposure	Condition of larvae after addition of water	Number of larvae used	Duration of exposure	Condition of larvae after addition of water
	Minutes			*Minutes*	
10	2	All active.	10	10	9 dead; 1 showed slight movement after 10 minutes, but was dead after 24 hours.
10	5	8 dead; 2 showed slight movement after 10 minutes, but were dead after 24 hours.	15	20	All dead.

REACTION TO COLD

Observations were made on the effects of various low temperatures on the third-stage larvae of *Strongyloides ransomi*. Each record is based on observations involving about 1,000 larvae. During the course of the experiment the larvae were kept in glass tubes containing moist animal charcoal. The results are given in table 36, which shows that exposure of 6 hours to −15° C. destroyed the vitality of some larvae, and an exposure of 25 hours to that temperature destroyed all the larvae.

TABLE 36.—*Effects of low temperature (−15° C.) on the infective larvae of Strongyloides ransomi, each culture involving about 1,000 larvae*

Culture no.	Period of refrigeration	Condition of larvae at termination of exposure	Culture no.	Period of refrigeration	Condition of larvae at termination of exposure
	Hours			*Hours*	
1	6	Majority of larvae moved slowly.	3	22	About 50 percent showed slight movement; others dead.
2	18	About 50 percent showed slight movement; others dead.	4	25	All larvae dead.
			5	30	Do.

LONGEVITY OF LARVAE IN FECES AT ROOM TEMPERATURE

General observations have indicated that the life of third-stage larvae of *Strongyloides* is somewhat short. Bruns (*14*) found that he could keep third-stage larvae of *Strongyloides* alive for 20 days. Schuurmans Stekhoven (*107*) reported that under favorable conditions of humidity and temperature, the longevity of *S. stercoralis* did not exceed 3 to 4 weeks. In 2 cases the writer has noted that the length of life of *S. ransomi* in swine feces kept in 1-quart glass jars did not exceed 11 to 13 days. This indicates that the life of the third-stage larva under field conditions is probably short. Such short duration of life is not surprising, however, since these larvae are not protected by a sheath as are third-stage larvae of most strongyle nematodes.

COMPARATIVE MORPHOLOGY OF EGGS AND THIRD-STAGE LARVAE OF SOME NEMATODES OCCURRING IN SWINE

As already indicated, the eggs and third-stage larvae of each of the various species of nematodes parasitic in swine can be differentiated from one another in most cases. The various differential characters are given in tables 37 and 38.

TABLE 37.—*Comparison of eggs of some swine nematodes soon after eggs are deposited*

EGGS WHICH USUALLY DO NOT HATCH OUTSIDE OF HOST

Nematode	Measurements of egg	Characteristics of eggshell	Degree of development and characteristics of egg when deposited
	Microns		
Gongylonema pulchrum	57–60 by 30–34	Slightly thick, smooth, colorless.	With fully formed embryo. Cephalic portion with rows of spines; anterior end of ventral portion with 2 small hooks; posterior hook about 3μ long.
Ascarops strongylina	41–45 by 22–26	Thick; surface with small punctations; colorless.	As in *G. pulchrum*, except posterior hook about 1.7μ long.

TABLE 37.—*Comparison of eggs of some swine nematodes soon after eggs are deposited*—Continued

EGGS WHICH USUALLY DO NOT HATCH OUTSIDE OF HOST—Continued

Nematode	Measurements of egg	Characteristics of eggshell	Degree of development and characteristics of egg when deposited
	Microns		
Physocephalus sexalatus	41–45 by 22–26	Thick; surface with small punctations; colorless.	As in *G. pulchrum*.
Metastrongylus salmi	43–57 by 38–41	Thick; surface slightly mammilated; dark grayish.	With fully formed embryo; spines or hooks absent.
Metastrongylus elongatus	45–57 by 38–41	do	Do.
Choerostrongylus pudendotectus	60–64 by 43–45	do	Do.
Ascaris suum	68–84 by 50–76	Thick; surface usually coarsely mammilated; brownish yellow.	Usually in 1-cell stage.
Trichuris suis	60–68 by 28–31	Thick; surface smooth; lemon shaped; dark brown.	In 1-cell stage.

EGGS WHICH HATCH OUTSIDE OF HOST

Hyostrongylus rubidus	60–76 by 31–38	Thin; surface smooth, colorless.	In early tadpole stage.
Oesophagostomum dentatum	61–84 by 38–53	Thin; surface smooth, colorless.	Usually with 8 to 16 cells.
Stephanurus dentatus	91–114 by 53–65	do	With about 32 to 64 cells.
Strongyloides ransomi	53–57 by 30–34	do	In late tadpole stage to early vermiform embryo stage.

TABLE 38.—*Comparison of third-stage (infective) larvae of some nematodes occurring in swine*

Nematode	Size	Differential characters	Location
Gongylonema pulchrum	1.90–2.45 mm by 50–68μ.	Lateral border of mouth projects outward; esophagus divided into 2 parts; tail with 2 or 4 small digitiform processes.	Encysted in insects or accidentally, possibly in vertebrates.
Ascarops strongylina	1.91–2.32 mm by 53–91μ.	Head with 2 dorsoventral liplike elevations; esophagus divided into 2 parts; tip of tail with smooth knob.	Encysted in insects or, accidentally, in vertebrates.
Physocephalus sexalatus	1.35–1.60 mm by 60–68μ.	Head and esophagus as in *A. strongylina;* tip of tail with many small digitiform processes.	Do.
Metastrongylus salmi	550–630μ by 26μ	Head rounded; esophagus strongyliform; near end of tail, 2 somewhat indistinct notches.	Usually in esophageal wall or blood vessels of annelid s ensheathed.
Metastrongylus elongatus	625–665μ by 26μ	Head and esophagus as in *M. salmi;* near end of tail, usually 2 deep notches.	Do.
Choerostrongylus pudendotectus	600–655μ by 26μ	As in *M. salmi*	Do.
Hyostrongylus rubidus	715–735μ by 22μ	Head rounded; esophagus strongyliform; buccal cavity spearhead-shaped; tip of tail with a digitiform process.	Usually in feces; ensheathed.
Oesophagostomum dentatum	500–532μ by 26μ	Head and esophagus as in *H. rubidus;* buccal cavity with lining drawn out into thin strands of interlacing fibers; tail somewhat pointed.	Do.
Stephanurus dentatus	518–610μ by 24–26μ	Head and esophagus as in *H. rubidus;* buccal cavity spindle-shaped; tip of tail rounded.	Do.
Strongyloides ransomi	501–635μ by 15–19μ	Head rounded; esophagus slender, occupying about one-half length of worm; tip of tail with 3 small pointed processes.	Usually in feces; ensheathed.

SUMMARY

A study was made of the early developmental stages of the following nematodes parasitic in swine: *Gongylonema pulchrum*, *Ascarops strongylina*, *Physocephalus sexalatus*, *Metastrongylus salmi*, *M. elongatus*, *Choerostrongylus pudendotectus*, *Ascaris suum*, *Trichuris suis*, *Hyostrongylus rubidus*, *Oesophagostomum dentatum*, *Stephanurus dentatus*, and *Strongyloides ransomi*. For two of the nematodes, however, *G. pulchrum* and *H. rubidus*, observations on the stages in the definitive host are also discussed briefly. The nomenclature of each parasite, its host relationship, geographic distribution, morphology, and bionomics of its early stages are given.

When fed to roaches, eggs of *Gongylonema pulchrum* hatched within 24 hours and the larvae developed to the infective or third stage in about 32 days. These larvae encysted within the sarcoplasm of the muscle fibers of the insect host. When fed to guinea pigs, third-stage larvae promptly penetrated the tissue at the junction of the stomach and esophagus, usually entering the wall of the esophagus in this region, and migrated under the linings of the esophagus and of the oral cavity. Under these linings the worms were found sexually mature 70 days after experimental infection. Eggs of *G. pulchrum* contained viable embryos after 4 months' exposure to outdoor-conditions at from —6.6° to 37.7° C., and after being kept 4 months at room temperature (22° to 24°). Three new intermediate hosts for this parasite are reported in this bulletin, namely, *Aphodius lividus*, *Dermestes vulpinus*, and *Parcoblatta* sp.

When fed to beetles, eggs of *Ascarops strongylina* hatched within 24 hours, and the larvae developed to the infective stage in about 29 days. Eggs of this parasite contained viable embryos after 20 days' exposure at from —4° to 2° C., and after being kept 4 months at room temperature (22° to 24°). Two new intermediate hosts are reported in this bulletin for this parasite, namely, *Aphodius granarius* and *Passalus cornutus*.

When fed to beetles, eggs of *Physocephalus sexalatus* hatched within 24 hours and developed to the infective stage in 36 days. Eggs of this parasite contained viable embryos after being kept at a temperature of from —4° to 2° C. for 20 days. Two new intermediate hosts for this parasite are reported in this bulletin, namely, *Ataenius cognatus* and *Passalus cornutus*.

The larvae of *Metastrongylus salmi* have been successfully reared to the third stage in earthworms, *Lumbricus terrestris* and *Helodrilus caliginosus* var. *trapezoides*. Third-stage larvae may remain alive in the body of the earthworm for at least 4 months.

In a study of the development of *Metastrongylus elongatus* and *Choerostrongylus pudendotectus*, third-stage larvae of these parasites have been found in the bodies of earthworms as late as 9 months after experimental infection. Lungworm eggs eliminated with the feces of swine do not hatch outside the host. The surface of the eggshell is corrugated.

Eggs of *Ascaris suum* were found to reach the infective stage in 16 days at 33° C., in 18 days at 30°, and in 28 days at 22° to 24°. The infectivity of the egg was determined by the molting of the embryo within the shell and by the ability of the eggs to produce

an infestation when fed to guinea pigs. Nonmolted embryos were not infective to guinea pigs.

Eggs of *Trichuris suis* were found to contain fully developed embryos in 18 days when kept at 37.5° C., in 22 days at 33°, in 54 days at room temperature (22° to 24°), and in about 7 months outdoors when kept underground; the temperature outdoors during the 7 months was from 6.1° to 24.5°.

Eggs of *Hyostrongylus rubidus* cultured at room temperature hatched and the larvae developed to the infective stage in 7 days. Third-stage larvae fed to guinea pigs developed in the stomach without undergoing further migration and reached the fifth stage in from 17 to 19 days. In the preparasitic larval stages of *H. rubidus* sex was differentiated by the position of the posterior cell of four giant cells in the body cavity of the larva. Preparasitic larvae in which this giant cell is anterior to the genital primordium develop into males, whereas those in which this cell is slightly lateral or posterior to the genital primordium develop into females. The general development of the male and female reproductive systems was traced in the four larval stages of this parasite. Third-stage larvae of *H. rubidus* showed the following characteristics: (1) They failed to penetrate the skin of young rats or guinea pigs; (2) they were resistant to a temperature of 3° to 5° C. for 6 days, but not to a temperature of −5° to 1° for 30 days; (3) their vitality was destroyed when they were kept at −20° for 9 hours; (4) they were killed when subjected to air drying for 4 hours; (5) in water-charcoal culture, they survived for 2½ months but not for 3½ months.

Eggs of *Oesophagostomum dentatum* cultured at room temperature hatched, and the larvae developed to the infective stage in about 6 days. Third-stage larvae of *O. dentatum* showed the following characteristics: (1) They failed to penetrate the skin of young rats under experimental condition; (2) some larvae showed signs of life when exposed to −19° to −29° C. for 10 days; however, their vitality was destroyed when kept at a temperature of −15° to −29° for 31 days; (3) larvae were killed when subjected to air drying for 22 hours; (4) larvae kept in water-charcoal culture for about 10 months appeared normal.

Eggs of *Stephanurus dentatus* cultured at room temperature (22° to 24° C.), hatched and the larvae developed to the infective stage in about 5 days. Third-stage larvae have been found to possess the following characteristics: (1) They failed to penetrate the skin of 2-day-old rats; (2) they succumbed after 1 hour's exposure to air drying at room temperature; (3) their vitality was not destroyed when they were exposed for 6 hours at −19° but an exposure for 9 hours at that temperature proved fatal; (4) they were found to be normal after being kept in water-and-charcoal media for 40 days.

The life of *Strongyloides ransomi* in feces-and-charcoal cultures kept in bottles at room temperature did not exceed 11 to 13 days. Strongyliform larvae were found to perish when subjected to air drying for about 20 minutes, and when subjected to −15° C. for 25 days.

It was determined that the shape of the buccal cavity usually serves to differentiate between the third-stage larvae of the various parasites discussed in this bulletin.

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